Influence of Electrical Axis of Stimulation on Excitation of Cardiac Muscle Cells

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Orthogonal sequential shock can defibrillate the heart with greater efficacy compared with single shock defibrillation. In this study we tested the hypothesis that cardiac cells have a preferred orientation in their response to excitatory extracellular electric fields, so that orthogonal shocks may stimulate distinct populations of cells. A micropaddle electrode system was used to deliver rectangular pulses for extracellular field stimulation of individual heart cells. We found that single frog and guinea pig ventricular myocytes are excitable with rectangular pulse field stimulation over a wide range of pulse durations, ranging from 10 msec to as little as 20 μsec. The excitation field strength varies inversely with pulse duration as described by the Weiss-Lapicque equation, although the frog myocytes show a significant "notch" at pulse durations of ~1–2 msec, and the guinea pig myocytes are more excitable than predicted for pulse durations of <0.2 msec. Every myocyte tested was more excitable when the long axis of the cell was oriented parallel to the stimulating field than when perpendicular to the field. For 2-msec pulses, the difference in field strength was a factor of 5.8±2.0 (n=30) for frog and 2.6±0.5 (n=23) for guinea pig myocytes. Complete excitation strength-duration curves were obtained in seven frog and 14 guinea pig cells for both parallel and perpendicular cell orientations. The increase in electric field required for stimulation of cells in a perpendicular orientation over a parallel orientation was approximately constant for pulse durations between 0.2 and 10 msec, by a factor of 5.5±2.2 (seven cells, six durations) for frog and 2.5±0.7 (14 cells, six durations) for guinea pig, but it was less at shorter durations. These results suggest that the efficacy of orthogonal sequential shock may be regulated in part by tissue excitability at the cellular level. (Circulation Research 1991;69:722–730)

Electrical defibrillation of cardiac muscle is commonplace today in the management of certain types of arrhythmia. An increase in the efficacy of the electric shock and subsequent reduction of the total energy delivered to the myocardium has several advantages. Injury to the myocardium that occurs in regions of high current density can be minimized. Also, the size of implanted cardioverter-defibrillators (determined by battery and capacitor size) can be reduced, and their lifetime can be prolonged. Several methods have been pursued to reduce the total energy required for defibrillation. Improved electrode design and placement3–5 and sequential shock with multiple current pathways4–7 are intended to distribute the shock current more evenly throughout the heart volume, a strategy in keeping with various mechanisms proposed for defibrillation.8 The use of multiphasic waveform shapes5,9,10 may take advantage of the electrophysiological properties of the cell membrane11 to lower the defibrillation threshold.

It has also been observed that during sequential shock with multiple current pathways, current pathways that are perpendicular (i.e., orthogonal sequential shock) substantially reduce the total energy required for defibrillation.12,13 Orthogonal sequential shock could be particularly efficacious if it were the case that cardiac cells respond to the defibrillating field in a manner dependent on their orientation with respect to the electrical axis of the applied field. In this way, orthogonal sequential shock could defibrillate a subpopulation of heart cells with the first shock and then a separate, orthogonally oriented subpopulation of heart cells with the succeeding orthogonal shock. Indeed, cardiac fibers in the intact heart have been shown to have directional sensitivity to stimulation by extracellular electric fields.14 The aim of this...
study was to investigate this hypothesis on a cellular level.

With whole-heart preparations, the tissue is anisotropic, and the electric field (potential gradient) is, in general, three-dimensional and nonuniform throughout the heart volume. Therefore, electric fields are not known unless measured directly. In contrast, uniform fields with known intensity and orientation can be applied to stimulate single-cell preparations. The excitability of heart cells can be used as an index of defibrillation threshold, even though fibrillation and defibrillation generally apply to tissue systems and not to single cells. In this report we show that cell excitation is highly dependent on the electrical axis of stimulation in a significant and reproducible manner.

