Cellular Uncoupling Can Unmask Dispersion of Action Potential Duration in Ventricular Myocardium
A Computer Modeling Study

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Although slow conduction is a requirement for the preparation of sustained reentry, it alone is not sufficient for the initiation of reentry. Additionally, unidirectional block and recovery of excitability distal to the site of block must occur. Thus, a comprehensive description of the electrophysiological determinants of reentry must explain both slow conduction and unidirectional block. Although there is a growing body of research exploring the influence of axial resistivity and anisotropy on slow conduction, somewhat less is known about the relation of axial resistivity to spatial dispersion of action potential duration, a condition favorable to the development of unidirectional block. We hypothesized that when cells are well coupled, local differences in intrinsic action potential duration are not evident and that, as axial resistivity increases, local variation in action potential duration becomes manifest. We tested this hypothesis in a numerical model of electrical propagation in a grid of resistively coupled ionic current sources simulating a sheet of ventricular myocardium. Spatial dispersion of intrinsic action potential duration was simulated by varying the magnitude of the fully activated slow inward conductance in Beeler-Reuter membrane ionic kinetics. By then altering coupling resistance, we showed that dispersion of manifest action potential duration is masked in the setting of normal low-resistance cellular coupling and unmasked by increased axial resistance. When nonuniform anisotropy was simulated, dramatic pacing-site-dependent changes in both the pattern of activation and dispersion of action potential duration were noted. These findings may be important in understanding the mechanism of reentrant tachycardia initiation in the border zone of chronic, healed myocardial infarctions where evidence suggests that abnormal cellular coupling is the predominant electrophysiological derangement. In this study, we have shown, using a detailed ionic current-based model of cardiac electrical propagation, that changes in axial resistivity can modulate how spatial dispersion of intrinsic action potential duration is manifest. (Circulation Research 1989;65:1426-1440)

Since early in this century,1-3 experimental evidence has supported the hypothesis that a reentrant mechanism is responsible for many clinical cardiac arrhythmias. For perpetuation of a reentrant arrhythmia to occur, conduction within the reentrant circuit must be slow enough so that the circulating impulse encounters excitable tissue ahead of it. In addition to slow conduction somewhere within the circuit, unidirectional block must occur at the initiation of reentry. Some degree of spatial variation or dispersion in recovery of excitability generally favors the development of unidirectional block. For example, nonuniform recovery of excitability enhances initiation of reentry in both atrial4,5 and ventricular myocardium.6,7 Such nonuniform recovery of excitability is necessary whether reentry is present as the usual circus movement variety or as localized reflected reentry.8-11

Substantial effort in basic electrophysiology has been focused on how modification of active membrane ionic properties and, specifically, alterations...
FIGURE 1. Diagram of electrical equivalent circuit of anisotropic sheet of ventricular myocardium. Membrane patches obeying Beeler-Reuter28 ionic kinetics are coupled with axial resistors. A representative patch at node i,j is shown diagrammatically on the right. $R_x$ and $R_y$, longitudinal and transverse resistivity; $V_m$, transmembrane voltage; $C_m$, membrane capacitance; $I_{ion}$, ionic current; $I_{Na}$, sodium current; $I_K$, time-independent inward rectifying potassium current; $I_K^*$, voltage- and time-dependent outward potassium current; $I_{Ca}$, slow inward current carried primarily by calcium. All resistor values are independently programmable to simulate uniform or nonuniform anisotropy. Parameters modifying Beeler-Reuter kinetics are also independently programmable for each membrane patch to simulate dispersion of intrinsic action potential duration. See text for further discussion.

in the fast inward sodium current lead to slowing of electrical propagation in cardiac tissue.12 More recently, there has been increasing recognition that alterations that lead to an increase in the passive resistivity encountered by a propagating impulse can lead to conduction slowing that is independent of changes in active membrane ionic properties.13-15 For example, because of the geometry of myocardial fibers and the distribution of specialized gap junctions, the higher axial resistivity encountered by an impulse propagating transversely to fiber orientation results in slower conduction than when an impulse travels longitudinally to the fiber axis.16-19 Although there is now a growing body of research work exploring the role of cellular uncoupling and anisotropy in the slow conduction required for reentry,20,21 less is known about the possible role of cellular uncoupling on dispersion of action potential duration.

