Cellular Mechanism of the Functional Refractory Period in Ventricular Muscle

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A premature action potential elicited in ventricular muscle during the functional refractory period of a preceding action potential requires an increased stimulus intensity for successful propagation. We measured the cellular basis for these relative decreases in tissue excitability during the recovery phase by performing parallel experiments on rabbit left papillary muscle and isolated rabbit ventricular cells in addition to conducting theoretical studies with numerical simulations of action potential initiation. For each experimental preparation, the pacing protocol consisted of a train of 10 stimuli (S1) at an S1-S1 interval of 500 msec with a premature stimulus (S2) of variable S1-S2 intervals following the tenth S1 action potential. The stimulus threshold for initiation of an S2 action potential (I2) was then measured as a function of the time of occurrence of the S2 stimulus relative to the time of 95% repolarization of the tenth S1 action potential (stimulus delay [SD] time). In the tissue preparation, the I2 increased sharply for SD times < 0 msec to a value that was 100% above the S1 stimulus threshold for SD time = -5 ± 2.4 msec (n = 8). Similar experiments on the isolated ventricular cell showed no increases in I2 as a function of SD time but rather significant decreases in both the action potential amplitude (APA) and the maximum rate of rise of the action potential upstroke (Vmax) of the S2 action potential. The APA and Vmax for the S2 action potential were decreased to 50% of the S1 action potential values for SD time = -5.2 ± 2.1 msec and SD time = 0.3 ± 1.6 msec, respectively (n = 8). Both parameters reached 100% recovery by SD time = 10 msec. These results and our numerical simulations are consistent with the hypothesis that the decreases in tissue excitability that occur with premature stimulation have a cellular mechanism as a result of a decrease in cellular responsiveness (APA, Vmax) rather than an intrinsic decrease in cellular excitability. (Circulation Research 1990;66:147–162)

Initiation and propagation of cardiac action potentials involves a complex interaction between the excitability properties of the individual cells and the multidimensional flow of intercellular current. Our goal in this paper is to present data from papillary muscles, isolated ventricular cells, and numerical simulations that clarify the roles of the cellular and syncytial properties in determination of the time course of recovery of tissue excitability during premature excitation.

The definition of the functional refractory period for the cardiac action potential has been well established.1,2 The functional refractory period can be described as the time interval from the upstroke of the action potential to the point during phase 3 repolarization in which a second propagating action potential can be elicited by use of a current stimulus intensity that is 1.5–2.0 times the diastolic threshold. It is during this phase of the action potential that the current threshold decreases at a relatively rapid rate for action potentials elicited at progressively later intervals. Previous studies in both the canine endocardium and epicardium have shown threefold to fivefold decreases in the current threshold over a 10–15 msec interval during phase 3 repolarization.3 This measurement of current threshold provides quantitative assessment of relative changes in tissue excitability, which is defined as the inverse of the current threshold.3,4 With this relation, the time course of recovery of tissue excitability during phase 3 repolarization can be expressed in terms of a return of the current threshold to its diastolic level. These assessments of current threshold or tissue excitability are a reflection of those properties or events that are responsible for the all-or-none phenomenon in the successful initiation of a propagating action potential.

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Supported in part by National Institutes of Health grants HL-22562 and HL-41851 and the Children’s Research Center, Department of Pediatrics, Emory University.

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Received November 25, 1988; accepted August 1, 1989.
During the period of recovery of tissue excitability, there is also recovery of tissue responsiveness, defined as those properties that reflect the magnitude of the tissue's response to a current stimulus after the threshold voltage for a successful propagating action potential is achieved. The maximum rate of rise of the action potential upstroke (V

max

), the action potential amplitude (APA), and the conduction velocity are, in a manner similar to tissue excitability, decreased during repolarization and follow a time course of recovery that includes phase 3 repolarization and the early part of phase 4. Since these properties are related to the magnitude of the net inward current generated by each cell in the tissue, the decreases in tissue responsiveness during this period are related to the incomplete recovery from inactivation of the time- and voltage-dependent sodium channels.

The time course of recovery of tissue excitability during phase 3 repolarization may be dependent on the tissue geometry of the preparation. In the nearly one-dimensional canine Purkinje strand, Spear and Moore demonstrated a period of supernormal excitability during phase 3 repolarization in which the required current for successful initiation of a propagating action potential along the strand was, on the average, 17% less than the diastolic threshold. At more depolarized levels during the earlier part of phase 3 repolarization, current requirements were increased several times until the tissue became inexcitable. In multidimensional ventricular muscle preparations, there was no period of supernormal excitability, such that for more premature action potentials, the current requirement continually increased from the diastolic level until the tissue became inexcitable.

Few studies have focused on the distinction between the intrinsic properties of excitability and those properties that can be attributed to the tissue geometry. While these studies have attempted to eliminate the effects of tissue geometry by use of a small, approximately isopotential tissue preparation, the properties of excitability in such preparations have not been examined. Instead, these studies have closely examined the properties of responsiveness in the small preparation by determination of the relative rates of recovery for V

max

 and APA, using both premature and elevated [K

+]i, as a way of decreasing the takeoff potential. In addition, it should be noted that the action potentials from these small preparations have exhibited some degree of propagation, suggesting that they may possess properties that are still syncytial in nature. Thus, they are not ideal preparations for distinguishing properties of excitability from tissue geometry.

One goal of this paper, therefore, is measurement of the recovery of properties at the cellular level when these properties are isolated from the influence of tissue geometry. By expressing these cellular properties in terms of either cellular excitability or cellular responsiveness, we can then compare the relative influence of each property on the recovery of tissue excitability during the repolarization phase of an action potential. We propose two hypotheses as possible mechanisms for the decreased tissue excitability during phase 3 repolarization. Hypothesis 1 states that there is a general decrease in excitability for each cell that, in turn, directly lowers tissue excitability. One possible explanation for this decrease in tissue excitability would be a general decrease in the input resistance of each cell during phase 3 repolarization because of the increase in K

+ conductance. Since each cell would require a larger stimulus current to elicit an action potential, the cells as a group would require a corresponding increase in the current stimulus strength to elicit a propagating action potential. From single-cell studies, we can directly measure cellular excitability during phase 3 repolarization in the same manner that we measure tissue excitability by defining it as the inverse of the cellular current threshold. Therefore, in single-cell studies that compare the current threshold for an action potential elicited during diastole with the current threshold for an action potential elicited during the repolarization phase of a preceding action potential, this hypothesis would predict that the time course of changes in the current threshold for the isolated cell would be similar to those determined for the intact tissue.