Materials and Methods

Experimental Preparations

Single heart cells were isolated from the ventricle of frog (Rana pipiens) ventricle or from guinea pig (Hartley strain) ventricle using a retrograde-perfused, whole-heart enzymatic dissociation technique. In brief, the heart was rinsed in Ringer's (for frog) or Tyrode's (for guinea pig) solution containing 1) 1 mM calcium ion for 3–5 minutes, 2) nominally zero calcium (60 minutes for frog, 5 minutes for guinea pig), 3) ~330 units/ml collagenase (type IA, Sigma Chemical Co., St. Louis, Mo.) and 0.53–0.65 units/ml protease (type XIV, Sigma) (20 minutes for frog, 5 minutes for guinea pig), and 4) nominally zero calcium (5 minutes for guinea pig). The frog cells selected for study (Figure 1, top panel) were generally 200–400 µm in length and 5–10 µm in width when stretched out with glass micropipettes (although experiments were performed with non-stretched, slack cells); guinea pig cells (Figure 1, bottom panel) were 110–200 µm in length and 20–40 µm in width at slack conditions. Cells that lacked clear striations, contracted spontaneously, or had an abundance of blebbing of the surface membrane were rejected and not used in this study.

Solutions

All solutions were prepared using type I (18 MΩ) deionized water and contained the following: for frog, Ringer's solution consisting of (mM) NaCl 110, KCl 4.7, CaCl₂ 1.8, MgCl₂ 1.2, NaHCO₃ 26.0, and glucose 5.5.
KCl 3, CaCl\(_2\) 2, HEPES 10, and glucose 10, along with 0.5 mg/ml bovine albumin (fraction V, Sigma), pH 7.2 using NaOH; for guinea pig, Tyrode’s solution consisting of (mM) NaCl 135, KCl 5.4, CaCl\(_2\) 0.2, MgCl\(_2\) 1, NaH\(_2\)PO\(_4\) 0.33, HEPES 10, and glucose 5, pH 7.35 using NaOH. Because cell contraction was used as an index of cell excitability, Ca\(_s\) was reduced to a low level for experiments with guinea pig myocytes to reduce the likelihood of spontaneous contractions and, therefore, is a possible limitation of this study. We would expect that a reduction of Ca\(_s\) would result in a simple voltage shift of the gating parameters in a negative direction (by \(\sim\)16 mV in skeletal muscle when Ca\(_s\) is lowered from 5 to 0.5 mM\(^{17}\)), which would be largely negated, however, by our inclusion of 1.0 mM MgCl\(_2\). Nevertheless, we do not expect that any change in activation kinetics, if present, would provide a systematic bias in the data in terms of the relative effect of electrical axis of stimulation. All experiments were conducted at room temperature (23–25°C).

**Stimulation**

Cells were placed in an experimental chamber constructed from plexiglass and a glass cover slip, sometimes pretreated with poly-L-lysine to promote cell adhesion. After the cells had settled and attached to the cover slip, a pair of parallel-plate, rectangular micropaddle electrodes (described elsewhere\(^{18}\)) were gently lowered as a unit to straddle a test cell for the purposes of field stimulation (Figure 2A). These electrodes originally were developed to apply very high intensity (>100 V/cm) pulsed electric fields to cardiac myocytes.\(^{19}\) Each paddle was constructed by compressing the exposed end of a Teflon-coated, 0.010-in.-diameter platinum wire in a vise. Typical dimensions for the paddles were 800–1,200 \(\mu\)m in length, 20–40 \(\mu\)m in width, 100–200 \(\mu\)m in height, and 400–600 \(\mu\)m separation. The paddles were coated with platinum black to increase the effective surface area to minimize electrode polarization and bubble formation, and typical paddle impedance was 300–800 \(\Omega\). Even with the platinum black surface, significant polarization and nonlinearities in impedance can occur in the micropaddle electrodes. If a constant voltage waveform is used, the electrodes are effectively shorted together after the stimulus pulse, during which time significant current continues to flow.\(^{18}\) Since the electric field in the bath is equal to the density of current times the resistivity of the solution,\(^{20}\) a significant electric field may persist after the stimulus pulse. Therefore, we used constant current waveforms, generated either with a stimulator custom-built in our laboratory or by a commercial stimulator (model SD9, Grass Instrument Co., Quincy, Mass.). In this way, the electrodes were effectively open-circuited after the stimulus pulse, shutting off the electric field.

