Optical Measurements of Transmembrane Potential Changes During Electric Field Stimulation of Ventricular Cells

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We evaluated transmembrane potential changes at the ends of isolated rabbit ventricular myocytes during defibrillation-strength shocks given in the cellular refractory period. The myocytes were stimulated (S1 pulse) to produce an action potential. Then a constant-field shock (S2 pulse) with an electric field of 20 or 40 V/cm was given at an S1–S2 interval of 50 msec. The cells were stained with photometric dye (di-4-ANEPPS), and the cell end facing the S2 anode or cathode was illuminated with a laser while the fluorescence was recorded. During S2, the cell end facing the S2 cathode became more positive intracellularly, whereas the cell end facing the S2 anode became more negative intracellularly. The S2-induced transmembrane potential change at the cell end (ΔV_m) was determined relative to the amplitude of the S1-induced action potential (APA) in each recording (i.e., ΔV_m/APA). In Tyrode’s solution containing 4.5 mM potassium, ΔV_m/APA for 40-V/cm S2 was 1.36±0.34 at the cell end facing the S2 cathode and -1.65±0.61 at the cell end facing the S2 anode (n=9). For the 20-V/cm S2, ΔV_m/APA was 0.61±0.33 at the cell end facing the S2 cathode and -0.71±0.33 at the cell end facing the S2 anode (n=6). The ΔV_m/APA was not significantly influenced by 20 mM diacetyl monoxime. These results indicate that large ΔV_m values occurred at the ends of the cells during S2. The calculated values of ΔV_m, assuming a nominal APA of 130 mV, were 177 and -214 mV for the 40-V/cm S2 and 79 and -93 mV for the 20-V/cm S2. The ΔV_m was correlated with cell size (r=0.95) and agreed with values predicted by the S2 electric field strength multiplied by half of the cell length to within 27%. When the potassium concentration was increased to 20 mM, ΔV_m/APA for 40 V/cm S2 increased 85% and 67% at the cell ends facing the S2 cathode and anode, respectively (n=9, p<0.005 versus 4.5 mM potassium), consistent with reduced APA. Thus, with normal or elevated extracellular potassium, transmembrane potential changes at the ends of cells during defibrillation-type stimulation are large enough to produce activation or recovery of voltage-dependent ion channels and may produce the effects responsible for defibrillation. (Circulation Research 1993;72:255–270)

KEY WORDS • transmembrane action potentials • ventricular myocytes • myocardial stimulation • defibrillation • voltage-sensitive fluorescent dye • potassium • diacetyl monoxime

Cellular excitation or refractoriness prolongation, effects that can occur after a defibrillation-type shock,1–7 are thought to result from changes in the transmembrane potential during the shock. Understanding the mechanisms for the effects after a shock, and hence defibrillation, will require knowledge of the transmembrane potential change during the shock. Theoretical models predict that electric field stimulation hyperpolarizes one end of the cell and depolarizes the other end.8–10 This idea has been used to explain experimentally observed effects of electric fields on myocardial cells11,12 and the heart.5,13 However, there is only a small amount of experimental evidence describing transmembrane potential changes at the ends of cells exposed to electric fields,14,15 and no evidence has been previously reported for myocardial cells or for shocks given in the cellular refractory period, which is the cellular state at most instants during fibrillation.16,17 This article describes measurements of the transmembrane potentials at the ends of ventricular myocytes during electric field stimulation applied in the cellular refractory period.

Materials and Methods

Experimental Preparation

Ventricular myocytes were isolated from rabbit hearts with methods previously described.18,19 Cells were typically used on the same day that the isolation was...
performed. In two experiments, cells were used on the following day and gave similar results. The cells were prepared on glass coverslips, and laminin was added to the solution to stabilize the cells on the glass. The cells were washed with approximately 2 ml Tyrode's solution while the coverslip was slightly tilted to reduce the number of cells on each slide. The remaining cells were then placed in an experimental chamber with approximately 4 ml solution supplied either as a single bolus with a syringe or at a continuous rate of 0.2 ml/min with a roller pump. HEPES-buffered Tyrode's solution was used at room temperature equilibrated with air and contained (mM) glucose 5, CaCl\textsubscript{2} 1.5, NaCl 150, KCl 4.5, MgCl\textsubscript{2} 1, HEPES 10, and NaH\textsubscript{2}PO\textsubscript{4} 1.2, which was titrated with NaOH to a pH of 7.4 and contained 0.5% ethanol saturated with potentiometric dye (di-4-ANEPPS; Molecular Probes, Inc., Eugene, Ore.).\textsuperscript{20} The final concentration of di-4-ANEPPS was 0.005 mM. In some experiments the solution potassium concentration was increased to 20 mM by adding KCl and removing an equivalent amount of NaCl to maintain the same total ionic strength. The solutions were checked by measuring the Na\textsuperscript{+}, Ca\textsuperscript{2+}, and K\textsuperscript{+} content with an electrolyte analyzer (Nova Biomedical, Waltham, Mass.) and measuring the osmolarity with a vapor pressure osmometer (model 5500, Wescor Inc., Logan, Utah). The solution osmolarity was 288±2.8 mos. In some experiments the electromechanical uncoupler diacetyl monoxime was added to the solution at a concentration of 20 mM.\textsuperscript{21} Experimental trials were begun after 10 minutes of exposure to di-4-ANEPPS and were completed after 28±11 minutes of exposure. The cells chosen for experimentation had an approximate rod shape and cross striations and were oriented with the long axis in line with the stimulation electric field. A total of 82 cells were studied. Fluorescence recordings of the effects of field stimulation during the action potential were obtained from 65 cells. Acceptable recordings (criteria for acceptance in 4.5 mM potassium or 20 mM potassium are described in “Results”) were obtained from 21 cells, which were 119±29 \(\mu\)m long and 22±4 \(\mu\)m wide.

**Experimental Setup**

Figure 1 shows a diagram of the custom-built apparatus to apply electric field stimulation to a myocardial cell, record the electric field strength, view the cell microscopically, stain the cell with transmembrane voltage-sensitive fluorescent dye, illuminate a selected end of the cell, and record the fluorescence from the cell end. The apparatus was assembled on a 12×47-in. optical table (Newport, Fountain Valley, Calif.) that was mechanically isolated with air cushions.

**Cell Illumination**

The cell image was obtained with transillumination by a miniature battery-powered lamp positioned below the experimental chamber. The image was reflected by dichroic and nondichroic beamsplitters and then passed upward through focusing lenses (L6 and L7, Figure 1) and an aperture to a video camera (model XC-77, Sony Component Products, Cypress, Calif.). The image was viewed on a video monitor (diagonal size, 9 in.) with an overall magnification of ×350 to determine the cell...
orientation with respect to the electric field, the cell size, the cell contraction after stimulation, and the location and size of the epi-illumination spot at the cell ends. In some instances the video image was photographed with a Polaroid camera (model C-5C, Tektronix, Beaverton, Ore.). At the end of the experiment, the size scale on the video monitor was calibrated by positioning a glass reticle with 100 μm divisions in place of the cell.