Hypothesis 2 states that the decreased responsiveness of each cell, as a result of the incomplete recovery from inactivation of sodium channels during the repolarization phase, is responsible for the decreased tissue excitability. We have defined cellular responsiveness in the same manner as for tissue responsiveness, expressing it in terms of the V

max

 and APA as functions of time during the repolarization process. This hypothesis can be explained by the liminal length theory for a one-dimensional strand of cells. According to the liminal length theory, after the injection of a threshold current pulse into the strand, there are a number of cells, located at a distance from the stimulus but within a liminal length, whose membrane potentials are more positive than the cellular voltage threshold and thus have an inward membrane current. The remaining cells of the strand, located outside the liminal length, have a subthreshold potential and produce a net outward membrane current. The spatial integral of the inward and outward currents from each cell results in a net inward current for the strand that is sufficient for initiation of a propagating action potential along the strand. Therefore, any decrease in the net inward current at the cellular level would result in a larger number of cells (or a larger liminal length) required for generation of a net inward current for the strand. This increase in liminal length would be reflected as an increase in tissue current threshold for action potential initiation because a larger stimulus current would be required to bring a larger number of cells above threshold. This hypothesis would predict essentially no changes in the single-cell current threshold for premature action potentials elicited either dur-
ing diastole or earlier during phase 3 repolarization; however, the action potentials elicited during phase 3 repolarization would have a decreased $V_{\text{max}}$ and APA.

Therefore, in this paper our first aim is quantitative definition of the recovery in tissue excitability for the multidimensional papillary muscle during phase 3 repolarization and the early part of phase 4 diastole in terms of the current threshold. To eliminate the influence of tissue geometry on the recovery of excitability, we also performed parallel studies in isolated rabbit ventricular cells. Based on the results from the isolated cells, our second goal is assessment of the mechanisms of these changes in tissue excitability in terms of two cellular properties: 1) changes in cellular excitability that occur during this same time interval, defined as changes in the current threshold for successful elicitation of a premature action potential; and 2) changes in cellular responsiveness that occur during this time interval, defined as the APA and $V_{\text{max}}$ for the same premature action potentials. Finally, we examined the dependence of liminal current on the sodium channel availability and the sensitivity of this dependence in both the one-dimensional and two-dimensional syncytium. Using the Beeler-Reuter (BR) model, we analyzed the alterations in liminal length produced by changes in sodium channel conductance ($G_{\text{Na}}$) for the one-dimensional strand and the two-dimensional disk. Thus, our third goal is 1) evaluation of the effect of $G_{\text{Na}}$ on the liminal length for a one-dimensional strand with a complete membrane model as a comparison with previous theoretical work done with a static polynomial approximation for the current-voltage relation, and 2) determination of whether the additional electrical loading effect of a multidimensional system would increase the dependence of liminal length on the availability of inward current.

Materials and Methods

Syncytial Preparation (Papillary Muscles)

Adult rabbits of either sex weighing 1–2 kg were anesthetized by intravenous administration of pentobarbital sodium (50 mg/kg) and heparin (1,000 units). The heart was quickly excised, and the left ventricular papillary muscles with some attached ventricular wall were placed in a Plexiglas chamber (15 ml vol). The tissue was superfused at a constant rate of 20 cc/min with Tyrode's solution composed of (mM) NaCl 120, NaH$_2$PO$_4$ 0.3, NaHCO$_3$ 24.2, KCl 4.0, MgCl$_2$ 0.5, CaCl$_2$ 1.8, and dextrose 5.0, aerated with 95% O$_2$ and 5% CO$_2$ and warmed to 36°–37°C.

Stimulating and recording techniques were similar to those previously used, and electrode placement was arranged according to the configuration shown in Figure 1. There were two different sites of stimulation (S1 and S2) on the papillary muscle. The S1 stimulating electrode consisted of a twisted pair of Teflon-coated silver wires (wire diameter 125 µm) and was located near the apex of the papillary muscle. The S2 electrode was also a twisted pair of Teflon-coated silver wires (wire diameter 75 µm), but one strand was slightly shorter (1 mm) than the other to provide monopolar cathodal stimulation to the S2 site. Since the Purkinje layer only covers the basal half of the papillary muscle in the rabbit left ventricle, as previously described, the site chosen for S2 stimulation was apical to this region to ensure direct stimulation of the ventricular layer by the S2 stimulating electrode. The extracellular recording electrode, a modified bipolar electrode (BE) composed of a twisted pair of Teflon-coated silver wires (wire diameter 75 µm), was lightly placed on the papillary muscle surface. Located at a distance of 3–4 mm from the stimulating electrodes, the BE was used to confirm successful propagation of action potentials from both stimulation sites. Because of the strong contractions produced by the papillary muscle, a floating microelectrode (ME) was used to maintain intracellular recordings at the S2 site for sustained periods. The ME, similar to those previously described, had a tip resistance of 10–15 MΩ after being filled with 2.5 M KCl. The recordings from the BE and ME were amplified and sampled at a rate of 10,000 samples/channel/sec by use of an IBM PC/AT computer.

The papillary muscle was paced from the S1 site by use of a stimulus duration of 5 msec and a stimulus intensity 1.5 times the diastolic threshold for initiation of a train of 10 conditioning action potentials at a basic cycle length of 500 msec. After the tenth S1 stimulus, the S2 stimulus, with a 2-msec duration and a variable intensity, was applied to the S2 site at variable stimulus-coupling intervals. A 1-second delay followed the tenth S1 stimulus before the protocol beginning with the S1 stimulus train was repeated. The reason for the use of two different sites of stimulation on the papillary muscle was the reduction
of any tissue fatigue or injury associated with prolonged repetitive stimulation at the site where excitability was measured. This procedure eliminated any drift in current threshold that may have occurred as a result of the use of the same site for both S1 and S2 stimuli. It was also important that the ME implemation be located as close to the S2 stimulation as possible. It has been shown that there are regional differences in action potential duration (APD) in ventricular tissue as well as APD changes that are electrotonically induced near the pacing site. Placement of the ME and S2 stimulating electrodes at the same site eliminated any errors in refractory period measurement due to these regional differences in APD.

Isolated Ventricular Cell Preparation

Adult rabbits of either sex weighing 1–1.5 kg were anesthetized with sodium pentobarbital (50 mg/kg i.v.) and heparin (1,000 units). The heart was rapidly excised, and the aorta was cannulated for Langendorff perfusion and isolation of single cells according to the methods of Taniguchi et al. The heart was first perfused at a rate of 4 ml/min/g with a nominally Ca2+-free Tyrode’s solution consisting of (mM) NaCl 148, KCl 4.0, MgCl₂ 0.5, Na₂HPO₄ 0.3, dextrose 5.0, and HEPES 5.0 with pH adjusted by 7.4 by use of NaOH. After all the blood was washed from the coronary arteries, the heart was perfused for 30–60 minutes with the same Tyrode’s solution containing 40 mg/100 cc Type I collagenase (Sigma Chemical, St. Louis). The collagenase was then washed from the heart with a high K+/low Cl⁻ storage solution composed of (mM) taurine 10, oxalic acid 10, glu-tamic acid 10, KCl 25, KH₂PO₄ 10, dextrose 11, and EGTA 0.5 with pH adjusted to 7.4 by use of KOH. After perfusion of the high K⁺ storage solution, the left ventricle was opened and small pieces of left ventricular endocardium were cut and gently agitated in an experimental chamber containing the same Tyrode’s solution as described above plus 1.8 mM CaCl₂. With a chamber volume of 2 cc, the Tyrode’s solution in the experimental chamber was perfused at 2 cc/min at a constant temperature of 36°–37°C.