Because of difficulties introduced by polarization of the paddle electrodes, the electric field could not be calculated simply by dividing the interpaddle potential by the interpaddle separation. The alternative calculation of current density times bath resistivity also was unreliable since large errors could arise in estimating the electrode surface area and conductivity of the solution as well as so-called “fringing,” or spread, of the current at the edge of the electrodes. Therefore, we measured directly the field between the paddles using a Teflon-coated, stainless-steel wire as an exploring electrode (tip size, 10–50 \(\mu\)m) along two sets of points on the glass floor of the chamber, with each set lying at a fixed distance between the electrodes (Figure 2B). This procedure provided a calibration in which the applied electric field could be calculated in terms of the current passed between the electrodes. Note that the applied electric field described in this report does not include the local perturbation introduced by the cell itself, which results from the imped-

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**FIGURE 2.** Diagram of micropaddle electrode system. Panel A: Miniature rectangular paddle electrodes were constructed by compressing the exposed ends of Teflon-coated platinum wires in a vise and then coating the exposed ends with platinum black (Pt black). Typical dimensions of the paddle electrodes are shown. The electrodes were mounted together as a pair on a micromanipulator and could be lowered into the bath as a single unit to straddle individual heart cells. The cell is shown in an orientation both parallel (solid image) and perpendicular (striped image) to the stimulating electric field (\(E_0\)). Panel B: The calibration procedure for a pair of electrodes is illustrated. A Teflon-coated, 50-\(\mu\)m-diameter stainless-steel wire was exposed only at the tip and used as an exploring electrode on the floor of the chamber between the two paddle electrodes. The view shown in the figure is looking down between the electrodes toward the floor of the chamber, and \(X\) refers to the site of measurement. Two sets of measurements were made, with each set parallel to the electrodes and thus along a theoretical equipotential line. The average potential drop between the two sets of measurements, divided by the measured distance, was used to calibrate the applied electric field in terms of the current passed between the electrodes.
ance mismatch between the conductances of the cell and bathing solution. For long thin cells, this perturbation occurs within a distance approximately equal to the cell width (~10 μm for frog cell) from the cell surface, as modeled for cells parallel\textsuperscript{22} or perpendicular\textsuperscript{22} to the applied electric field.

Excitation Threshold

It is possible to measure the intracellular potential with microelectrodes during extracellular field stimulation,\textsuperscript{9} but this is technically difficult, since the recording and stimulus systems must be fully isolated to prevent distortion of the electric field profile by the reference electrode of the recording system, to minimize voltage offsets of the ground electrode of the recording system owing to polarization, and to impede entry of current through the recording electrode into the cell. On the other hand, cell excitation can be readily detected by the absence or presence of cell contraction, an approach used previously by other investigators.\textsuperscript{9,15,23}

Stimuli were applied manually approximately every 5 seconds, and the cell was observed using an inverted microscope (model TMS, Nikon Instruments, Tokyo) as pulse amplitude was raised, keeping pulse duration constant. When contraction was observed, the amplitude threshold was noted. The variability in the measured amplitude threshold was checked by repeating the measurement 10 times at two durations (0.05 and 5 msec) on three frog heart cells and was <6.7% of the average reading, or approximately the height of the symbols used to display the data in Figures 4–6.

In one series of experiments, the chamber was initially rotated so that the long axis of the cell was perpendicular to the plane of the electrodes and therefore parallel (∥) to the stimulating electric field. The stimulus threshold for a 2-msec pulse was measured. The chamber was then rotated 90° so that the cell was parallel to the plane of the electrodes and therefore perpendicular (⊥) to the stimulating field, and the new stimulus threshold was measured. In a second series of experiments, the stimulus threshold was measured in the same cell for a series of pulse durations ranging from 20 μsec to 10 msec. In this way, the excitation strength–duration (ESD) curve was determined, for both the || and ⊥ orientations.

Since the absolute value for stimulus intensity can vary on a cell-to-cell basis from uncontrolled factors such as cell size and shape, the stimulus amplitude threshold was always measured for each cell at a pulse duration of 2 msec for the || orientation. This value was used to normalize the stimulus amplitude measured at other durations or with the ⊥ orientation, so that changes in the ESD curve could be attributed primarily to differences in cell excitability. The shift in the ESD curve was analyzed statistically by two methods: the first by a paired t-test at each test duration, and the second by analysis of covariance (ANCOVA) of the total population of data points, with cell orientation as the grouping factor and stimulus duration as the covariate.