Epi-illumination of a selected end of the cell was performed with a 488-nm argon laser (model 532, Omnichrome, Chino, Calif.). The laser cooling fan was mechanically isolated from the optical apparatus with a flexible air pipe containing a heavy iron midsection. The laser beam passed through beam expansion lenses (L1 and L2, Figure 1) and was reflected 180° by mirrors. The beam then passed through a vertical cylindrical lens (C1, Figure 1), a vertical acousto-optic deflector (model ADM150, Interaction, Bellwood, Ill.), a horizontal cylindrical lens (C2, Figure 1), a convex lens (L3, Figure 1), an aperture, and a focusing lens (L4, Figure 1). Approximately 10% of the laser light was then reflected by a beamsplitter downward through a focusing lens (L5, Figure 1) to a silicon photodiode (model HAD100A, EG & G Photon Devices, Salem, Mass.). Laser intensity fluctuations of approximately 3–4% were compensated by feeding the amplified and inverted photodiode signal to the acousto-optic deflector driver (model DE, Interaction). With the compensation, the intensity of light reaching the silicon photodiode was constant to within approximately 0.2%. The laser light that passed straight through the nondichroic beamsplitter was reflected downward by the dichroic beamsplitter to the objective lens (numerical aperture, 0.50; model 170/0.17 25/0.50, Leitz, Wetzlar, FRG) and to the cell.

The objective lens housing diameter was 20 mm, the lens diameter was 5.5 mm, and the distance from the lens to the cell was approximately 1 mm. The beamsplitters, lens holders, and focusing, cylindrical, and beam expansion lenses were obtained from Newport. The focal lengths of the lenses were 9.5 mm for L1, 205 mm for L2, 200 mm for C1 and C2, 20 mm for the combination of L6 and L7, 100 mm for L3, 58.8 mm for L4, 12.5 mm for L5, and 25 mm for focusing lens L8 (Figure 1).

In each experimental trial, a laser spot with the minimum intensity needed to monitor the spot was positioned at one end of the cell (Figure 2). In pilot experiments, the diameter of the laser spot was varied from approximately 2 μm, when the top surface of the cell was at the focal point of the laser spot, to a value larger than the cell diameter. The spot diameter was adjusted by moving the cell slightly above or below the focal point of the laser spot. When the spot diameter was increased to match the cell diameter, the total fluorescence and signal-to-noise ratio increased. In all later experiments, the spot diameters were adjusted to match the cell diameter.

The laser illumination in each trial began approximately 50 msec before the S1 stimulation and lasted approximately 500 msec. The onset and termination of the illumination were controlled by timers that were connected to the stimulation apparatus and the acousto-optic deflector driver.

Experimental trials were performed in which fluorescence was collected from a selected end of a cell. For each trial, the transillumination lamp was turned off, the high voltage to the photomultiplier tube (model R446, Hamamatsu Corp., Bridgewater, N.J.) was turned on, and the laser light passing through the acousto-optic deflector was set to a switched mode with no light before and after trials and with 0.1–0.5 mW of light illuminating the cell end during trials. The intensity of the laser illumination was measured in some experiments with a digital power meter (model 815, Newport) having a probe (model 818-st, Newport) positioned in place of the experimental chamber and cell.

Fluorescence Collection

The fluorescence emitted from the illuminated end of the cell was collected by the objective lens and passed upward through the dichroic beamsplitter, a focusing lens (L8), and a 645-nm long-pass filter (model RG645, Newport) to the photomultiplier tube. The photocathode high-voltage supply (model C956-04, Hamamatsu) was −1,000 V. The anode current was passed to an amplifier with a current-to-voltage gain of 10^5 V/A.

The fluorescence voltage signal was passed simultaneously through three parallel low-pass single-pole filters with −3-dB frequencies of 50, 500, and 5,000 Hz. The 50-Hz filter provided a sufficient signal-to-noise ratio to identify the action potential in recordings with solution containing 4.5 mM potassium, in which the fluorescence change during the action potential upstroke as a fraction of the total fluorescence (i.e., fractional fluorescence) was greater than 5%. For example, a trial that had a fractional fluorescence of 9% (the trial in Figure 3A) had ratios of the action potential amplitude to the peak-to-peak fluorescence noise of approximately 2.5, 1, and 0.31 with the 50-, 500-, and 5,000-Hz filters, respectively. The 50-Hz low-pass filter slowed the response time of the fluorescence recordings slightly, which can be seen by comparing the fast shifts in the 500-Hz-filtered data (individual data points in Figure 6) at the onset of the S2 stimulation with the shifts in the 50-Hz-filtered data (solid line). The 50-Hz filtering did not alter the action potential contour, the action potential amplitude, or the transmembrane potential changes induced during the S2 pulse.

In two experiments, the possibility that the electrical stimulation pulse introduced an artifact in the recording that was not related to fluorescence changes at the cell ends was eliminated by positioning the laser spot away from the cell. When trials with S1 and a 40-V/cm S2 of either polarity were performed, the photomultiplier signal during laser illumination was constant; i.e., S1 and S2 did not influence the recording.

Optical Alignment

The experimental chamber, the photomultiplier tube, the aperture in front of the video camera, the focusing lens L4, the photodiode, and the beam expansion lens (L1) were mounted on x-y-z micromanipulators (model M3301, World Precision Instruments, New Haven, Conn.). Before each experiment, the manipulators were adjusted to align the illumination and monitoring optics.

The position of the most sensitive part of the photomultiplier photocathode grid in relation to the image of the laser illumination spot was optimized with the
transillumination lamp off and the laser on continuous power. The photomultiplier tube position was adjusted while the photomultiplier output level was monitored with an oscilloscope. The adjustment was performed with a fluorescent crayon in place of the cell or with a cell. The cell was damaged by such continuous laser illumination; hence, a different cell was then chosen for experimentation. Once the apparatus was aligned, the desired cell end was illuminated by repositioning the experimental chamber and cell without altering the alignment of the optics.

The alignment of the optical components other than the photomultiplier tube and the positioning of the cell were performed with the transillumination lamp on, the laser light that passed through the acousto-optic deflector set at the lowest continuous power needed to observe the laser spot on the video monitor, and the high voltage of the photomultiplier tube turned off to prevent tube saturation by the transillumination lamp.

**Electrical Stimulation**

In each experimental trial, a 5-msec electric field stimulation pulse (S1) was applied at a strength of two to five times the diastolic threshold strength. The S1 and the test shock (S2), having an S2 electric field strength of 20 or 40 V/cm and an S2 duration of 50 msec, were given from large Ag/AgCl electrodes (S, Figure 1) at the ends of the experimental chamber. The interval from the onset of S1 to the onset of S2 was 50 msec. S1 and S2 were rectangular pulses produced by isolated voltage sources connected to the stimulating electrodes through photovoltaic relays (model PVR1301, International Rectifier, El Segundo, Calif.). The S2 current (0.2–1 A) passed through a custom-made current-limiting circuit that produced a constant S2 current (Figure 6) and a nearly constant S2 electric field in each trial. S1 and S2 timing and pulse duration were controlled by stimulators (models S44 and S88, Grass Instruments, Inc., Quincy, Mass.) connected to the control inputs of the photovoltaic relays. The turn-on time of the relays was less than 100 μsec.