Only those cells that were quiescent with preservation of rod-shaped appearance were studied. Relatively high-resistance patch pipettes were used to minimize intracellular dialysis. The electrodes were pulled from borosilicate glass and, after fire-polishing, had resistances of 4–7 MΩ when filled with an internal solution of (mM) KCl 120, Na₂ATP 5.0, EGTA 11, MgCl₂ 5.0, and HEPES 10, with pH adjusted to 7.2 with KOH. High-resistance seals (3–5 GΩ) were formed with the cell membrane by use of light suction, which was followed by disruption of the cell membrane with a transient suction. Both the S1 and S2 stimuli were derived from a digital stimulator.

The pacing protocols for the single cell were the same as those used for the synectical preparation. Each pacing protocol consisted of a train of 10 S1 conditioning stimuli with a 2-msec stimuli duration and a 500-msec basic cycle length followed by an S2 stimulus with a 2-msec stimulus duration and a variable S1-S2 coupling interval. As in the synectical preparation, there was a 1-second delay between the tenth S1 and the first S1 of the next train. By use of steady-state pacing at a basic cycle length of 500 msec, the S1 diastolic threshold was determined for 1:1 pacing. The S1 stimulus intensity was then set at 1.1 times this diastolic threshold for the pacing protocol, while the S2 intensity was set at a value low enough such that the stimulus turned off 1–2 msec before the time of occurrence of the maximum upstroke velocity (Vmax) of the S2 action potential. The minimum sampling rate for data acquisition was 5–10 kHz; the rate was increased to 66 kHz during the S2 action potential upstroke for accurate determinations of its Vmax.

Theoretical Methods

The numerical methods we use for simulation of action potential initiation and propagation have been previously published in detail for the one-dimensional strand and for a radially symmetrical disk of cells. In brief, we use either a linear or radial array of discrete elements (Figure 2) coupled by resistances. Each discrete element has a calculated membrane area that is used to scale the membrane current density of the BR membrane conductance model and also the membrane capacitance (1 μF/cm²). The set of equations that can be derived for membrane potential and membrane current as functions of distance and time can be solved at each time step by a modification of the method of Crank and Nicholson as developed by Lieberman et al. As we described previously, the equations derived for the disk model allow a one-dimensional solution while preserving the two-dimensional effects of the geometric model due to the radial symmetry.

Figure 2 illustrates the two geometric arrangements simulated in this study. The one-dimensional strand is assumed to be a rectangular solid with a width of 50 μm and a depth of 200 μm. Each discrete element of the strand has a length of 50 μm. The discrete elements do not correspond to single cells. The surface-to-volume area of the cells (S₀=5,000/ cm) is used for calculation of the membrane area of each element as S₀ (0.005)(0.005)(0.02)=0.0025 cm². Since the membrane resistivity of the BR model at rest is about 5,500 Ω cm², this calculation gives an input resistance for each element of 2.2 MΩ. The longitudinal resistivity is assumed to be 200 Ω cm, including the cytoplasmic and gap junctional resistances. Thus, the resistance between the centers of adjacent elements is (0.005)(200)/(0.005)(0.02)=10 KΩ. For these simulations, we used the central element of 101 elements for current injection, giving a distance of 5 mm from the stimulus point to either end of the strand.

For the disk model, we assumed radial symmetry about the central element into which current is applied. Each successive radial element from the center has a radius increase of 50 μm. We used the
same $S_n$, longitudinal resistivity, and membrane model for the disk as for the strand. The input resistance of each radial element depends on its position from the center, as does the resistance between adjacent elements.\textsuperscript{25}

The plot in Figure 2 was generated by injection of a steady current into either the one-dimensional or two-dimensional model sufficient to depolarize the central element by about 5 mV and then by calculation of the relative depolarization as a function of distance from the stimulus by dividing each membrane depolarization by the steady potential of the element into which current was injected. The numerical simulations gave the result expected from analytical approximations\textsuperscript{29} that the voltage decay for small depolarizations is nearly exponential for the one-dimensional strand, with a length constant of 0.80 mm, while for the two-dimensional model the steady-state voltage distribution is steeper, with an effective length constant (defined simply as the distance at which the depolarization has declined to 63\% of the central value) of 0.26 mm.

The numerical simulations were performed in FORTRAN on a Compaq 386/20 personal computer by use of an integrating time step of 2 \textmu s.

**Results**

In syncytial preparations the minimum current (I2) required for initiation of a propagating S2 action potential after a train of S1 conditioning action potentials depends on the coupling interval of the S1 and S2 stimuli (S1-S2 interval). With this I2 parameter as a criterion for determination of relative tissue refractoriness, we measured the I2 at variable S1-S2 intervals and investigated tissue refractoriness as a function of the time of occurrence of S2 relative to the repolarization phase of the action potential elicited by the tenth S1 for superfused rabbit papillary muscles. An example of these data is illustrated in Figure 3, which shows two sets of microelectrode recordings of action potentials from the tenth S1 and the following S2. We have added a dashed vertical line to indicate the time at which the S1 action potential has repolarized to within 5 mV of the resting membrane potential and to identify this point as time=0 msec in the figure. On the horizontal axis we define the stimulus delay (SD) time as the time of the S2 stimulus relative to this time of repolarization of the S2 action potential to within 5 mV of the resting membrane potential. In the top example, the S2 stimulus occurs after the time of SD=0 msec, resulting in a positive SD time. In the bottom example, the S2 intensity has been increased to elicit an earlier S2 response, resulting in an SD time with an approximate value of 0 msec. This definition of SD time is similar to the “diastolic interval” or “test interval” defined by others.\textsuperscript{5,7,20} We also defined the APD\textsubscript{50} of the S1 action potential as the time interval from the action potential upstroke to this time of repolarization to within 5 mV of the resting membrane potential.