Results

The stimulus thresholds at a 2-msec pulse duration were measured for 30 frog ventricular and 23 guinea pig ventricular myocytes for both || and ⊥ orientations and are summarized in Figure 3. In every cell tested, the stimulus threshold increased when the cell was rotated from a || to a ⊥ orientation. The mean±SD applied field strength in the || configuration was 2.4±0.6 V/cm for frog and 2.8±0.4 V/cm for guinea pig. The average applied field strength in the ⊥ orientation was 13.8±5.8 V/cm for frog and 7.3±1.5 V/cm for guinea pig. Considering the paired data for each cell, the field strength for excitation increased by a factor of 5.8±2.0 for frog and 2.6±0.5 for guinea pig when the cell orientation was changed from || to ⊥. A paired t-test showed significance in both cases at p<0.001.

An ESD curve for a single frog ventricular myocyte is shown on linear axes (Figure 4, top panel). By
plotting the same curve on log-log axes (Figure 4, bottom panel), scale factor changes appear as upward or downward shifts in the curve. Several characteristics typical of the ESD are worth noting. First, the ESD followed an approximately hyperbolic relation as expected for excitable tissues. However, a notch at a duration of 1–2 msec was seen in this cell, as well as in the majority of frog ventricular cells tested. Since this finding has not been reported previously, we double-checked the stimulation thresholds for the durations in question to verify that the notch was not an instrumentation artifact. Second, a stimulus pulse with a duration as short as 20 μsec was sufficient to stimulate the cell. Finally, the stimulus intensity at all durations tested was always higher when the cell was oriented ⊥ to the field compared with when it was || to the field, similar to the result shown in Figure 3 for the 2-msec pulse.

Complete ESDs were obtained for seven cells (Figure 5, top panel) and normalized as described in “Materials and Methods.” The average absolute field intensity corresponding to the relative field intensity of 1 was 2.2±0.6 V/cm. As before in Figure 4, the

**FIGURE 4.** Excitation strength–duration curve. Top panel: Excitation of myocytes was monitored as a cell shortening in response to the rectangular field stimulus pulse. The threshold for excitation of a frog ventricular myocyte was measured for both parallel (||) and perpendicular (⊥) cell orientations, over pulse durations ranging from 20 μsec to 10 msec and plotted on linear axes. Bottom panel: The data of the top panel are replotted on log–log axes to clarify the shift in the excitation strength–duration curve with change in cell orientation. An upward, approximately parallel shift in the excitation threshold can be seen.

**FIGURE 5.** Dependence of excitation strength–duration curve of frog ventricular myocytes on direction of stimulating field. Top panel: Excitation strength–duration curves were measured for seven cells at eight durations for both parallel (||) and perpendicular (⊥) orientations. The curves are normalized to the excitation strength at 2 msec (average of 2.2±0.6 V/cm) in the parallel orientation to reduce variability in the data owing to cell shape; 112 measurements are summarized. Bottom panel: The same data are replotted as the scale factor increase in field strength required to stimulate the cell as the cell was rotated from a position parallel to the electric field to one perpendicular to the field. In all cases the ratio was >1 (indicated by the dashed line). The increase in field intensity was approximately constant for durations ≥0.2 msec and was a factor of 5.5±2.2 (n=42). Paired t test analysis showed the scale factor increase to be significant at each duration (p<0.001).