The electric field strength during stimulation was measured from 25- or 50-μm-diameter enameled tungsten wire recording electrodes (R, Figure 1) on either side of the cell. The wires were cemented to the objective lens housing. The free ends extended 1 mm through the solution radially inward from either side of the lens toward the focal point. The distance from the wire tips to the lens surface was 0.2–0.3 mm. The tips of the wires were 2.8 or 3.4 mm apart. The distance between the tips was measured before the experiments with a dissecting microscope and reticle having graduations every 100 μm. The lens was oriented so that the wire tips were aligned with the stimulation electric field. The potential difference between the tungsten electrodes was recorded with a custom-made differential amplifier having an input resistance of 10^12 Ω and a gain of 1. The stimulation electric field strength was determined by dividing the potential difference by the distance between the recording electrodes. In some experiments, the stimulation current and voltage at the stimulation electrodes were also recorded with isolation amplifiers (model AD210AN, Analog Devices, Norwood, Mass.) and a 1-Ω current-measuring resistor.

Pilot experiments indicated that the S1 stimulation threshold strength required to excite the cells, as indicated by the cell contraction, underwent large reversible increases when an uninsulated objective lens housing was brought within 1–2 mm of the cells. The conductive surface of the objective lens housing was then covered with a thin insulating layer of epoxy that extended onto the edge bevel of the lens. The insulation prevented shunting of the electric field in the region of the focal point by the conductive lens housing. With the objective housing insulated, the S1 stimulation threshold was not sensitive to changes in the distance from the objective lens to the cell.

**Data Recording, Analysis, and Display**

The stimulation electric field and 50-Hz fluorescence signal were monitored during the experiments with a storage oscilloscope (model 5D10, Tektronix). The stimulation and fluorescence signals were sampled at a rate of 2,000 samples per second per channel with a computer (Macintosh model IIf, Apple Computer Corp., Cupertino, Calif.) containing an analog-to-digital converter (model MIO16, National Instruments, Austin, Tex.) and data acquisition and analysis software (LABview, National Instruments).

For each recording with S2, the fluorescence intensity 25 msec before S1, the decrease in the fluorescence intensity corresponding to the amplitude of the S1-induced action potential, and the magnitude and direction of the change in the fluorescence intensity 25 msec after the onset of S2 applied during the action potential plateau were measured. The change in the fluorescence intensity due to S2 during the action potential plateau was determined by subtracting the change in the fluorescence intensity corresponding in time to recordings having an S1-induced action potential but no S2. Such changes occur because of normal repolarization of the S1-induced action potential and photobleaching of the dye. The effect of shot noise was reduced in each measurement by determining the average fluorescence over a 25-msec interval. Statistical significance was determined with two-tailed nonpaired t tests. Values of p<0.05 were considered significant. Numerical results are given as the mean±1 SD.

The laser illumination produced an increase in the fluorescence, whereas depolarization of the cell membrane produced a decrease in the fluorescence. The inverted fluorescence signal is shown in the figures so that membrane depolarization corresponds to an upward deflection.

**Results**

**Cellular Tolerance of Laser Illumination**

In pilot experiments with solution containing 4.5 mM potassium, laser illumination with sufficient intensity to obtain fluorescence action potential recordings with an action potential amplitude-to-noise ratio greater than 2 produced irreversible cell contracture after approximately 5 seconds of continuous illumination. The time of laser exposure was then lessened by flashing the laser illumination on and off automatically before and after each trial. The effect of repeated flashes was evaluated in 17 dye-stained cells. A series of laser flashes having a duration of 500 msec was given at one end of the cell at
1-minute intervals between flashes. The cells tolerated 5.8±1.6 flashes without undergoing irreversible cell contracture. Because of the cellular sensitivity to the laser illumination, the number of laser flashes used on a given cell was then limited to five, and in most instances only three flashes were used.

**Magnitude of Fluorescence Change During the Action Potential Upstroke**

In 40 cells bathed in solution containing 4.5 mM potassium, the change in the fluorescence corresponding to the amplitude of the S1-induced action potential at a cell end was determined as a fraction of the baseline fluorescence before S1, i.e., fractional fluorescence. Of the 40 cells, 29 (73%) gave recordings with fractional fluorescence of 3% or greater, 12 (30%) gave recordings with fractional fluorescence of 5% or greater, and 7 (18%) gave recordings with fractional fluorescence of 7% or greater. Results from the 12 cells that gave recordings with fractional fluorescence of 5% or greater were accepted into the analysis for experiments with 4.5 mM potassium. These cells were 108±29 μm long and 22±4 μm wide.

**S1-Induced Excitation at the Cell Ends**

Figure 2 shows photographs of a cell and the laser illumination spot. The S1 and S2 electric fields were oriented from left to right. In Figure 2A the laser spot was positioned at the left end of the cell, which was the end facing the electric field anodal electrode. In Figure 2B the laser spot was positioned at the opposite end of the same cell, i.e., the end facing the cathodal electrode.

Figure 3 shows the S1 electric field strength (top tracing) and fluorescence (bottom tracing) at the ends of a cell during and after application of the S1 stimulation. The cell was bathed in solution containing 4.5 mM potassium. S1 had an electric field strength of 17 V/cm. The S1 stimulation elicited an action potential that is seen as a decrease in the fluorescence (i.e., an upward deflection in the fluorescence recording).

The upward deflection of the fluorescence at the cell end facing the S1 anode (Figure 3A) occurred later and was faster than the deflection at the cell end facing the S1 cathode (Figure 3B). At the cell end facing the S1 anode, the transmembrane potential became hyperpolarized during the S1 pulse and then depolarized at the end of the S1 pulse. The transition from the hyperpolarized state during the S1 pulse to the depolarized state of the action potential after S1 increased the size and speed of the deflection of the depolarization. On the other hand, at the cell end facing the S1 cathode (Figure 3B), the transmembrane potential was depolarized during the S1 pulse and reached the level of the action potential plateau by the end of the S1 pulse.

**Correlation of Fluorescence Change With Cell Movement**

In experiments without diacetyl monoxime, the cell mechanical contraction occurred after S1 stimulation. The contraction was observed while monitoring the cells at high magnification with the video camera and transillumination. The S1 end was attached to an audio indicator that allowed the relation of S1 and cell contraction to be observed in each cell. The contraction after S1 verified that the S1 strength was greater than the excitation threshold strength.

The contraction was also apparent in the fluorescence recordings from cells without diacetyl monoxime. The contraction produced a motion artifact that increased or decreased the fluorescence intensity after the initial plateau of the action potential induced by S1.

It was observed with the video camera that the displacement of the cell end during the contraction was often much greater at one end of a cell than the other end. This is consistent with one end of the cell being attached to the glass beneath the cell. Likewise, the motion artifact in the fluorescence recording was often greater at one end of the cell than at the other end.

**Correlation of Fluorescence Change With Excitation**

That the fluorescence decrease during or immediately after S1 indicated membrane excitation was verified by correlating the fluorescence decrease with the cell contraction that occurred after S1 and by examining the temporal relation between the fluorescence decrease and S1. In two experiments without diacetyl monoxime, the contraction after S1 was observed repeatedly with the video camera. The S1 strength was then increased approximately 50% to ensure that S1 remained superthreshold while the transillumination lamp was turned off and a fluorescence recording was obtained. After the recording, the contraction corresponding to S1 was again observed with the video camera, thus confirming that the cell underwent excitation during the fluorescence recording. Examination of the recording confirmed that the fluorescence exhibited an abrupt decrease during or immediately after S1.