Figure 4 summarizes eight experiments reflecting the means and standard deviations of the earliest SD times for variable values of I2. In each preparation, we first determined I2 for a relatively long S1-S2 interval, which for each case was equal to the basic
cycle length (500 msec) of the S1 conditioning stimuli (I2500). By successively increasing the S2 current amplitude, we then determined the shortest S1-S2 interval that produced successful S2 action potential propagation for each I2 value. We then defined the relative S2 threshold (relative I2) as the ratio of the S2 current amplitude at that S1-S2 interval (I2) and the I2500. Finally, for each of these S1-S2 intervals, we computed the corresponding SD time as shown in Figure 3. Figure 4 graphs the relative I2 as a function of SD time and illustrates the relation for the increased current requirement in initiation of an S2 action potential with respect to its time of stimulation during phase 3 repolarization. It is evident that, as we decrease the S1-S2 interval in the syncytial preparation, the relative I2 begins to increase at SD times less than approximately 25 msec and that for SD times between 25 and 0 msec the relative I2 increases by 50%. For SD times between 0 and −8 msec, the relative I2 sharply increases from a value of 1.5 to 3.0. In these syncytial preparations the APD−5 of the tenth S1 action potential remained constant throughout each experiment. Thus, for those I2 values that elicited an S2 action potential before the 5-mV repolarization point of the S1 action potential (SD time < 0 msec), we computed the SD time using APD−5 values from other tenth S1 action potentials showing complete repolarization.

The use of SD time rather than S1-S2 interval allowed us to compare the time courses for recovery of tissue excitability among preparations with different APDs as well as to examine interventions that simultaneously affect both APD and the time course of recovery of excitability. This comparison is shown in Table 1, which tabulates the individual values used in calculation of all of the SD times summarized in Figure 4 for a relative I2 value equal to 2.0. The values for the S1 conduction time represent the arrival time of the S1 action potential at the S2 site, as recorded by the ME, with respect to its time of initiation at the S1 site. The point during the S1-S2 interval at which the SD time is equal to 0 msec is the

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**Figure 3.** Two sets of action potentials recorded from S2 stimulation site for rabbit papillary muscle preparation. Each set consists of tenth S1 and S2 action potential plotted with respect to stimulus delay (SD) time. Dashed vertical line indicates time at which S1 action potential has repolarized to within 5 mV of resting membrane potential and identifies this point as 0. SD time for each set of action potentials is time of occurrence of S2 stimulus relative to this time of repolarization. Position of S2 action potential in each trace represents shortest S1-S2 interval obtained for a specific S2 stimulus intensity. Top Panel: S2 stimulus intensity was sufficient to elicit an S2 action potential with an SD time of approximately 20 msec. Bottom Panel: S2 stimulus intensity was increased to elicit an earlier S2 action potential with an SD time near 0 msec.

**Figure 4.** Relative recovery of tissue excitability as a function of stimulus delay (SD) time for rabbit papillary muscle preparation. Relative S2 intensity is plotted on ordinate and is calculated as ratio of stimulus current threshold for premature response (I2) and rheobase stimulus current threshold for an S2 action potential with a stimulus coupling interval equal to basic cycle length for S1 conditioning action potentials (I2500). ▲, average SD time (±SD) for successive increases in relative S2 intensity (n=8).
sum of the S1 conduction time and the APD₅. Therefore, we can calculate the SD time of the S2 action potential as the S1-S2 interval minus the sum of the S1 conduction time and the APD₅. In the comparison of the measured S1-S2 intervals with the calculated SD times, it is apparent that even though the APD₅ varies from 139 to 212 msec (187±23.5 msec), the determination of the time at which I₂ is increased by a factor of 2.0 is quite constant when expressed as an SD time (−5±2.4 msec). The values for the S1-S2 interval, on the other hand, range from 147 to 227 msec (199±26.6 msec), a variation similar to that observed with the APD₅ values.

The methods for determination of the time course of recovery of tissue excitability for the papillary muscle were applied with slight modification in the single-cell experiments for determination of the time course of recovery for cellular excitability. One fundamental difference observed between the single-cell and the syncytial preparations is illustrated in Figure 5A, which shows four pairs of action potentials recorded from a single ventricular cell, with each pair consisting of the tenth S1 action potential and the action potential resulting from the S2 stimulus. Unlike the APDs in the syncytial preparations, which remained constant at steady-state pacing, the single-cell action potentials had APDs that varied from beat to beat over a 25-msec range. It is important to note that the APD variations we observed were beat-to-beat fluctuations rather than continual increases or decreases occurring over a short period, such as those decreases in APD that are often attributed to

**Table 1. Rabbit Left Ventricular Papillary Muscle Preparations**

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>S1 conduction time (msec)</th>
<th>APD₅ (msec)</th>
<th>S1-S2 interval* (msec)</th>
<th>SD time† (msec)</th>
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<td>188</td>
<td>204</td>
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<td>139</td>
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<tr>
<td>Mean±SD</td>
<td></td>
<td>187±23.5</td>
<td>199±26.6</td>
<td>−5±2.4</td>
</tr>
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</table>

APD₅, action potential duration.
*For all values in this column, S2 stimulus intensity was set to a value of two times the I₂₀₀ (relative I₂=2.0). Each value represents shortest S1=S2 interval obtained in which S2 stimulus intensity was sufficient for initiation of a propagating S2 action potential.
†Stimulus delay (SD) time = S1-S2 interval minus S1 conduction time minus APD₅.

**Figure 5.** Left Panel: *Four sets of superimposed action potentials from an isolated rabbit ventricular cell, illustrating effect of decreasing stimulus delay (SD) time on resulting S2 action potential response. Each set, labeled 1 through 4, consists of tenth S1 action potential and corresponding S2 action potential. For all four sets of action potentials, the S1-S2 interval was 180 msec and the S2 stimulus intensity was set equal to S1 stimulus current threshold for 1:1 steady-state pacing. This figure is also a typical example in which S1-S2 interval remained constant while action potential duration was allowed to fluctuate and, therefore, generate a range of SD times. Right Panel: *Data from a typical experiment describing relative change in cellular response as a function of SD time. Open symbols represent relative changes in amplitude of S2 action potential (APA), and closed symbols represent relative changes in maximum rate of rise of upstroke of S2 action potential (V_m). Relative values for both parameters are calculated as ratio of value measured for S2 action potential to value measured for S1 action potential during steady-state pacing at basic cycle length of 500 msec. S2 stimulus intensity for all S2 action potentials represented in this graph was set to a value 5% above S1 current threshold for steady-state pacing.
cell “rundown” or deterioration. The four sets of action potentials in Figure 5A were obtained from the protocol described earlier using an S1-S2 interval of 180 msec and an S2 intensity equal to the S1 threshold (I1) for all four sets. Unlike the syncytial preparation, in which we varied the S1-S2 interval to generate a range of SD times for a constant APD, for the single-cell preparation with its variable APD we kept the S1-S2 interval constant and allowed the fluctuating APD to generate a series of SD times. In Figure 5A, the S1 action potential with the shortest APD and, therefore, the most positive SD time results in a corresponding S2 action potential that is similar in shape to the S1 action potential. For S1 action potentials with longer APDs (2, 3, and 4), corresponding to shorter SD times, the resulting S2 action potentials respond with smaller amplitudes and slower rates of rise. From Figure 5A it is apparent that although the four S2 action potentials illustrate a wide spectrum of cellular responsiveness as a function of SD time, the cellular excitability in terms of the current threshold I2 was not decreased during the terminal repolarization. Despite being equal in magnitude to the I1, the S2 stimulus was sufficient to successfully elicit all four premature action potentials. This result is in sharp contrast with the twofold and threefold decreases observed in the tissue excitability of the syncytial preparations.