The aim of this study is to investigate the ability of alterations in cellular coupling to mask or unmask intrinsic differences in action potential duration in a sheet of ventricular myocardium. We hypothesize that when cells are well coupled, differences in intrinsic action potential duration are not evident and that as axial resistivity increases, local variation in action potential duration becomes manifest.

We have tested this hypothesis using a computerized numerical model of electrical propagation in a sheet of ventricular myocardium containing a grid of resistively coupled elements. Spatial dispersion of action potential duration was simulated by varying the magnitude of the fully activated slow inward conductance. We found that dispersion of action potential duration was masked in the setting of normal low-resistance cellular coupling and unmasked when increased axial resistance was present. We speculate that these findings may be important in understanding how reentrant tachycardia is initiated in the border zone of chronic, healed myocardial infarctions where cellular coupling is deranged.22-27

Methods

Description of Propagation Model

Our model is meant to simulate a thin sheet of ventricular myocardium. The sheet can be thought of as representing the surviving cell layer of a myocardial infarct in an experimental canine preparation (epicardial border zone), in humans (subendocardial layer), or in an isolated strip of ventricular muscle superfused in the tissue bath. A constant ratio of cell surface area to tissue volume ($S_v$) is assumed throughout the sheet. The extracellular space is assumed to be isotropic and unbounded and to have negligible resistivity when compared with intracellular space. The model is shown diagrammatically in Figure 1. Our implementation of a two-dimensional propagation model is similar to models used by previous investigators29-31 with several important differences. First, axial resistivity is not necessarily assumed to be constant in either direction but can vary heterogeneously throughout the sheet. Similarly, for each membrane patch, parameters modifying Beeler-Reuter ionic current kinetics28 can be individually specified. Second, since we need to simulate complete action potentials throughout the sheet rather than just the rising
phase to measure action potential duration, we have implemented features to enhance execution speed, which are described below. Typically, 500 msec was calculated for each experiment. This is as compared with the two-dimensional simulations of Roberge et al.29 or Barr and Plonsey31 in which only the rising phase of the action potential was calculated (approximately 40 msec).

At each location on the sheet, conservation of total current implies that the transmembrane current \( I_m \) is the sum of capacitive current \( I_{cap} \) and ionic currents \( I_{ionic} \) and the possible addition of an external pacing current \( I_{pace} \):

\[
I_m = I_{cap} + I_{ionic} + I_{pace}
\]  

(1)

For simplicity, we will drop the \( I_{pace} \) term from the following derivations. In simulation runs, it is added to the calculated ionic currents at specified times and locations on the sheet. The capacitive term is given by

\[
I_{cap} = \frac{\delta V_m}{\delta t}
\]

where \( C_m \) is the membrane capacitance per square centimeter. Transmembrane current is derived by taking the divergence of the intracellular, or axial, current density. Axial current, in turn, is obtained from the divergence of intracellular voltage potential \( <\phi> \) divided by the axial resistivity, an application of Ohm's law:

\[
I_m = \frac{1}{S_v} \left[ \nabla \cdot \left( \frac{1}{\rho_a} \cdot \nabla <\phi> \right) \right]
\]  

(2)

where \( \rho_a \) is the intracellular resistivity, which, in general, is a tensor function of space and, for a given experiment, assumed constant over time. \( S_v \) is the ratio of cell surface to tissue volume and division by it allows referencing transmembrane current to a unit area of membrane, that is, microamperes per square centimeter. Since extracellular resistivity is assumed small, we can approximate \( <\phi> \) by transmembrane voltage \( V_m \). If we align the resistivity tensor with the \( x \) and \( y \) axes, that is, along myocardial fibers, and substitute Equation 2 into Equation 1, we obtain the following expression for electrical propagation in arbitrarily nonuniform anisotropic two-dimensional myocardium:

\[
\frac{1}{S_v} \left[ \frac{\delta}{\delta x} \left( \frac{1}{\rho_{ax}} \cdot \frac{\delta V_m}{\delta x} \right) + \frac{\delta}{\delta y} \left( \frac{1}{\rho_{ay}} \cdot \frac{\delta V_m}{\delta y} \right) \right] = C_m \frac{\delta V_m}{\delta t} + I_{ionic}
\]  