In other cells in solutions containing 4.5 mM potassium with (n=9) or without (n=3) 20 mM diacetyl monoxime, a 5–10% fluorescence decrease associated with S1-induced membrane excitation occurred. In contrast, in trials in which no S1 was given, the fluorescence did not decrease. The fluorescence decrease with superthreshold S1 occurred during or immediately after the S1 pulse (Figures 3–7), indicating the temporal relation of the fluorescence decrease with the S1-induced excitation. Finally, the direction of the change in the fluorescence after the superthreshold S1 was always a decrease in fluorescence regardless of the polarity of the S1 stimulation electric field or which end of the cell was illuminated. These observations indicate that the fluorescence decrease represented S1-induced excitation of the cell membrane.

**Effect of Diacetyl Monoxime on Cell Contraction**

In two cells bathed in solution containing 4.5 mM potassium, the ability of diacetyl monoxime to reduce the cell contraction after S1 was evaluated. Without the diacetyl monoxime, the S1 strength was adjusted to approximately two times the threshold for stimulation. The cell was stimulated regularly at an S1 rate of 0.5 Hz. Contraction was observed with the video camera after each S1 stimulation. The S1 stimulation strength was then increased to ensure that S1 remained superthreshold, and the bathing solution was aspirated from the experimental chamber and replaced with Tyrode’s solution containing 20 mM diacetyl monoxime. The visible cell contraction induced by each S1 decreased steadily during the next beats and became negligible within 30
seconds after adding the diacetyl monoxime. The solution was again aspirated and replaced with normal Tyrode’s solution. The cell contraction after the S1 stimulation then increased steadily.

Effect of Electric Field Stimulation During the Action Potential

Figure 4 shows recordings of the electric field and fluorescence for a cell bathed in solution containing 20 mM diacetyl monoxime and 4.5 mM potassium. The motion artifact was reduced sufficiently so that the action potential contour including repolarization could be seen (Figure 4A). The action potential depolarization occurred after S1, as it did without the diacetyl monoxime.

The effects of S2 having a strength of 40 V/cm on the fluorescence recordings at each of the ends of the cell are shown in Figures 4B and 4C. The transmembrane potential at the cell end facing the S2 anode became more negative during S2 (Figure 4B), whereas the transmembrane potential at the cell end facing the S2 cathode became more positive during S2 (Figure 4C). The magnitudes of these changes in transmembrane potentials at the ends of the cell during S2 were considerably greater than the amplitude of the S1-induced action potential.

Effect of Changes in Strength and Polarity of Electric Field Stimulation

Figure 5 shows results obtained with the laser spot at one end of a myocyte in solution containing 20 mM
diacetyl monoxime and 4.5 mM potassium. In the fluorescence recording in Figure 5A, no S2 was given, and the normal S1-induced action potential is seen. In the other recordings, S2 having an electric field strength of 20 or 40 V/cm was given during the action potential. When the illuminated cell end faced the S2 anode (Figure 5B), the 20-V/cm S2 produced a negative deflection in the transmembrane potential. When the illuminated cell end faced the S2 cathode after reversing the S2 polarity (Figure 5D), S2 produced a positive deflection. The 40-V/cm S2 produced deflections in the same directions as the 20-V/cm S2; however, the magnitudes of the deflections were approximately twice as large (Figures 5C and 5E). Again, the magnitudes of these changes in transmembrane potentials at the ends of the cell during S2 were large in comparison with the amplitude of the S1-induced action potential.

Rapid Transmembrane Potential Changes Induced by Field Stimulation

Figure 6 shows the transmembrane potential changes induced by S1 and S2 in a cell bathed with solution containing 20 mM diacetyl monoxime and 4.5 mM potassium. The time base is expanded to show the time courses of the transmembrane potential changes at the onset of S1 and S2 stimulation. The data points from the fluorescence recording that was filtered at 500 Hz are superimposed with the fluorescence recording filtered

FIGURE 3. Recordings of S1 electric field strength and the fluorescence emitted from the ends of the cell of Figure 2. Fluorescence recordings are shown with the 50-Hz low-pass filter (solid lines) and the 500-Hz low-pass filter (individual data points). At the end of the cell facing the S1 anode (panel A), the transition from a hyperpolarized state during S1 pulse to the depolarized state of the action potential after S1 produced a larger total deflection of the depolarization than seen at the cell end facing the S1 cathode (panel B). The baseline fluorescence recorded at the cell end facing the anode was approximately 30% greater than the baseline fluorescence at the cell end facing the cathode. The percent changes in the fluorescence during the action potential depolarizations were 10.4% and 9% at the cell ends facing the stimulation anode and cathode, respectively. The solution contained 4.5 mM potassium. The calibration bars on the left side of the recordings represent an electric field of 10 V/cm or a fluorescence change of 10% of the baseline fluorescence.

FIGURE 4. Recordings of S1 and S2 electric field strength and fluorescence emitted from the ends of a single cell in a solution containing 20 mM diacetyl monoxime to reduce the cell movement. Trials were performed with laser illumination at the cell end facing the stimulation anode (panels A and B) and at the cell end facing the cathode (panel C). In panel A, only an S1 stimulation pulse was given. In panels B and C, a 40-V/cm S2 field stimulation pulse having a duration of 50 msec was applied during the S1-induced action potential. The action potential produced by S1 alone exhibited depolarization, plateau and repolarization phases. Panel B shows that the S2 hyperpolarized the transmembrane potential at the cell end facing the S2 anode. Panel C shows that the S2 having the same strength and polarity as in panel B produced a positive shift in the transmembrane potential at the cell end facing the S2 cathode. The magnitudes of the transmembrane changes induced by S2 were greater than the amplitude of the S1-induced action potential. The cell was 130 μm long and 20 μm wide and is a different cell from the cells of the other figures. The solution contained 4.5 mM potassium. The calibration bars on the left side of the recordings represent an electric field of 40 V/cm or a fluorescence change of 10% of the baseline fluorescence.
Measurements of the Transmembrane Potential Changes Induced by S2

Figure 7 shows the average of the S1-induced action potential recordings from eight cells bathed in solution containing 20 mM diacetyl monoxime to reduce the motion artifact during the repolarization phase. The solution contained 4.5 mM potassium. The averaging reduced the noise sufficiently to discern the action potential phases. The averaged action potential did not return fully to the baseline because of slight photobleaching of the fluorescent dye.

The numbered bars in Figure 7 indicate when measurements of the fluorescence were performed. Each measurement was an average over a 25-msec interval centered approximately at the bars shown. The measurement at bar 1 indicated the baseline fluorescence before S1 was given. The measurement at bar 2, obtained 25 msec after the S1 stimulation, represented the overshoot of the action potential. The difference in the values at bars 1 and 2 indicated the decrease in the fluorescence during the S1-induced action potential depolarization. The ratio of this difference to the value at bar 1 was the fractional fluorescence. The value corresponding to bar 3 was determined 75 msec after S1, i.e., at the midpoint of S2 in the experimental trials. With no S2, the difference in the values at bars 2 and 3 indicated the change in fluorescence due to the normal repolarization of the S1-induced action potential and any photobleaching of the dye (e.g., see Figures 4A and 5A). The difference in the values at bars 2 and 3 in the trials with no S2 for cells bathed in solution containing 4.5 mM potassium was 22±15% of the action potential amplitude (n=7). For the S2 trials, the fluorescence change during S2 that was due to S2 was obtained by subtracting the mean change in the fluorescence in the trials with no S2. The transmembrane potential changes induced by S2, i.e., due to S2, are given as a multiple of the amplitude of the S1-induced action potential in each trial.