Because the cellular excitability was not significantly altered with corresponding changes in the SD time, we instead measured changes in the cellular responsiveness with the S2 action potential as a function of its time of occurrence during the repolarization phase of the S1 action potential by plotting the APA and the Vmax of the S2 action potential as a function of the SD time. However, unlike the syncytial preparation, in which we assumed a constant value for the APD_{50} when determining a value for the SD time <0 msec, our results from Figure 5A showed that this assumption could not be made for the single cell. As an alternative, we established for each cell the APD_{50} as a function of the duration in which the action potential has repolarized to within 40 mV of the resting membrane potential (APD_{40}). The linearity of this relation allowed us to measure the APD_{40} and then use this value, multiplied by the mean ratio of the APD_{50} to the APD_{40}, for determination of the time at which the action potential would have repolarized to within 5 mV of the resting membrane potential (SD time=0). Using this modification as a way of extrapolating the APD_{50} for all SD times <0 msec, we determined the relation of cellular response for a wide range of SD times for a single ventricular cell (Figure 5B). In this experiment the S2 stimulus intensity was set such that the stimulus turned off 1–2 msec before the maximum upstroke of the S2 action potential. For every cell studied, this value of I2 was less than a 5% increase above the S1 current threshold (I1) for steady-state pacing at a basic cycle length of 500 msec. For SD times <0 msec in which the cellular response was significantly decreased and it was difficult to distinguish between an actual S2 action potential and a passive membrane depolarization produced by current injection, we defined an action potential as the membrane depolarization that continued to produce a positive Vmax after the S2 stimulus turned off. Both the APA and Vmax in Figure 5B are expressed as a fraction of the values determined for the S1 action potential. It is evident that the Vmax relation is not only affected at more positive SD times than the APA relation, but also has a greater percent decrease at SD time=0 msec. Despite this apparent difference, both relations showed a decreased cellular responsiveness during the same period of repolarization in which the syncytial preparations showed a decreased tissue excitability.

Table 2 summarizes the results of graphs similar to Figure 5B from six different cells, measuring the time course of recovery for both APA and Vmax. For each

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Action potential amplitude (msec)</th>
<th>Vmax (V/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD time Rel APA=0.50* Rel APA=0.82†</td>
<td>SD time Rel Vmax=0.50* Rel Vmax=0.82†</td>
</tr>
<tr>
<td>1</td>
<td>-3.5 +0.2</td>
<td>+2.8 +8.2</td>
</tr>
<tr>
<td>2</td>
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<td>-1.0 +3.0</td>
</tr>
<tr>
<td>3</td>
<td>-3.0 0.0</td>
<td>+1.0 +4.3</td>
</tr>
<tr>
<td>4</td>
<td>-8.0 -4.8</td>
<td>-1.5 +2.0</td>
</tr>
<tr>
<td>5</td>
<td>-3.5 -1.0</td>
<td>+1.0 +4.8</td>
</tr>
<tr>
<td>6</td>
<td>-6.2 -2.5</td>
<td>-0.4 +4.6</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>-5.2±2.1§ -1.9±1.9†</td>
<td>3.3±0.5§ +0.3±1.6 +4.5±2.1 4.2±0.8</td>
</tr>
</tbody>
</table>

Vmax maximum rate of rise of upstroke of S2 action potential; SD, stimulus delay; APA, action potential amplitude; Rel, relative.

*SD time in which parameter has recovered to 50% of S1 value.
†SD time in which parameter has recovered to 82% of S1 value.
§Approximate time constant of recovery to S1 value, calculated as SD time difference from 50% to 82% recovery.
¶p=0.00004 vs. relative Vmax at 50% recovery.
\*p=0.00006 vs. relative Vmax at 82% recovery.
\p=0.03 vs. T for Vmax.
experiment we determined the corresponding SD times for the value of S2 APA that were 50% and 82% of the value for the S1 action potential (relative APA). The mean SD times corresponding with a relative APA of 50% and 82% were $-5.2 \pm 2.1$ and $-1.9 \pm 1.9$ msec, respectively. Similarly, we measured the SD times corresponding with S2 action potentials having $V_{\text{max}}$ values that were 50% and 82% of those of the S1 action potential (relative $V_{\text{max}}$). The mean SD time was $0.3 \pm 1.6$ msec for a relative $V_{\text{max}}$ of 50% and $4.5 \pm 2.1$ msec for a relative $V_{\text{max}}$ of 82%. The time course from 50% to 82% recovery for each parameter was calculated as the SD time difference between the relative values for 50% and 82%; this value, $T$, represents the approximate time constant of recovery of the parameter. The mean $T$ values for APA and $V_{\text{max}}$ were $3.3 \pm 0.5$ and $4.2 \pm 0.8$ msec, respectively (Table 2). It is important to note that, while each of the relative values for APA and $V_{\text{max}}$ have standard deviations that are approximately 2 msec, the standard deviations for the T values are considerably smaller. This difference suggests minor shifts of 1–2 msec in the recovery curves with relatively constant rates of recovery. Using paired $t$ tests, we also calculated the statistical significance of the SD times for 50% recovery of relative APA versus relative $V_{\text{max}}$, for 82% recovery of relative APA versus relative $V_{\text{max}}$, and for the respective T values of each parameter. The respective $p$ values are shown in Table 2. The $p$ values comparing the SD times for both 50% and 82% maximum recovery of relative APA and relative $V_{\text{max}}$ were less than 0.001. For the T values, the $p$ value comparing the two relative time constants was 0.03.

When we closely examined the current threshold for isolated rabbit ventricular cells during late phase 3 repolarization, we found that, like the near one-dimensional Purkinje strand, the single cells did indeed have a period of supernormal cellular excitability, in contrast with the intact ventricular tissue, which had no period of supernormal tissue excitability. Figure 6 shows an example of this phenomenon. We measured the current threshold (I1) for regular stimulation and then set the I2 current level at 0.9 times this I1 threshold. As in previous figures, the traces start just before the tenth S1 stimulus of a train. During late phase 3 repolarization, this smaller I2 current was able to elicit a premature action potential, as shown by the trace with the earlier premature stimulus. When we raised the I2 current to 1.0 times the I1 threshold and delayed the S2 stimulus beyond the period of supernormal cellular excitability, a failure of premature excitation occurred, as shown by the trace with the later premature stimulus.