(3)

To solve Equation 3 numerically, we discretize on a rectangular grid as shown in Figure 1. Where \( x \) and \( y \) grid lines intersect, we define a node \( i,j \) with transmembrane voltage \( V_{m_{i,j}} \). Each membrane patch of dimension \( \Delta x \) by \( \Delta y \) is assumed to be isopotential. The interelement resistance along the \( x \) axis between nodes \( i-1,j \) and \( i,j \) is written as \( R_{ax,i} \) along the \( x \) axis between nodes \( i,j \) and \( i+1,j \) is written as \( R_{ax,i+1} \); along the \( y \) axis between node \( i,j-1 \) and node \( i,j \) is written as \( R_{ay,i} \) and along the \( y \) axis between node \( i,j \) and node \( i,j+1 \) is written as \( R_{ay,i+1} \) (see Figure 1).

To convert from resistivity, \( \rho \), to resistance per element, \( R \), we use the following relations:

\[
R_x = \frac{\Delta x}{z \Delta x \rho_{ax}} \quad R_y = \frac{\Delta y}{z \Delta y \rho_{ay}}
\]  

(4)

where \( z \) is the thickness of the tissue sheet.

Thus, applying the finite element approximation to Equation 3 we obtain

\[
\frac{1}{S_v} \left( \frac{V_{m_{i-1,j}} - V_{m_{i,j}}}{R_{ax,i}} + \frac{V_{m_{i+1,j}} - V_{m_{i,j}}}{R_{ax,i+1}} + \frac{V_{m_{i,j-1}} - V_{m_{i,j}}}{R_{ay,i}} + \frac{V_{m_{i,j+1}} - V_{m_{i,j}}}{R_{ay,i+1}} \right) = C_m \frac{\delta V_m}{\delta t} + I_{ionic}
\]  

(5)

where \( S_v \) is the surface area of membrane per element:

\[
S_v = S_v \cdot \Delta x \cdot \Delta y \cdot z
\]

Note that we have not assumed a constant resistance for a given direction but, rather, have allowed resistance to vary from element to element. All terms in Equation 5 are in units of microamperes per square centimeter.

Equation 5 can be viewed as a finite element approximation of the continuous case (Equation 3), though not rigorously equivalent since it imposes possible discontinuities at the interface between elements. However, this is not wholly inappropriate, since the geometric arrangement of normal myocardial fibers results in a discrete, discontinuous substrate for conduction and these discontinuities may have even greater relevance in the setting of chronic infarction. Since the model allows independent programmability of the resistance values in Equation 5, the effect of spatial heterogeneity of coupling can be explored.

The Crank-Nicholson method is often used to numerically solve Equation 5 by approximating

\[
\frac{\delta V_m}{\delta t} = \frac{V_{m_{i,j}} - V_{m_{i,j}^t}}{\Delta t}
\]

by the first-order forward difference in time

\[
V_{m_{i,j}^t+1} = V_{m_{i,j}^t} + \frac{\Delta t}{\Delta x} \left( \frac{1}{\rho_{ax}} \cdot \frac{\delta V_m}{\delta x} \right) + \frac{\Delta t}{\Delta y} \left( \frac{1}{\rho_{ay}} \cdot \frac{\delta V_m}{\delta y} \right)
\]

and replacing the left side, the second central difference, by the average values at times \( t \) and \( t + \Delta t \). Substituting and rearranging we obtain:
Note that this formulation is independent of sheet thickness since $z$ cancels from the numerator and denominator of the terms involving $S_{AR}$, which is expected given the assumption that each element is isopotential and that therefore no current flows within a given element. Because $I_{	ext{ionic}}$ is calculated based on voltage values and integrated kinetics from the previous time step, at time $t+\Delta t$, the right-hand side is known for all $i,j$, and Equation 6 is a system of linear equations that can be solved once boundary conditions are specified. We assume von Neumann conditions, $V_{m}=0$; that is, no current flows off the edge of the sheet.