In the solution containing 4.5 mM potassium, the transmembrane potential change induced by the 40-V/cm S2 as a multiple of the action potential amplitude (i.e., the transmembrane potential change divided by the action potential amplitude) was 1.36±0.34 at the cell end facing the S2 cathode and -1.65±0.61 at the
Figure 6. Recordings of the stimulation current and fluorescence, indicating the timing of the transmembrane potential changes at the end of a single cell induced by S1 and S2 pulses. Fluorescence recordings are shown with the 50-Hz low-pass filter (solid lines) and the 500-Hz low-pass filter (individual data points). When the illuminated end of the cell faced the stimulation anode (panel A), membrane depolarization corresponding to an S1-induced action potential occurred within the first millisecond after the end of the S1 pulse. After the stimulation polarity was reversed so that the illuminated end of the cell faced the cathodal electrode (panel B), the depolarization corresponding to the S1-induced action potential occurred within the first few milliseconds after the onset of the S1 pulse. The S2 pulses of different polarities induced hyperpolarization at the cell end facing the S2 anode and a positive change in the transmembrane potential at the cell end facing the S2 cathode. After the end of the S2 pulse, the transmembrane fluorescence recordings returned approximately to the action potential plateau level. The cell was 115 μm long and 18 μm wide and is a different cell from the cells of the other figures. The bathing solution contained 20 mM diacetyl monoxime to reduce cell movement and 4.5 mM potassium. The calibration bars on the left side of the recordings represent a stimulation current of 0.4 A or a fluorescence change of 10% of the baseline fluorescence.

cell end facing the S2 anode. For the 20-V/cm S2, these values were 0.61±0.33 at the cell end facing the S2 cathode and −0.71±0.33 at the cell end facing the S2 anode. For a cell end facing a given S2 electrode, the transmembrane potential changes induced by the 40-V/cm S2 as a multiple of the action potential amplitude were not significantly different in cells with (n=3) diacetyl monoxime. The combined results from cells bathed in solution containing 4.5 mM potassium are shown in Figure 8 for

Figure 7. Averaged fluorescence recordings from the ends of eight cells. The bathing solution contained 20 mM diacetyl monoxime to reduce the motion during the repolarization phase. The action potentials were produced by an S1 electric field stimulation pulse with a duration of 5 msec. The recordings were rescaled before averaging so that all of the action potential recordings contributed equally. The averaging reduced the noise sufficiently so that the action potential phases could be discerned. The fluorescence change during the S1-induced action potentials as a percentage of the baseline fluorescence was 7.5±1.9%. The numbered bars indicate the times when the measurements of fluorescence were performed in trials with and without S2. Bar 1 corresponds to the baseline fluorescence. The change in the fluorescence between bars 1 and 2 corresponds to the amplitude of the S1-induced action potential. Bar 3 corresponds to the time when S2 was given in other trials (e.g., see Figures 4 and 5). Without S2, the difference in the values at bars 2 and 3 indicates the change in fluorescence due to the normal repolarization of the S1-induced action potential. The normal repolarization in trials with no S2 was subtracted to determine the transmembrane potential changes at the cell ends induced by S2. The result was expressed as a multiple of the amplitude of the S1-induced action potential. The bathing solution contained 4.5 mM potassium.

the 20-V/cm S2 (filled circles) and the 40-V/cm S2 (crosses). The transmembrane potential changes induced by S2 are shown as multiples of the action potential amplitude (right ordinate) and as approximate voltage (left ordinate) by assuming that the S1-induced action potential in each recording had an amplitude of 130 mV.22–27 The abscissa shows the position of the cell ends relative to the center of the cell. Negative positions indicate cell ends facing the S2 anodal electrode, and positive positions indicate cell ends facing the S2 cathodal electrode. The linear regressions of voltage versus position for S2 values of 20 V/cm (solid line) and 40 V/cm (dashed line) indicate the increase in the magnitudes of the S2-induced transmembrane potential changes with increasing cell length. The slopes indicate the greater magnitude of the S2-induced transmembrane potential change for the 40-V/cm S2 compared with the 20-V/cm S2.

Effect of Depolarization With 20 mM Potassium

The approximate voltages on the left ordinate in Figure 8 require an assumption of the value of the
Figure 8. Graph showing changes in the transmembrane potential (V_m) at the ends of cells induced by electric field stimulation (S2) pulses. The cells were aligned with the S2 electric field, and the recordings were obtained at the cell end facing the S2 anode or cathode. The data are from 12 cells. The effect of a 20-V/cm S2 was determined for six cells (filled circles), and the effect of a 40 V/cm was determined for nine cells (crosses). The changes in V_m during the S2 pulse that were due to S2 were determined after subtracting the normal action potential repolarization that occurred in trials with no S2 (Figure 7). The changes in V_m due to S2 are shown as multiples of the S1-induced action potential amplitude in the same recordings (right ordinate). The changes are also shown as approximate voltage (left ordinate) by assuming that the S1-induced action potential in each recording had an amplitude of 130 mV,22-27 The abscissa shows the position of the cell ends relative to the center of the cell. Negative positions indicate cell ends facing the S2 anode, and positive positions indicate cell ends facing the S2 cathode. The linear regressions of voltage vs. position for S2 of 20 V/cm (solid line) and 40 V/cm (dashed line) had slopes of 18 and 33 V/cm, respectively (r=0.95 and 0.97). The solution contained 4.5 mM potassium.

S1-induced action potential amplitude in millivolts. The reliability of this method was tested in further experiments with a solution containing 20 mM potassium to decrease the amplitude of the S1-induced action potential.26,28-33 The ionic strength of the solution was held constant to prevent changes in the solution conductivity that may alter the ability of the S2 electric field pulse to influence the transmembrane potentials at the cell ends.15 Provided that the values of transmembrane potential changes at the ends of the cells produced by S2 did not change, these values divided by the amplitude of the S1-induced action potential should increase in the 20 mM potassium solution compared with the 4.5 mM potassium.

To ensure that S1 produced action potentials in cells exposed to high potassium, the excitation thresholds were determined by observing cell movement as described previously. In two cells that were 141±16 μm long in solution containing 4.5 mM potassium, the diastolic excitation threshold for a 5-msec S1 stimulation pulse was 4.0±0.4 V/cm, whereas in two different cells 144±23 μm long in 20 mM potassium, the diastolic excitation threshold for a 5-msec S1 stimulation pulse was 3.6±0.1 V/cm. In the subsequent experiments with 20 mM potassium, an S1 strength of 15–20 V/cm, i.e., four to five times the diastolic threshold strength, was used.

Fluorescence recordings were obtained at the ends of cells exposed to 20 mM potassium and 20 mM diacetyl monoxime by the same method as the experiments with 4.5 mM potassium. S2 having an electric field strength of 40 V/cm was used. For each cell, recordings with the laser illumination at one end of the cell were obtained with S1 stimulation alone and then with S1 and S2 stimulation having either S2 polarity.