ESD when the cells were ⊥ to the stimulating field was higher than the ESD obtained when the cells were || to the field. The data were reevaluated on a cell-by-cell basis as the relative scale factor increase required at each test pulse duration to stimulate the cell in the ⊥ compared with the || configuration (Figure 5, bottom panel). In every one of the 56 pairs of data summarized in this figure, the scale factor change was greater than unity. A paired t test was performed at each duration, and in each case, the change in stimulus strength was significant (p<0.001). The change in stimulus intensity was approximately constant for durations ≥0.2 msec and was a factor of 5.5±2.2 (n=42) greater for excitation in the ⊥ orientation than for the || orientation.
The major result of this investigation is that the threshold for field stimulation of single cardiac myocytes is always greater when the cell is \( \perp \) to the stimulating field than when it is \( \parallel \) to the field. This relation has been implied previously from observations of whole heart\(^{12}\) and groups of cardiac myocytes\(^{25}\) and also has been described in a recent study of guinea pig cardiomyocytes.\(^{26}\) The ESD relation for the single cardiac myocyte is hyperbolic, as observed previously in single frog atrial cells,\(^{23}\) cardiac cell aggregates,\(^{9}\) Purkinje fibers,\(^{27}\) and whole heart.\(^{14}\) The ESD shifts toward greater field strengths over the entire range of pulse durations tested (from 20 \( \mu \)sec to 10 msec) when the cell is changed from the \( \parallel \) to the \( \perp \) orientation. The shift is approximately constant for durations between 0.2 and 10 msec (Figures 5 and 6). The threshold change may be explained in part by theoretical predictions of excitation of prolate spheroidal cells.\(^{22}\) When the cell is either \( \parallel \) or \( \perp \) to the electric field, the maximum change in membrane potential occurs at the ends of the cell closest to the two electrodes. The magnitude of the potential change depends in part on the projection of the cell length along the electric field, so that the change in potential will be less for the \( \perp \) orientation. Therefore, an increased electric field is needed to produce the same degree of membrane depolarization as for the \( \parallel \) orientation, which results in an apparent increase in excitation threshold.

Given typical widths and lengths for frog and guinea pig cells of 10\( \times \)300 and 25\( \times \)135 \( \mu \)m, respectively, one would theoretically expect a scale factor difference of \( \approx 15 \) for frog and \( \approx 3 \) for guinea pig (Equation 9 from Reference 22). However, our results indicate an average scale factor of \( \approx 5.8 \) for frog and \( \approx 2.6 \) for guinea pig at a pulse duration of 2 msec (Figure 3). Although the scale factor is close to that expected for the guinea pig myocyte, the scale factor for the frog myocyte data is not. This discrepancy may arise in part because the Klee–Plonsey model\(^{22}\) is inappropriate for our frog cell preparation, which exhibits tortuosity along its major axis in its isolated slack condition (Figure 1, top panel). The effective

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**Figure 6.** Dependence of excitation strength–duration curve of guinea pig ventricular myocytes on direction of stimulating field. Top panel: Excitation strength–duration curves were measured for 14 cells at nine durations for both parallel (\( \parallel \)) and perpendicular (\( \perp \)) orientations and normalized to the excitation strength at 2 msec (average of 2.5\( \pm \)0.4 V/cm) in the parallel orientation. Values were not measured for one cell at two durations, so that 248 measurements are summarized. The data for both orientations at durations \( \geq 0.2 \) msec have been fit by the Weiss–Lapicque equation\(^{24}\) (dotted lines; see text for description. Bottom panel: The same data are replotted as the scale factor increase in field strength required to stimulate the cell as the cell was rotated from a position parallel to the electric field to one perpendicular to the field. In all cases the ratio was \( > 1 \). The increase in field intensity was approximately constant for durations \( \geq 0.2 \) msec and was a factor of 2.5\( \pm \)0.7 (\( n = 84 \)). Paired t test analysis showed the scale factor increase to be significant at each duration (\( p < 0.001 \)) and also to be significant overall (\( p < 0.0001 \)) by analysis of covariance, with cell orientation as the discrete and pulse duration as the continuous covariates.

Similar experiments were carried out on single guinea pig ventricular myocytes. Unlike the ESDs of the frog ventricular cells, the ESDs of these myocytes were smooth and did not contain any notch. Data pooled from 14 myocytes are shown in the top panel of Figure 6 as ESDs. The dotted lines are theoretical curve fits to the data, as described later in “Discussion.” The average absolute field intensity corresponding to the relative field intensity of 1 was 2.5\( \pm \)0.4 V/cm. As in the case for the frog myocytes, guinea pig myocytes in the \( \perp \) orientation had a higher stimulus threshold than in the \( \parallel \) orientation. The data were replotted in the bottom panel of Figure 6 as a scale factor increase in stimulus threshold for each duration tested. In each of the 124 pairs of data summarized in this figure, the scale factor change was greater than unity. As before, a paired t test was performed at each duration, and in each case, the change in stimulus strength was significant (\( p < 0.001 \)). Since the guinea pig data were reasonably linear and parallel, ANCOVA was performed on the total data set, using cell orientation as the grouping factor, and showed the change in stimulus strength with cell orientation to be highly significant (\( p < 0.0001 \)). The stimulus intensity necessary for excitation of cells in the \( \perp \) orientation was a factor of 2.5\( \pm \)0.7 (\( n = 84 \)) greater than that for the \( \parallel \) orientation at durations \( \geq 0.2 \) msec.