Each membrane patch is represented by a current source with nonlinear current-voltage relation as described by Beeler and Reuter. The Beeler-Reuter (BR) membrane model consists of a system of simultaneous differential equations of the type described originally by Hodgkin and Huxley for nerve axons and then modified to fit experimental data for ventricular myocardium. At each node, total transmembrane ionic current is the sum of components due to the fast inward sodium current, a slow inward calcium current, and a voltage and time-dependent outward current carried primarily by potassium. Initial conditions used for the BR model based on steady-state values were: $m=0.01126$, $h=0.9871$, $j=0.9927$, $x_i=0.0241$, $d=0.0030$, $f=1.0$, $[\text{Ca}]=1.792 \times 10^{-7} \text{M}$, $V_m=-84.35$ mV, where $m$, $h$, $j$, $x_i$, $d$, and $f$ are gating variables and $[\text{Ca}]$ is intracellular calcium concentration. These parameters were uniform throughout the sheet.

Since publication of the BR model of the ventricular action potential, models that incorporate newer concepts of potassium and calcium current kinetics during the plateau and which include a quantitative description of ionic exchange pumps have become available. However, we felt justified in using the BR membrane since we were not examining the role of specific ionic currents during repolarization but, rather, wished to examine the overall effect of cellular coupling (and uncoupling) on action potential duration and dispersion thereof. Despite its limitations, the BR membrane exhibits a realistic ventricular action potential shape, responds appropriately to such interventions as premature beats (which shorten action potential duration), and has been usefully incorporated in a number of studies in which electrical propagation through ventricular myocardium has been stimulated.

**Computational Methods**

In implementations of one-dimensional propagation models, the matrix representing the left side of the system of equations, analogous to our Equation 6, is tridiagonal and can be solved using efficient techniques for such matrices. However, in the two- (or three-) dimensional case, the matrix is no longer tridiagonal but is tridiagonal with side bands, and its inversion requires a greater amount of computation. Fortunately, a numerical method known as operator splitting or alternate-direct-implicit (ADI) is available for the solution of systems of elliptic and parabolic partial differential equations, such as represented by our model. In brief, this method involves "splitting" each time step into two half steps, each of which reduces to a tridiagonal system. Rather than the traditional Crank-Nicholson formulation, we rewrite Equation 6 as half steps:

$$[AV_{m,i,j}^{t+\Delta t/2} + BV_{m,i,j}^{t+\Delta t/2} + CV_{m,i,j}^{t+\Delta t/2}]$$

$$+ [DV_{m,i,j}^{t+\Delta t/2} + EV_{m,i,j}^{t+\Delta t/2} + FV_{m,i,j}^{t+\Delta t/2}] = G$$

In the first half step, $t+\Delta t/2$, the $y$-axis voltage terms, those multiplied by $D$, $E$, and $F$, are held constant, and a tridiagonal matrix results for the solution of the $x$-axis terms. Similarly, the $x$-axis terms are held fixed for the second half step, and a tridiagonal system is solved for the $y$-axis terms. Though apparently doubling the number of time steps, ADI has a great advantage in reduced memory requirements and computation time.

Another area in which computational speed can be improved involves adaptive control of time-step size. In implementing integration of a single action potential, such adaptive step control is accomplished with relative ease. However, when simulating propagation in fibers or two- (or three-) dimensional systems of discrete elements, adaptive step-size control gains little if all elements must be integrated in synchrony with the same step-size, since that step will always be limited by the element with the most rapidly changing $V_m$. A technique is needed to individually adjust step-size for each element while maintaining proper synchronization between elements so that the voltage at each node reflects both the currents generated from within a given element (right side of Equation 5) and the current flowing between elements (left side of equation...
We have developed a method for adaptive control of the both ADI step-size (Δt in Equation 7), based on the maximum error over the grid of the values of $V_{\text{mem}}$ projected by BR, and each individual BR step-size. In essence, each BR membrane patch is allowed to integrate over smaller (or sometimes larger) time-steps than ADI. By “uncoupling” local BR integration from global control of ADI, the BR integration for each element can undergo independent adaptive step-size control. During BR integration, an estimate of the contribution of total axial current for each element (the sum of axial currents from the neighboring elements in the longitudinal and transverse directions), obtained from the previous ADI step, is included in the derivative of $V_{\text{m}}$, so that relatively large steps can be taken without the introduction of errors from that source. The ionic charge movements during BR integration are accumulated and summed (or interpolated when a BR step is larger than an ADI step) to report $I_{\text{elem}}$ to ADI. Then, when an ADI step is taken, corrected values of $V_{\text{mem}}$ are calculated according to Equation 7, and the ADI step-size is controlled by restricting the discrepancy between the BR-projected and ADI-corrected $V_{\text{mem}}$ for all elements to be below a minimum upper bound. When a pacing event (pacing-on or pacing-off) is imminent, ADI and BR time-steps are constrained to reach it exactly, since a pacing event will result in a sudden change in current and the voltages being predicted by ADI and the summed currents from BR integration will not have accounted for this. BR integrations were performed using an extension of the hybrid integration method that included provisions to calculate accurately the state of the m-gate over relatively large steps.