The values of fractional fluorescence change during the S1-induced action potential of 25 cells studied in the solution containing 20 mM potassium were typically 1–2%, i.e., markedly smaller than the fractional fluorescence of cells in 4.5 mM potassium. Recordings were accepted from nine cells that had a peak-to-peak noise in the 50-Hz-filtered recording that was less than half of the fluorescence change at the cell ends induced during the 40-V/cm S2. These cells were 134±20 μm long and 23±4 μm wide, i.e., approximately 25% longer than the cells that gave acceptable recordings in experiments with 4.5 mM potassium (p=0.041).

Figure 9 shows a representative recording obtained when the solution potassium concentration was increased to 20 mM. As before, for cells in the solution containing 4.5 mM potassium, S2 hyperpolarized the cell end facing the S2 anode and depolarized the cell end facing the S2 cathode. With the 20 mM potassium solution, the magnitudes of the S2-induced transmembrane potential changes at the cell ends were a larger multiple of the amplitude of the S1-induced action potential than occurred for cells in 4.5 mM potassium. This is consistent with the known ability of elevated potassium to decrease the amplitude of the S1-induced action potential.

In the combined results for nine cells in the solution containing 20 mM potassium, the transmembrane potential change induced by the 40-V/cm S2 as a multiple of the S1-induced action potential amplitude was 2.52±0.31 at the cell end facing the S2 cathode and −2.75±0.67 at the cell end facing the S2 anode. These values were larger (p<0.005) than the corresponding values in cells bathed in 4.5 mM potassium by 85% for the cell end facing the S2 cathode and 67% for the cell end facing the S2 anode. Provided that the increase in the solution potassium decreased the action potential amplitude by a nominal value of 40%, the transmembrane potential changes induced by S2 as a multiple of the action potential amplitude should be larger by approximately 67%, a value that agrees with the values determined experimentally.

Comparison With Theoretical Transmembrane Potential Changes

For the 20-V/cm S2, the transmembrane potential changes due to S2 calculated from the optical measurements in cells bathed in the solution containing 4.5 mM potassium, assuming that the action potential upstroke in each recording had an amplitude of 130 mV,22-27 were 79±43 mV at the cell end facing the S2 cathode and −93±43 mV at the cell end facing the S2 anode (p<0.005 versus 0 mV, p=0.58 for cathode versus anode, n=6). For the 40-V/cm S2, the transmembrane
action potential amplitude of the potassium depolarized myocytes was decreased by 40%, i.e., to 78 mV. The approximate transmembrane potential changes induced by the 40-V/cm S2 with the 20 mM potassium were $-215 \pm 52$ mV at the cell end facing the S2 anode and $197 \pm 24$ mV at the cell end facing the S2 cathode ($p<0.005$ versus 0 mV, $n=9$). These values are comparable to the values given above for the 40-V/cm S2 in 4.5 mM potassium (i.e., with the normal action potential amplitude).

In the solution containing 20 mM potassium, the theoretical magnitude of transmembrane potential changes at the cell ends (i.e., the S2 electric field strength times half of the cell length) was 269 ± 40 mV. This exceeded the values calculated from the recordings (assuming that the S1-induced action potential amplitude was 78 mV) by 25% at the cell end facing the S2 anode and 37% at the cell end facing the S2 cathode ($p<0.05$).

**Discussion**

The results demonstrate for the first time in ventricular myocytes that electric field stimulation depolarizes one end of the cell aligned with the field while it hyperpolarizes the opposite end. This agrees with previous theory concerning the effects at the cell ends during electric field stimulation. The effects at the cell ends have been assumed in most explanations of excitation of isolated myocardial cells or the heart by electric field stimulation in diastole and prolongation of repolarization time by electric field stimulation during the refractory period.

The transmembrane potential changes at the cell ends are roughly predicted by the product of the electric field strength and half of the length of the cell in the direction of the field. This is seen in the comparison of the mean values of the measured and predicted transmembrane potential changes and in the slopes of the regression lines of transmembrane potential changes versus cell length for the 20- and 40-V/cm S2 in the solution containing 4.5 mM potassium, which were 18 and 33 V/cm, respectively.

That the transmembrane potential changes agree with the product of the electric field strength and half of the cell length in the direction of the field explains the higher electric field threshold for excitation of cardiac myocytes by a field oriented transverse to the axis of the cell compared with an orientation along the axis. Since the distance in the direction of the field that is occupied by the cell is reduced for a transverse field, a larger electric field is required to attain the transmembrane threshold potential for excitation. The results also explain the 2.8-V/cm diastolic field stimulation threshold for guinea pig myocytes (median length, 155 μm) aligned with the field. A 2.8-V/cm field would be predicted to induce a 22-mV depolarization at the end of the myocyte facing the stimulation cathode, which agrees with the 18-mV depolarization required to excite myocardium.

A stimulation pulse having an electric field strength of 20 V/cm given during the action potential can hyperpolarize the end of a cell facing the stimulation anode nearly to its diastolic potential (Figure 5B). This is consistent with the hypothesis that the voltage-dependent sodium current at that end of the cell may recover...
sufficiently during the stimulation pulse to allow a large inward sodium current after the pulse, which may reinitiate the action potential or produce a graded response.6 This would prolong the time until the cell undergoes repolarization, an effect that occurs with electric field strengths comparable to those studied here.4,24 The prolongation is thought to contribute to either transient block leading to reentry or enhanced refractoriness leading to defibrillation.1,2,3,35

The mean magnitude of the negative change in the transmembrane potential during S2 at the cell end facing the S2 anode was slightly greater than the mean magnitude of the positive change at the cell end facing the S2 cathode (e.g., see Figures 4–6). A greater magnitude of hyperpolarization compared with depolarization by current having a given strength but opposite polarities applied during the action potential plateau is consistent with previous measurements of transmembrane potential changes induced by hyperpolarizing and depolarizing current pulses applied during the action potential of dog and cat ventricular fibers.36 Such a difference in the magnitudes may result from nonlinear properties of the membrane. Our findings that the differences in the magnitudes of hyperpolarization and depolarization at the cell ends for a given S2 strength of 20 or 40 V/cm were not statistically significant and that the magnitudes became approximately twice as large when the S2 strength was increased from 20 to 40 V/cm suggest that nonlinear membrane properties may play only a minor role in determining the magnitudes of the hyperpolarization and depolarization at the cell ends.

**Validity of the Optical Measurements of Transmembrane Potential Changes Induced During Field Stimulation**

The fluorescence emitted from the illuminated end of the cell followed the transmembrane potential both during the S1-induced action potential depolarization and the subsequent repolarization (Figures 3–7). That the optical recordings represent transmembrane action potentials is evident from the 5–10% decrease in the fluorescence that occurred during or soon after S1 stimulation. Such large decreases were previously observed during transmembrane depolarization in myocardium and isolated cells stained with di-4-ANEPPS and with dye excitation and fluorescence wavelengths comparable to the wavelengths we used.37,38 The correlation of the rapid decrease in the fluorescence after S1 stimulation with both the cellular contraction in experiments without the electromechanical uncoupler diacetyl monoamine and the S1 stimulation in experiments with or without diacetyl monoamine further indicate that the optical recordings represent transmembrane action potentials.