**Discussion**

The major result of this investigation is that the threshold for field stimulation of single cardiac myocytes is always greater when the cell is \( \perp \) to the stimulating field than when it is \( \parallel \) to the field. This relation has been implied previously from observations of whole heart\(^{12}\) and groups of cardiac myocytes\(^{25}\) and also has been described in a recent study of guinea pig cardiomyocytes.\(^{26}\) The ESD relation for the single cardiac myocyte is hyperbolic, as observed previously in single frog atrial cells,\(^{23}\) cardiac cell aggregates,\(^{9}\) Purkinje fibers,\(^{27}\) and whole heart.\(^{14}\) The ESD shifts toward greater field strengths over the entire range of pulse durations tested (from 20 \( \mu \)sec to 10 msec) when the cell is changed from the \( \parallel \) to the \( \perp \) orientation. The shift is approximately constant for durations between 0.2 and 10 msec (Figures 5 and 6). The threshold change may be explained in part by theoretical predictions of excitation of prolate spheroidal cells.\(^{22}\) When the cell is either \( \parallel \) or \( \perp \) to the electric field, the maximum change in membrane potential occurs at the ends of the cell closest to the two electrodes. The magnitude of the potential change depends in part on the projection of the cell length along the electric field, so that the change in potential will be less for the \( \perp \) orientation. Therefore, an increased electric field is needed to produce the same degree of membrane depolarization as for the \( \parallel \) orientation, which results in an apparent increase in excitation threshold.

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cell length (with respect to excitation) may have been less than the anatomic length, and the effective cell width may have been greater than the anatomic width. Another possibility is that, because only a fraction of the total surface area of the cell is depolarized by field stimulation,22 the remaining passive membrane presents a load on the excited membrane patch, so that there will be a minimal, or "liminal" length of membrane required for stimulation.27,28 If this were the case, long and thin cells with a parallel orientation would have a much lower percentage surface area of excited membrane compared with those with a perpendicular orientation22 and, therefore, might exhibit a higher excitation threshold, offsetting the reduction associated with the parallel cell orientation. The same effect may also explain why the excitation threshold for frog cells is not much lower than that for guinea pig cells when both are in the parallel orientation, whereas the threshold for frog cells is almost twofold higher than that for guinea pig cells when both are in the perpendicular orientation (Figure 3).

Values of electric field stimulation have been reported in dog ventricle (estimated using a linear discriminant function) to be 0.64 V/cm for 3-msec pulses along the longitudinal fiber direction14 and in cultured chick cell aggregates to be 2.4 V/cm for 5-msec rectangular pulses with random orientation at low pacing rates9 (although the value depends in part on aggregate size) and can be compared with the mean value of 2.6 V/cm obtained in our isolated guinea pig myocytes for 2-msec pulses in the parallel orientation. The somewhat higher absolute levels of stimulation required for our myocytes compared with intact tissue may be explained by theoretical considerations. When the extracellular space available to a cell is limited, as in the case of tissue where each cell is surrounded by other (relatively nonconducting) cells, the transmembrane potential induced at the ends of the cell increases,29 so that the level of extracellular field required for stimulation would be lower than that for a single cell in isolation. Another possibility is that the well-known electrical coupling between cardiac cells results in an effective electrical length that is greater than the anatomic length of the cell, as suggested by the recent periodic structure model of cardiac muscle,30 which again serves to increase the transmembrane potential induced in tissue by the extracellular field and to lower the effective stimulus threshold.