**Theoretical Results**

The influence of $G_{Na}$ on the threshold for the isopotential BR model is shown in the simulations in Figure 7. For each value of $G_{Na}$, we found the threshold stimulus current for initiation of an action potential and plotted the resulting action potentials for $G_{Na} \times 1$, $\times 2$, and $\times 4$. We defined the membrane potential at the end of the just-threshold stimulus pulse as the threshold potential for action potential initiation. For $G_{Na} \times 1$, $\times 2$, and $\times 4$ the threshold stimulus currents were 0.0137, 0.0122, and 0.0107 mA/cm$^2$, and the threshold potentials, expressed as depolarizations from the resting potential, were 25.2, 22.4, and 19.8 mV, respectively. Note that this fourfold range of $G_{Na}$ had only a small effect on either the threshold stimulus current or the threshold potential for the isolated cell.

For the one-dimensional and two-dimensional simulations, we defined the threshold stimulus current as the magnitude of a 2-msec current pulse that was just adequate to initiate a propagated action potential. Results for this threshold current application are shown in Figure 8, using $G_{Na} \times 2$ for both the one-dimensional and two-dimensional models. In each case, we plotted the membrane potential for every
fifth element (a spatial increment of 250 μm), starting at the element into which current was injected. Successive plots have been displaced vertically for clarity. For the one-dimensional strand, the membrane potential achieves a depolarization of 39 mV at the end of the stimulus current and then starts to decline. After a delay of a few milliseconds, the action potential begins at the site of current injection and propagates toward the ends of the strand. Results for the two-dimensional disk show several striking differences. For the same membrane model, the required depolarization of the element where current is injected is much greater for the two-dimensional disk than for the one-dimensional strand (71 mV for the disk). There is also more of a drop in membrane potential at the central element at the end of the stimulus current, before activation begins.

This effect of a radial electrical load also accentuates the sensitivity of the initiation process to changes in $G_{Na}$. Figure 9 illustrates this difference in sensitivity for the two-dimensional model versus the one-dimensional model by comparison of the changes in the magnitude of the membrane current integral at the end of a just-threshold for decreasing values of
\(G_{Na}\). For low values of stimulus current, the membrane current integral at the end of the stimulus is an increasingly positive value. However, as sodium conductance starts to turn on during the larger stimulus currents, the current integral crosses zero and becomes strongly negative. As \(G_{Na}\) is increased, the poststimulus current integral shifts to the left. The actual magnitude of the poststimulus current integral achieved for the just-threshold stimulus current is larger for lower values of \(G_{Na}\). This result comes from the increased demand for inward current at lower values of \(G_{Na}\) for initiation of a propagated response. The differences between the one-dimensional model and the two-dimensional model are substantial. The effect of \(G_{Na}\) on the magnitude of the poststimulus current integral is much larger for the two-dimensional model than for the one-dimensional model. The results for both models illustrate that the conditions specified for the definition of a liminal length predict a threshold stimulus current somewhat lower than that actually required. For successful initiation of a propagating action potential, the membrane current integral at the end of the stimulus has to be not merely negative but actually negative with a substantial magnitude, and this magnitude is very sensitive to the value of \(G_{Na}\).

The differences in the poststimulus membrane current integrals required for action potential initiation in the one- and two-dimensional models cannot be explained entirely on the differences in input resistance for the two models. For the one-dimensional strand the input resistance for small depolarizations is 79 kΩ, while for the two-dimensional disk the input resistance is 4.9 kΩ, giving an input resistance ratio of 16. For \(G_{Na}\) \(\times 1\), \(\times 2\), and \(\times 4\) the ratios of the required membrane current integral for the two-dimensional disk versus the one-dimensional strand were 38, 30, and 25, respectively.

We can compute the actual value of liminal length for our two geometric models as functions of \(G_{Na}\) in two different ways. Figure 10 illustrates the procedure used by Fozzard and Schoenberg\(^{31}\) on sheep Purkinje strands. For the one-dimensional strand, we plotted the spatial distribution of membrane potential at the end of a just-threshold current pulse for \(G_{Na} \times 1\), \(\times 2\), and \(\times 4\). We used the intrinsic (or isopotential) membrane depolarization threshold (obtained from Figure 7) to distinguish the length over which the cells should have an inward membrane current. The arrows in Figure 10 indicate the length of the strand over which the membrane depolarization exceeds the intrinsic depolarization threshold. For the one-dimensional strand, the two arrows are at nearly the same length, indicating essentially no effect of \(G_{Na}\) on the liminal length for action potential initiation. The actual value of liminal length, about 200 µm, is short compared with the space constant for this one-dimensional strand (Figure 2), in agreement with the experimental results. For the two-dimensional disk, a very different result is obtained. The same procedure of adding arrows at a vertical height corresponding to the values of intrin-
sic membrane depolarization threshold is used. However, the arrows indicate a very substantial effect of $G_{Na}$ on this determination of liminal length, and both values of liminal length are as large as or larger than the effective space constant for the two-dimensional disk.

Figure 11 illustrates a determination of the effects of $G_{Na}$ on liminal length by a procedure that more closely corresponds to the original definition of a length over which inward membrane current occurs at the end of a just-threshold stimulus current. For this plot, we computed the poststimulus current density (in milliamperes per square centimeter) for each element at the end of the stimulus current and plotted these current densities as a function of distance from the stimulus site. For the one-dimensional strand, the three curves for $G_{Na}$ × 1, × 2, and × 4 are nearly indistinguishable, with all three curves intersecting the horizontal axis at a length of 250 μm. For the two-dimensional disk, the three curves are very different. For high $G_{Na}$ (×4) the curve has the same general shape as for the one-dimensional strand, with a monotonic decrease in the magnitude of inward current with distance from the stimulus site. For

**Figure 10.** For one-dimensional strand (top panel) and two-dimensional disk (bottom panel), we plotted spatial distribution of membrane potential at a time at end of a just-threshold current stimulus for sodium channel conductance ($G_{Na}$) ×1, ×2, and ×4. Arrows are drawn at a level of potential (vertical axis) that corresponds to intrinsic membrane voltage threshold (from Figure 2) appropriate to value for $G_{Na}$ ×1 and $G_{Na}$ × 4. Arrows are extended to intersect corresponding membrane potential distribution. Length of arrows is a measure of liminal length for action potential initiation at stated value of $G_{Na}$.