The fluorescence emitted from the dye di-4-ANEPPS reportedly changes approximately in proportion to the applied electric field strength in isolated nonmyocardial cells.14 The dye’s fluorescence also changes in proportion to changes in the transmembrane potential in cultured HeLa cells.13 Similarly, our results with electric field strengths of 20 and 40 V/cm (Figure 5) suggest that the dye’s fluorescence changes in proportion to changes in both the electric field strength and the transmembrane potential in isolated ventricular myocytes.

The baseline fluorescence alone does not reliably indicate the transmembrane potential because it depends on variables such as the precise illumination power, amount of dye incorporated into the membrane, area of membrane from which fluorescence is collected, and details of the fluorescence apparatus such as the numerical aperture of the collecting lens and the cutoff of the dichroic beamsplitter and long-pass filter. The fractional fluorescence change may qualitatively indicate the change in transmembrane potential, since the fractional fluorescence was only a few percent for cells that had their action potential amplitudes decreased by 20 mM potassium, whereas the fractional fluorescence was larger for cells in 4.5 mM potassium. However, the fractional fluorescence alone cannot quantitatively indicate the change in transmembrane potential. For example, in the 4.5 mM potassium solution, the fractional fluorescence corresponding to the action potential depolarization varied from less than 3% to greater than 7%, which is too large a variation to account for by variations in the action potential amplitude. Therefore, we measured the fluorescence change induced by S2 as a multiple of the fluorescence change during the action potential amplitude in each recording. This method gave results that were quantitatively consistent from one cell to the next. The method allowed comparisons of transmembrane potential changes at opposite ends of a cell (Figures 3 and 4), at a given cell end with different electric field polarities and strengths (Figures 5 and 6), and in cells bathed in solution with a different potassium concentration to alter the action potential amplitude (Figure 9).

**Advantage of the Optical Method of Recording Transmembrane Potential Changes During Electric Field Stimulation**

The fluorescence emitted from the voltage-sensitive dye di-4-ANEPPS incorporated into the cell membrane and appropriately excited was previously shown to change in response to changes in the transmembrane potential.20 This reportedly occurs through an electrochromic mechanism based on a charge shift in the dye molecule.20

Although the optical method we used did not provide the fine voltage resolution obtainable with microelectrodes, the method provided a crucial advantage over electrode techniques to determine transmembrane potential changes during electric field stimulation. Transmembrane potentials can be measured with electrodes in isolated myocardial preparations during weak electric field stimulation pulses of approximately 0.5 V/cm.7 However, transmembrane potential measurements with electrodes during a strong electrical stimulation pulse such as the pulses studied here are highly sensitive to the location of the extracellular reference electrode. For example, when the field is 40 V/cm, a value within the range of values that occurs in the heart during defibrillation,35,40 an error of only ±100 μm in the position of the reference electrode could introduce a ±400-mV difference between the potential at the reference electrode and the extracellular space outside of the end of the cell. This potential is not a part of the true transmembrane potential at the cell end, although it would be present in the potential difference between the intracellular and extracellular electrodes. With such an
error, electrode recordings cannot reliably indicate the magnitude or even the direction of the transmembrane potential change at the ends of the cells induced by an electric field stimulation pulse.

For syncytial myocardial preparations, the membrane time constant is large enough that the instantaneous offset error introduced at the onset of a rectangular stimulation pulse by the position of the reference electrode can be distinguished from the slower transmembrane potential change.\(^{7,41,42}\) The time constant of the hyperpolarization and depolarization at the ends of cells induced by electric field stimulation is probably much faster (e.g., 20 \(\mu\)sec\(^{43}\)) than the time constant for a myocardial fiber. For example, a short time constant of 11 \(\mu\)sec that depended on the extracellular solution conductivity was reported for the rise in potential induced in a hemispherical bilayer system by electric field stimulation.\(^{15}\) Such a short time constant can explain the fast transmembrane potential changes at the ends of cells at the onset of S2 in our results (data points in Figure 6). Fast transmembrane potential changes cannot be distinguished from the fast offset error introduced by the position of the extracellular reference electrode; hence, the technique of distinguishing the transmembrane potential change from the offset error in electrode recordings by their time dependence cannot be applied to transmembrane potential changes that occur at the ends of single cells.

The fluorescence recordings with voltage-sensitive dye follow fast changes in the transmembrane potential\(^{37}\) and are not directly affected by the extracellular field, as evidenced by the absence of an effect of S2 in trials in which the laser spot was positioned in the solution away from the cell during the application of a 40-V/cm S2 electric field. Therefore optical recordings can clearly and reliably indicate the direction of the change in the transmembrane potential during an electrical stimulation pulse, a capability demonstrated in hearts\(^{44}\) and isolated cells.\(^{14}\) Furthermore, our method of determining the fluorescence change induced by S2 as a multiple of the fluorescence change for the S1-induced action potential amplitude in the same recording indicated the approximate magnitude of the transmembrane potential changes in millivolts that were induced at the ends of the cells during S2.

**Limitations of the Optical Method of Recording Transmembrane Potential Changes During Electric Field Stimulation**

The optical recording method to determine transmembrane potential changes at the ends of cells has the limitations of sensitivity to tissue movement, shot noise in the fluorescence recording that is due to the low level of light collected, and no fixed relation that holds for all recordings between fluorescence intensity and transmembrane potential. Because of the later limitation, determining the transmembrane potential changes in millivolts requires that an indication of the fluorescence change corresponding to a given transmembrane potential change be present in each recording.

The sensitivity to movement did not influence the measurements of the amplitude of the S1-induced action potential or the transmembrane potential changes during S2 because these measurements preceded the cell movement. The movement influenced the recordings of repolarization; hence, the recordings give only the approximate shape of the action potential repolarization phase. When diacetyl monoxime was added to reduce cell movement and the recordings from different cells were averaged, the repolarization phase became apparent (Figure 7).

Electrical noise due to the small amount of light that was collected limited the resolution of the transmembrane potential changes. The methods used low-pass filtering of the fluorescence signal, a long S2 pulse duration that allowed averaging of the fluorescence over 25 msec for each measurement, and large S2 electric fields. With these methods, measurements above the noise level were obtained.

The determination of the effects of S2 in millivolts is based on the fluorescence change corresponding to a given transmembrane potential change produced by the S1-induced action potential in each recording. The same action potential amplitude may not have occurred in every cell, which may contribute to the scatter of points in Figure 8. The mean values of action potential amplitudes measured in isolated mammalian myocytes with normal extracellular potassium vary from 116 to 133 mV,\(^{22–27}\) suggesting that our determinations of the transmembrane potential changes in millivolts may contain approximately 10% error. The exact value of the transmembrane potential changes is not important for the conclusions we have drawn. For example, if the action potential amplitude in the solution containing 4.5 mM potassium was as high as 133 mV, the calculated transmembrane potential changes produced by the 40-V/cm S2 would be \(-219\) and 181 mV at the cell ends facing the S2 anode and cathode, respectively. If the action potential amplitude was only 116 mV, the calculated transmembrane potential changes would be \(-191\) and 158 mV at the cell ends, respectively. These values are still large enough to produce activation and recovery of voltage-dependent ion channels.