The ESD is generally explained in terms of a transmembrane current source that charges a uniformly polarized, resistive-capacitive membrane to a constant voltage threshold with a several-millisecond-long charging time constant.24,27 However, this process needs to be clarified for the case of extracellular field stimulation. When cells are excited by a rectangular field pulse, the extracellular field produces a spatially varying potential along the external surface of the cell membrane. Since the intracellular potential remains essentially isopotential, the transmembrane potential will vary, with one end of the cell depolarized and the other end hyperpolarized.22 The membrane potential distribution will be similar to that modeled for an insulating surface22 but will differ slightly as a consequence of the small current flow through the resting, inwardly rectifying potassium channel. The charging time constant for the evolution of the transmembrane potential is governed initially by the cell membrane capacitance and the effective series resistance (consisting of extracellular plus half the intracellular resistivities times the cell radius31) and is on the order of microseconds (assuming a cell capacitance of 1 μF/cm², sum of intracellular and extracellular resistivities of 500 Ω-cm, and cell radius of 10 μm). Such rapid kinetics for charging of the cell membrane have been observed in sea urchin eggs by using pulsed laser microscopy and voltage-sensitive dyes.32 Therefore, we would expect the membrane to be polarized (nonuniformly) virtually instantaneously by the extracellular field. However, if excitation threshold is attained at the depolarized end of the cell, sodium currents are activated and act as an inward current source to depolarize the remainder of the cell, with a charging time course now a function of membrane impedance and activation kinetics of the sodium channel. At this point, the classical theories for ESD curves, derived for transmembrane current sources, may become applicable but will need to account for a voltage-dependent current source that varies temporally and spatially. Another aspect of the data that remains to be explained is that ultrashort pulses as brief as 20 μsec are sufficient for stimulation (Figures 4–6), similar to observations reported in whole heart.33,34 It is unclear whether activation of the sodium current can occur during such brief time intervals. The level of field intensity at these durations can exceed 60 V/cm (Figures 4–6) and may be sufficiently high such that excitation may occur by a different mechanism, such as a transient electrophoremeabilization of the cell membrane (discussed later).

Nevertheless, assuming that the classical theories of excitation are applicable, the characteristic time constant described by these data is then of interest. The Lapicque-Hill equation27 based on theoretical considerations describes the ESD relation for excitable membranes:

\[ I = \frac{I_{rh}}{1 - e^{-\frac{t}{\tau}}} \]  

where \( I \) is the stimulus strength, \( I_{rh} \) is the rheobase, \( t \) is the pulse duration, and \( \tau \) is the ESD time constant. Time constant \( \tau \) can be determined by finding the limit extrapolated to time zero of the ratio of "charge" \( I_{rh} t \) divided by \( I_{rh} \); note that only under the restrictive conditions described earlier for uniformly polarized membranes will \( \tau \) be a measure of the resting membrane time constant.27,28 However, we found that our data were better fit by the empirical Weiss-Lapicque equation,27,28 which has a slightly smaller upward concavity:
The chronaxie $c$ corresponds to $\tau$ of Equation 1. The “charge” $I \times t$ is a linear function of $t$, and $c$ can be estimated as the ratio of $y$ intercept to the slope of the best fit line.$^{24}$ This procedure was applied to analyze the guinea pig data (Figure 6) but not to the frog data because of the confounding notch in the ESD curve (Figure 5).

We found that the theoretical ESD curve (Equation 1 or 2) of the guinea pig myocytes was consistently greater than the measured ESD curve at pulse durations <0.2 msec. Therefore, we used only the data for durations ≥0.2 msec for the curve fits. When Equation 2 was fit to the data of all 14 cells treated together as an ensemble, $c$ and $I_h$ had values of 2.2 msec and 0.46, respectively, for the || orientation and 2.3 msec and 1.10 for the ⊥ orientation, respectively (curve fits for the ensemble are shown in Figure 6 as dotted lines). When Equation 2 was repetitively fit to the data on a cell by cell basis, the mean±SD values for $c$ and $I_h$ were 2.5±1.2 msec and 0.46±0.11, respectively, for the || orientation and 2.6±1.3 msec and 1.10±0.33 for the ⊥ orientation, respectively. The difference in mean values for $c$ for the two orientations was not statistically significant, as determined by a paired t test ($p>0.76$).