**Figure 11.** For one-dimensional strand (top panel) and two-dimensional disk (bottom panel), we plotted spatial distribution of membrane current density (milliamperes per square centimeter) at a time just at end of a just-threshold current stimulus for sodium channel conductance ($G_{Na}$) ×1, ×2, and ×4. Arrows are drawn at point at which membrane current density switches from negative to positive. Horizontal location (distance from stimulus) of arrows is a measure of liminal length for action potential initiation at stated value of $G_{Na}$. 
lower values of $\bar{G}_{\text{Na}}$, there is a substantial reduction in the magnitude of the membrane current density near the stimulus site, leading to a significant increase in the required length over which membrane current must be negative to obtain the required value of inward current for initiation of a propagating response. Thus, the liminal length increases substantially with decreases in $\bar{G}_{\text{Na}}$ in the two-dimensional disk. The mechanism of this increase in liminal length with decreasing values of $\bar{G}_{\text{Na}}$ is that the large depolarizations required for action potential initiation (see Figure 8) are substantial with respect to the values of $E_{\text{Na}}$ (about 135 mV depolarization from rest). Therefore, there is a process by which the high depolarization turns on the sodium conductance but inhibits the flow of sodium current by decreasing the driving force for sodium ions, and this process is responsible for the increased liminal length for the two-dimensional model when $\bar{G}_{\text{Na}}$ is decreased.

With respect to the number of cells that must be depolarized above their intrinsic membrane voltage threshold, the differences between the one-dimensional strand and the two-dimensional disk are even larger. For the one-dimensional strand, the number of cells is linearly related to the length along the strand. For the two-dimensional disk, the number of cells included within a given liminal length varies as the square of this liminal length. Thus, an increase in liminal length of 80% for a fourfold decrease in $G_{\text{Na}}$ (comparing $G_{\text{Na}} \times 4$ with $G_{\text{Na}} \times 1$) corresponds to a tripling of the number of cells that must be depolarized above their intrinsic membrane voltage threshold.

**Discussion**

In these studies, we performed parallel experiments on both the left ventricular papillary muscle and the isolated rabbit ventricular cell to explain, on a cellular basis, the changes observed in tissue excitability during the repolarization phase of the action potential. We presented two hypotheses as a way of distinguishing two possible but fundamentally different mechanisms for this cellular basis. These two hypotheses focus on the definitions of two terms characterizing the events involved in eliciting an action potential in a single cell. We refer to cellular excitability as the inverse of the minimum current required to elicit an action potential in a single cell. Temporally defined, the determination of the cellular excitability is concerned with the events involved in sufficient depolarization of the cell to its threshold voltage. We refer to the cellular responsiveness as the magnitude of the response of the action potential as measured by the APA and $V_{\text{max}}$. The cellular responsiveness is temporally defined as the magnitude of the cellular response after the threshold voltage is achieved.

Hypothesis 1 states that there is a decrease in the cellular excitability whose time course of recovery should parallel the time course of recovery of excitability in the left ventricular papillary muscle preparation. According to this hypothesis, a decrease in excitability at the cellular level would influence tissue excitability in two ways: 1) The cells in the tissue preparation directly stimulated by the external source would require a larger current to reach their voltage threshold, and 2) the surrounding cells would also require an increase in the current generated from the central group to reach their voltage threshold. This increased current requirement would make it necessary for a larger group of cells to be externally stimulated through an increase in magnitude of the externally applied current. Our results from Figure 5 showed that, regardless of the SD time, there was no significant increase in the current threshold to elicit an action potential in the single cell. In fact, the results from Figure 6 showed that there is actually an increase in cellular excitability during this period. From these results, we can conclude that the decreases in tissue excitability observed during phase 3 repolarization do not occur as a result of changes in excitability at the cellular level.

Hypothesis 2 states that there is a decrease in the cellular responsiveness that occurs as a result of a reduction in the magnitude of the net inward current generated by each cell. In terms of Rushton’s liminal length theory, the mechanism as proposed for hypothesis 1 would be changed such that 1) the cells directly stimulated by an external source in a tissue preparation would require the same current to reach their voltage threshold, and 2) the decrease in net inward current generated by the first group of cells, as a result of the decreased cellular responsiveness, would be less than that required to bring the surrounding cells to their voltage threshold. The result of this overall decrease in net inward current is essentially equivalent to an effective reduction in the size of the group of cells being externally stimulated above their voltage threshold. Consequently, a larger external current is required for depolarization of a greater number of cells above their voltage threshold. The results from Figure 5 are consistent with this hypothesis in that there is a monotonically increasing relation between the magnitude of the cellular responsiveness from the S2 action potential and the SD time. With respect to successful action potential initiation, a decrease in the APA represents a decreased potential difference, or driving force, produced by those cells that have attained threshold. For $V_{\text{max}}$, the magnitude of the net inward current is directly proportional to the rate of change of voltage with respect to time. Therefore, since the peak net inward current corresponds to the $V_{\text{max}}$ any reductions in the $V_{\text{max}}$ for an isolated cell would reflect a relative decrease in the available net inward current necessary for action potential initiation in the tissue. In Figure 5 this relation is quantitatively expressed in terms of the time course of recovery for the cellular responsiveness as a function of SD time. These results, as well as those summarized in Table 2, show that the time course of recovery for cellular responsiveness closely parallels the time course of recovery for tissue excitability. It is this result coupled with the
lack of decreases observed in cellular excitability that show that changes in tissue excitability are consistent with a change in responsiveness at the cellular level.

Our experimental results for examination of changes in tissue excitability at the cellular level support hypothesis 2. Therefore, the second part of our results focused, from a theoretical standpoint, on the changes in tissue excitability that occur for preparations of zero, one, or two dimensions when sodium channel availability is decreased. More specifically, our goal was theoretical examination of how changes in sodium availability, or $G_{Na}$, affect the liminal length of the one-dimensional strand versus the two-dimensional syncytium. The liminal length was earlier defined as that length of cable at the end of a just-threshold stimulus that has a net inward current. Based on the analysis of Purkinje strands as a one-dimensional cable and the demonstration that short segments of Purkinje strands had a “healing-over” process that resulted in a nearly isopotential short region, Fozzard and Schoenberg performed experiments on sheep Purkinje strands for evaluation of the liminal length for action potential initiation. They showed that long strands had very different initiation properties from short strands. In particular, the time constant for the strength-duration curve for short strands was 30 msec, about the same as the membrane time constant, but the strength-duration time constant for long strands was only 4 msec. They could not directly measure the liminal length because the membrane current could not be measured at specific points along the strand; however, they estimated the liminal length by measuring the threshold voltage level for the short strands and then, using the long strands, measuring the distance from the stimulus location over which the membrane potential exceeded this threshold potential at the end of a just-threshold stimulus. The estimated liminal length was 100–200 $\mu$m, about 10–20% of the resting length constant. In their theoretical analysis, they showed that this short liminal length could explain the shortening of the strength-duration time constant for the long strands.