A recent report indicates that isolated rabbit ventricular myocytes with a rod shape and cross striations, like the cells in our study, have remarkably uniform action potential amplitudes even among cells that were exposed to 30 minutes of global ischemia or 75 minutes of control Tyrode’s perfusion before being isolated.\(^{43}\) In that study, the action potential amplitudes (mean \pm SEM) of isolated myocytes in Tyrode’s solution with an extracellular potassium concentration of 4.8 mM varied from 128 \pm 1 mV for control cells that were isolated without prior Tyrode’s perfusion or ischemia to 126 \pm 1 mV for cells isolated after ischemia and reperfusion. It is likely that the cells in our study, which were not exposed to global ischemia before isolation, had action potential amplitudes similar to the control value.

**Effect of 20 mM Potassium**

The results obtained when the solution potassium was elevated to 20 mM support our use of the S1-induced action potential amplitude to indicate the magnitudes of the transmembrane potential changes induced by S2. Elevating the extracellular potassium concentration decreases the resting membrane potential and the action potential amplitude in ventricular muscle\(^{26,29}\) and in isolated ventricular myocytes.\(^{26,30–33}\) In ventricular muscle, elevating the extracellular potassium from 5.4 to 20 mM decreased the resting membrane potential from...
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(values changing decreased from 105 mV, a 23% reduction. In isolated guinea pig ventricular myocytes at 28°C, changing the solution potassium from 4.4 to 8.8 mM decreased the action potential amplitude from 122 to 105 mV, a 14% reduction. Thus, elevated potassium decreases the action potential amplitude.

On the other hand, increasing the potassium probably does not alter the hyperpolarization and depolarization at the cell ends induced by S2 because these effects are largely determined by the extracellular electric field and the fact that the cell is surrounded with a membrane having a high electrical resistance. The results for S2 strengths of 20 and 40 V/cm in a given cell (Figure 5) and for cells of various lengths that were stimulated with a given S2 strength (Figure 8) indicate that these transmembrane potential changes are a function of the S2 electric field strength and the length of the cell in the direction of the field. This conclusion is further supported by the agreement of our measurements of hyperpolarization and depolarization at the cell ends, with the theoretical values calculated as the S2 electric field strength times half of the cell length. With a given S2 electric field strength and solution ionic strength, and hence conductivity, we would expect the effect of S2 on the transmembrane potentials at the cell ends to be independent of the solution potassium concentration. If true, then the decrease in the action potential amplitude produced by elevating the potassium should increase the ratio of the transmembrane potential change to the action potential amplitude. Indeed, this ratio increased significantly for cells in the solution containing 20 mM potassium compared with 4.5 mM potassium.

The similar S1 diastolic excitation threshold that we observed in cells bathed in Tyrode’s solution containing elevated potassium compared with normal potassium is consistent with another study showing no increase in the stimulation threshold for isolated myocytes when the takeoff potential was made less negative by giving the stimulation pulse early relative to the time of 95% repolarization of the action potential. However, the cellular responsiveness for action potentials elicited during the terminal repolarization, i.e., the amplitude of the action potential and maximum dV/dt of the action potential upstroke, was decreased compared with action potentials elicited after the myocyte was fully repolarized. The decreased action potential amplitude for action potentials beginning at a less negative takeoff potential is consistent with a decreased action potential amplitude for cells in which the resting membrane potential is made less negative by elevating the potassium.

The results show that markedly different transmembrane potential changes occur at different ends of the cell during defibrillation-type shocks. Understanding the cellular effects of such shocks that are responsible for defibrillation, e.g., effects of the shock on voltage-dependent membrane ion channels, requires knowledge of these transmembrane potential changes. In spite of the limitations of the optical method, it is the only method available to obtain information on transmembrane potential changes during field stimulation on a subcellular spatial scale.

Effect of Diacetyl Monoxime

The addition of 20 mM diacetyl monoxime eliminated the cell motion that occurred after the S1-induced action potential. Such an effect on cell motion is consistent with the effect of 20 mM diacetyl monoxime on contractile force in guinea pig papillary muscles. In those experiments, 10 mM diacetyl monoxime reduced contractile force to a few percent of its normal value, and 20 mM diacetyl monoxime reduced contractile force to zero. In our experiments with isolated myocytes, the effect of diacetyl monoxime was reversible, as it is in papillary muscles and porcine trabecula. Also, the decrease in cell movement developed rapidly after adding diacetyl monoxime, again consistent with previous results. The reduction in cell movement is also consistent with our previous experiments in which 20 mM diacetyl monoxime eliminated the motion artifact in optical action potential recordings obtained from perfused rabbit hearts.

Diacetyl monoxime does not have major effects on the sarcolemmal electrical properties. At the 20-mM concentration in guinea pig papillary muscles, diacetyl monoxime decreases the fast action potential amplitude from a control value of 128 mV in normal Tyrode’s solution to 123 mV, decreases dV/dt_{max} of the action potential phase-zero depolarization from 180 to 121 V/sec, and decreases the resting membrane potential by 5 mV. In our results, there were no significant differences in the transmembrane potential changes induced by S2 as a fraction of the action potential amplitude with diacetyl monoxime versus without diacetyl monoxime. Thus, our results are consistent with the absence of a major effect of the diacetyl monoxime on the action potential amplitude.

Conclusion

Changes in transmembrane potentials in myocardial cells during electric field stimulation were determined with fluorescence recordings. This method eliminated the shock “artifact” that occurs in microelectrode recordings during a stimulation pulse. Unlike previous optical studies of cellular field stimulation, excitable cells were used, which allowed the approximate magnitude of the transmembrane potential change during stimulation pulses to be determined. This method required the assumption of the amplitude of the action potential, which was based on previous measurements for isolated mammalian ventricular myocytes. In experiments with elevated extracellular potassium to decrease the amplitude of the action potential, the transmembrane potential changes during electric field stimulation were consistent with the values obtained in normal potassium.

The stimulation pulse duration and electric field strengths that were studied are comparable to those that occur in the heart during ventricular defibrillation. Also, the pulses were delivered while the cell was undergoing an action potential, which is the state of the cells in the heart at most instants during fibrillation. The measurements of the transmembrane potential change at the ends of myocardial cells during an electric field stimulation pulse were obtained from individual
recordings, i.e., without signal averaging, which indicates that the method may be used to study single events in cells. Finally, the measurements agree both with theoretical predictions based on the electric field strength and cell length and with previous measurements of excitation thresholds for ventricular myocytes and myocardium.

The observed transmembrane potential changes at the ends of ventricular myocytes during defibrillation-type stimulation pulses are large enough to produce voltage-dependent activation or recovery of ionic currents. Thus, the transmembrane potential changes may be responsible for excitation, prolongation of repolarization, and the consequences of these effects for arrhythmias. Whether hyperpolarization and depolarization during defibrillation pulses in the heart occurs at the ends of the cells, at the ends of larger electrical units such as fibers, or at the ends of both cells and fibers remains to be determined. The increased magnitude of the transmembrane potential changes with increasing cell length that we observed suggests that transmembrane potential changes at the ends of larger units would be greater than changes at the ends of cells.

References

41. Weidmann S: Electrical constants of trabecular muscle from mammalian heart. J Physiol (Lond) 1970;210:1041–1054
Optical measurements of transmembrane potential changes during electric field stimulation of ventricular cells.
S B Knisley, T F Blitchington, B C Hill, A O Grant, W M Smith, T C Pilkington and R E Ideker

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