For comparison, the data were also fit by Equation 1, which, because of its slightly greater concavity, yielded somewhat smaller estimates for $c$ ($=\tau$): 1.4±0.9 msec for the || and 1.3±0.6 msec for the ⊥ orientation (fitting the data on a linear scale) or 1.7±0.8 and 1.7±0.7 msec (fitting the data on a logarithmic scale). Despite the variability owing to the specific method used to estimate the chronaxie, our values for $c$ appear to be in the range of those measured previously, ~2.7 msec in dog ventricle$^{33}$ and ~1.6 msec in cultured chick cell aggregates, and are insensitive to cell orientation.

As described earlier, the stimulus threshold was consistently lower than that expected from Equation 2 at pulse durations <0.2 msec. This finding was not observed in myocardial stimulation of dog and turtle ventricle$^{33}$ even for stimuli as brief as 1 μsec. On the other hand, a drop-off in charge threshold from the predicted linear dependence on pulse duration (Equation 2) has been observed for durations <2–3 msec in shortened sheep Purkinje fibers$^{27}$ and attributed to nonuniformity in charge distribution along the cell membrane. Alternatively, other mechanisms may assist in cell excitation at ultrashort pulse durations, such as a transient electropermeabilization of the cell membrane$^{56}$ produced by the large amplitude electric field, resulting in a nonspecific leakage pathway through the membrane and leading to depolarization of the cell on the one hand and direct entry of calcium ion into the cell on the other.$^{18}$

A notch in the ESD curve was observed at durations of ~1–2 msec in the frog ventricular myocytes but not in the guinea pig myocytes. One possibility is related to the cell morphology: because the frog cells rarely were straight along their long axis (Figure 1, top panel), local maxima in transmembrane potential may have occurred in regions of the cell other than the ends. For the longer stimulus durations (≥2 msec), the membrane eventually may have depolarized with some delay and lowered the apparent stimulus threshold, analogous to “escape” sometimes observed with the voltage clamp. A second possibility is that the ESD curve levels off at durations <1 msec, perhaps owing to electropermeabilization of the cell membrane as suggested earlier. A third possibility is that the notch reflects the activation of all excitatory channels with the longer stimulus durations but only a fraction of the channels with the shorter pulses, owing to differences in the kinetics for activation of the various channels. For example, “threshold”-type sodium channels that have been found in squid giant axon$^{37}$ may exist in frog cardiac cells, although they are absent in mammalian cardiac cells.$^{38}$ All of these possibilities are highly speculative and require further experiments for verification.

Our results suggest that it is advantageous to apply excitatory currents in a direction || to, rather than ⊥ to, the long axis of the heart cell. Of course, these results should be extrapolated with caution to the intact tissue. As mentioned earlier, electrical coupling between cardiac cells (which differs in the longitudinal and transverse directions) may influence the response of the cell membrane to the externally applied electric field, so that the fiber axis in the tissue may not be fully equivalent in an electrical sense to cell axis. Nevertheless, our observations of the influence of the electrical axis of stimulation on cardiac cell excitability support earlier observations of the differences of potential gradient associated with stimulation of intact tissue, measured parallel and transverse to the fiber axis and estimated to be in the range of 1.9–2.9.$^{14}$

In sequential shock with multiple current pathways,$^{6,7}$ it is likely that the multiple pathways act primarily to distribute the shock current more evenly throughout the heart volume. However, placement of multiple electrodes in an orthogonal configuration may provide some further advantage. Fibers in the heart wall have a helix angle that varies transmurally from approximately −60° to +60° relative to the circumferential axis.$^{39}$ Therefore, regions of the heart exposed to two orthogonal fields may benefit in having one subpopulation of heart cells respond to one shock and a separate, orthogonally oriented subpopulation of heart cells respond to the other shock. We would expect that this improvement would be observed primarily when the two orthogonal fields are directed along the heart surface (e.g., with pairs of epicardial patch electrodes) and not transmurally across the heart wall (e.g., between epicardial patch and catheter electrodes). Even for regions subjected to fields along only one axis, the results of our study suggest that it would be beneficial to direct the field axis along, rather than across, the fiber axis. These guidelines may be of use in evaluating the efficacy of electrode placement when using computer simula-
tion, not only by calculating the spatial distribution of electric field throughout the heart volume but also by taking into account the relative alignment of the field with respect to fiber direction.

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**References**


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