All ionic kinetic parameters were as given in Beeler and Reuter, except for fully activated slow inward conductance ($g_{\text{in}}$) as described below. Model variables are listed in Table 1. Pacing was performed by intracellularly injected rectangular current pulses 1 msec in duration at approximately threshold intensity at a cycle length of 1,000 msec. When pacing was initiated from a corner of the sheet or a point along the edge, an area of 2x2 elements was paced simultaneously. When an entire edge was paced, the current was applied to a strip one element wide along the entire length or width of the sheet. Required current varied with resistivity but averaged approximately 30 μA/cm² for each element paced. Axial resistivity varied from experiment to experiment as will be described in “Results.”

### Table 1. Model Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longitudinal element size (Δx)</td>
<td>100 μm</td>
</tr>
<tr>
<td>Transverse element size (Δy)</td>
<td>100 μm</td>
</tr>
<tr>
<td>Number of elements</td>
<td>50×50</td>
</tr>
<tr>
<td>Cell surface area to tissue volume ratio</td>
<td>0.24 μm⁻¹</td>
</tr>
<tr>
<td>Membrane capacitance</td>
<td>1.0 μF/cm²</td>
</tr>
</tbody>
</table>

Activation time for each element was defined as the time when a given action potential first equaled or exceeded −60 mV. Action potential duration was defined as the time spent above −60 mV. For uniformly anisotropic sheets, conduction velocity in the longitudinal and transverse directions was calculated by pacing an entire transverse or longitudinal edge, respectively, and measuring the difference in activation times between elements 1 mm apart (10 elements) in the center of the sheet. $V_{\text{max}}$ was calculated as the maximum change in transmembrane voltage with respect to time, and $\tau_{\text{all}}$ was calculated from the exponent in a least-squares fit of an exponential function to $V_{\text{m}}$ taken from resting potential to −70 mV, in the manner described by Spear et al. Both were determined from the same element in the center of the sheet after pacing an entire transverse or longitudinal edge. All programs were written in FORTRAN and implemented on a VAX 8600 running VMS Operating System (Digital Equipment, Marlboro, Massachusetts). Simulation of 500 msec of propagation on a 50×50 grid required approximately 10 minutes of central processing unit time.

### Dispersion of Action Potential Duration

In the BR membrane, the time to repolarize, that is, the action potential duration, is primarily controlled by the magnitude of $g_{\text{in}}$, with the magnitude of the fully activated inward rectifying potassium conductance ($g_{\text{K}}$ from Reference 28) of secondary importance. We used this dependence on $g_{\text{in}}$ to simulate dispersion of intrinsic action potential duration. The intrinsic action potential duration is that which would occur if a given membrane patch could be studied in isolation, in the absence of propagation, and without resistive loading by neighboring cells. The manifest action potential duration is that which is observed when an impulse propagates through the resistively coupled sheet. In our model, we varied intrinsic action potential duration by multiplying the nominal value given by Beeler and Reuter for $g_{\text{in}}$ (0.09 mmhos/cm²) by a Gaussian-distributed random variable with a mean of 1.0 and a standard deviation of 0.1. Intrinsic action potential duration thus varied over the 50×50 element grid with a mean of 263.2 msec. To examine the effect of cellular coupling on manifest action potential duration, the same random distribution of intrinsic action potential duration was used for all experiments with only cellular coupling and pacing site varied.