Further theoretical analysis dealt with the influence of excitability membrane properties on the liminal length for one-dimensional cables. These workers approximated the current-voltage relation for short-term depolarizations from rest (no inactivation) as a cubic polynomial that included a positive slope conductance ($G_\psi$) near the resting potential and a negative slope conductance ($G_\Delta$) positive for the membrane voltage threshold for inward current. One prediction of this analysis is that the liminal length for activation of a one-dimensional strand of cells would increase as the maximum sodium conductance was decreased. Another prediction is that since the effective length constant for a two-dimensional disk is shorter than for a one-dimensional strand, the liminal length for a two-dimensional disk would be shorter than for a one-dimensional strand if the same relation between length constant and liminal length were to apply.

In our recent study we measured the effect of lowering sodium current availability on the excitability of isolated rabbit ventricular cells and on intact rabbit papillary muscles. We found that for isolated cells, 100 $\mu$m lidocaine hydrochloride and 8 mM K$^+$ decreased the $V_{max}$ (an estimate of sodium channel availability) by 80%, but this intervention had less than a 5% effect on the cellular current threshold for activation even though the tissue excitability was reduced twofold to threefold. We simulated the effects of alterations in maximal sodium conductance in the BR model for an isopotential cell and for a two-dimensional disk of cells and found that we could recreate the basic features of our experimental work with lidocaine hydrochloride.

There are two major limitations in our mathematical representation of the cardiac syncytium. The geometric description we use disregards the effects of the discrete arrangement of the cardiac cells and cell bundles, assuming a one- or two-dimensional homogeneous distribution that can be approximated by discrete elements with a spatial increment of 50 $\mu$m. The effects of the discrete anatomy of cell lengths and cell bundles has been previously evaluated. Our earlier work showed that as long as the effective longitudinal resistivity was low, then a spatial increment of 50 $\mu$m with a lumped representation of cytoplasmic and junctional resistances produced the same result as a simulation with a spatial increment of 10 $\mu$m using a discretely localized junctional resistance for every fifth integration element. Thus, the effects of the discrete spatial distribution of coupling resistance should be minimal in the present results, for which we always used a low value of longitudinal resistivity.

For the two-dimensional disk, we ignored the directional differences in cell-cell coupling resistivity that have been demonstrated in cardiac tissue. Inclusion of these effects requires a complete two-dimensional numerical simulation. We anticipate that the inclusion of a high intercellular resistance in one axis would decrease the electrical loading effect of the two-dimensional disk, resulting in somewhat less effect of $G_{Na}$ on the liminal area of cells for initiation.

The second limitation of these studies is the uncertainty in the applicability of the BR model as a quantitative representation of the membrane properties of ventricular cells. Voltage-clamp analysis of sodium currents in mammalian cells at physiological temperature remains technically difficult due to the fast activation and large conductance involved. With respect to action potential initiation in isolated rabbit ventricular cells, the BR model has an intrinsic membrane voltage threshold about 10 mV too low and a maximum rate of rise severalfold too low. We do not feel that the present experimental evidence justifies specific modifications in the BR model to fit these parameters.
To the extent that our simulations do recreate the effects of tissue geometry on cardiac action potential initiation, we can state several

1) For the one-dimensional strand, the model predicts that the liminal length is much shorter than the passive space constant, as shown experimentally by Fozzard and Schoenberg.\textsuperscript{31} Several mechanisms are apparent from our results. First, the actual spatial distribution of membrane potential just after the stimulus pulse (Figure 10) is not a steady-state relation predicted by the passive cable properties. The nonlinearities of partial activation of elements near the stimulus location make the spatial decay of potential more steep at this point than the steady-state passive results of Figure 2. Second, the required depolarization at the stimulus location is less than twice the intrinsic membrane potential threshold, making the location at which the membrane potential is equal to the intrinsic threshold close to the stimulus location. Thus, the liminal length is short compared with the passive space constant.

2) For the two-dimensional disk, the liminal length is as long as or longer than the effective space constant. The extensive electrical loading of the radial geometry makes the required depolarization at the stimulus location threefold to fourfold higher than the intrinsic membrane potential threshold. Thus, the spatial distribution of membrane potential at the end of a just-threshold stimulus (Figure 10) is extended beyond that expected from a passive distribution (Figure 2), and the location at which the membrane potential is equal to the intrinsic membrane potential threshold is shifted out from the center.

3) For the one-dimensional strand, the liminal length is not significantly affected by changes in $\mathcal{G}_{Na}$. This result seems paradoxical and is not predicted by the polynomial approximation model.\textsuperscript{32} As shown in Figure 9, the necessary condition for initiation of an action potential is not merely that the spatial integral of membrane current be negative at the end of a stimulus pulse; there must actually be a substantial inward net current integral for continuation of the process of depolarization that leads to action potential initiation. Figure 9 shows that the amount of current required varies very little with $\mathcal{G}_{Na}$ for the one-dimensional strand. Figures 10 and 11 show that this required current can be obtained from the same liminal length of membrane for wide variations in $\mathcal{G}_{Na}$.

4) For the two-dimensional disk, decreases in $\mathcal{G}_{Na}$ produce large increases in the liminal length for action potential initiation. From Figure 9, it is apparent that a very large spatial integral of current at the end of the stimulus pulse is required for successful continuation of the activation process and that the magnitude of this required spatial integral of membrane current increases substantially with decreases in $\mathcal{G}_{Na}$. Figure 11 illustrates that the spatial distribution of inward current is very different for the disk than for the strand. The large local depolarization required for lower values of $\mathcal{G}_{Na}$ suppresses the contribution of inward current near the stimulus by approaching the value of $E_{Ks}$.

Our results show that the process of action potential initiation in a two-dimensional syncytium is fundamentally different from initiation in a one-dimensional strand. This is unfortunate from the standpoint of designing experiments with a minimum of geometric complexity, as has been done with many experiments in the use of Purkinje strands or long, thin papillary muscles. The very significant effects of tissue geometry on the sensitivity of the initiation process to alterations in $\mathcal{G}_{Na}$ may help explain the actions of antiarrhythmic drugs on blocking the effects of an ectopic focus while preserving the normal excitation sequence. With specific reference to the present experimental studies, the simulations support the concept presented in our hypothesis 2 that the decreased ability of cells to respond with inward current after excitation has occurred is the primary determinant of the functional refractory period in ventricular muscle. The dominance of this effect makes the supernormal excitability period present at the single-cell level disappear when the stimulus is applied to a multidimensional syncytium. For the one-dimensional strand, the liminal length changes are not as great as for the multidimensional tissue, thus allowing the supernormal excitability period to be present.

References


Key Words: functional refractory period • rabbit • ventricular muscle • isolated cell.
Cellular mechanism of the functional refractory period in ventricular muscle.
B M Ramza, R C Tan, T Osaka and R W Joyner

doi: 10.1161/01.RES.66.1.147

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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