### Quantification of Dispersion of Manifest Action Potential Duration

To avoid edge effects, which tend to lengthen action potential duration at the pacing site and shorten it at the center of the sheet beyond what is actually the case, the following analysis was performed on the 100 elements (10×10) in the middle of the sheet. Several measures of dispersion of manifest action potential duration were defined.
Maximum local difference of action potential duration. For each element, the maximum value of the difference between the given element and its eight closest surrounding neighbors is calculated. The mean of these 100 values is reported as one index of dispersion of manifest action potential duration for each experiment.

Average local difference of action potential duration. For each element, the average value of the difference between the given element and its eight closest surrounding neighbors is computed. The mean of these 100 values is reported for each experiment.

Overall dispersion of action potential duration. This is calculated as the standard deviation of the manifest action potential duration for the entire 10×10 central region.

Longitudinal and transverse dispersion of action potential duration. The standard deviation of action potential duration for each of the 10 rows (i.e., longitudinal direction) and 10 columns (i.e., transverse direction) in the central 10×10-element area was calculated. The root mean square of these standard deviations gives a measure of directional dependence of dispersion of action potential duration.

Results

Intrinsic Action Potential

The intrinsic action potential duration for each element of our 50×50 grid was varied by multiplying $g_{in}$ (nominal value, 0.09 mmhos/cm$^2$) by a Gaussian random variable with a mean of 1.0 and a standard deviation of 0.1. Intrinsic action potential duration was assessed by setting all coupling resistors to infinite values and then pacing each individual membrane patch. Since no axial current spread was possible in completely uncoupled cells, the resulting action potential duration is that which would occur in an isolated membrane patch. The numbers labeling each action potential correspond to the random variable multiplying the fully activated slow inward conductance ($g_{in}$ in Reference 28; nominal value, 0.09 mmhos/cm$^2$), which resulted in variation in action potential duration.

Figure 2 shows four typical intrinsic BR action potentials from adjacent membrane patches at the center of the sheet. Intrinsic action potential duration was assessed by setting all coupling resistors to infinite values and then pacing each individual membrane patch. Since no axial current spread was possible in completely uncoupled cells, the resulting action potential duration is that which would occur in an isolated membrane patch. These were recorded from four adjacent membrane patches in the center of the grid.

Effect of Uncoupling on Manifest Action Potential Duration

For all experiments reported below using either isotropic or uniformly anisotropic coupling, control experiments in which $g_{in}$ was constant throughout the sheet yielded values for dispersion of manifest action potential duration of less than 0.1 msec.

Effect of Uncoupling on Manifest Action Potential Duration

Isotropy. Figure 3A shows the effect of pacing the bottom left corner of a sheet with axial resistivity of 5,000 Ω-cm in both longitudinal and transverse directions. Since no current flows off the edge of the sheet, the 50×50-element grid is equivalent to one quadrant of a 100×100-element grid with pacing initiated at the center. In Figure 3, we have plotted activation isochrones on the base plane, with the corresponding action potential duration at each point in space depicted as a surface above that plane (i.e., the vertical axis corresponds to action potential duration). Note the marked flattening of variation in manifest action potential duration that occurs with well-coupled cells (Figure 3A). In contrast, Figure 3B shows the effect of increasing axial resistivity to 20,000 Ω-cm. As expected, activation isochrones are more closely spaced since conduction is slowed uniformly by the uniform increase in axial resistivity. However, we now see a "rippling" in the action potential duration surface plot, indicating that spatial dispersion of action potential duration has become manifest.

The effect of increasing axial resistivity on dispersion of action potential duration for the central 10×10 region is shown quantitatively in Figures 4 and 5. In all cases, pacing was initiated from the lower left corner as depicted in Figure 3, and the
FIGURE 3. Activation maps showing effect of changes in cellular coupling to unmask spatial variation in intrinsic action potential duration (APD). These experiments were performed using the computer model as described in the text and represented in Figure 1. In each case isochronal activation maps are plotted on the x-y plane. Pacing at a cycle length of 1,000 msec was initiated from one corner of the sheet as indicated by the asterisk. Solid curves represent 10-msec isochrones, and dotted curves represent interspersed 5-msec isochrones. The grid size was 50x50 elements with each element representing a segment of ventricular myocardium 100μm x 100μm. For a given element, the height of the “floating” surface represents APD in milliseconds at that site. In each case, the same dispersion of intrinsic APD was simulated by multiplying the fully activated slow inward conductance in the Beeler-Reuter kinetic equations by a random value.

Panel A: Isotropic coupling (5,000 Ω·cm) in both directions. Note “masking” of variation in manifest APD that occurs when the cells are well coupled. Panel B: Isotropic slow conduction produced by increasing the effective axial resistivity to 20,000 Ω·cm in both directions. The spatial variation of intrinsic APD is now manifest. Panel C: Anisotropic coupling with Rx equals 5,000 Ω·cm and with Ry equals 20,000 Ω·cm. Note elliptical activation isochrones with conduction slower in the transverse (y) direction. A greater degree of heterogeneity of manifest APD across than along fiber axis is demonstrated.
FIGURE 4. Graph showing the effect of uniformly increasing axial resistivity on dispersion of action potential duration (APD) for the central 10×10-element region. In all cases, pacing was initiated from the lower left corner as depicted in Figure 3, and the identical random variation of fully activated slow inward conductance (gNa in Reference 28) was used. Overall dispersion of APD, defined as the standard deviation of the manifest APD for the entire 10×10 central region, is plotted as a function of axial resistivity that was varied in separate simulations from 2,500 Ω·cm to 22,500 Ω·cm. There is a significant increase in overall dispersion of APD as axial resistivity increases. The inset shows that the mean APD is not altered as resistivity is changed.

axial resistivity. Note also that there is greater heterogeneity of action potential duration across than along fibers, which is manifested by the greater "rippling" of the action potential duration surface in that direction. The effect of degree of anisotropy was examined quantitatively by holding longitudinal resistivity at 5,000 Ω·cm and allowing transverse resistivity to vary up to 20,000 Ω·cm (Figure 6). As transverse resistivity was increased, there was a small increase in longitudinal dispersion and a larger increase in transverse dispersion of action potential duration. Thus, the effect of anisotropic coupling is to unmask intrinsic differences in action potential duration to a greater degree in the direction of relatively poorer coupling.

We also tested whether the direction of the activation wavefront had any significant effect on overall and directional (longitudinal and transverse) dispersion of action potential duration in the setting of uniformly anisotropic coupling. Pacing sites on the entire longitudinal edge (x axis), the entire transverse edge (y axis), and the lower left corner are compared in Figure 7 for two experiments in which longitudinal resistivity (ρL) and transverse resistivity (ρT) were 5,000 and 20,000 Ω·cm (ρL:ρT=4:1; Figure 7A) and 2,500 and 20,000 Ω·cm (ρL:ρT=8:1; Figure 7B).

FIGURE 5. Graph showing two additional measures of dispersion of action potential duration (APD). The set of experiments is the same as described in Figure 4. The average and maximum value of the difference between each element and its eight closest surrounding neighbors is calculated for the central 10×10-element region and the mean taken. Both these measures of local dispersion of APD significantly correlated to axial resistivity. In two-dimensional myocardium, as axial resistivity is increased, dispersion of APD becomes manifest.
Figure 7B). As we can see from Figure 7, in the case of uniform anisotropic axial resistivity the direction of the activation wavefront (longitudinal, transverse, or elliptical) does not significantly alter the greater unmasking of differences in intrinsic action potential duration transverse to fiber axis. In Figure 7B as compared with 7A, the lower longitudinal resistivity more closely "binds" repolarization; thus, all measures of dispersion are lower despite the same transverse resistivity. However, in both cases the transverse dispersion remains greater than longitudinal dispersion.

**The Effect of Heterogeneous Uncoupling**

Chronically infarcted myocardium, such as that which provides the substrate for reentrant ventricular tachycardia, is characterized by regions of relatively normal muscle with interdigitating collagenous septa that act to electrically uncouple myocardial fibers. In Figure 8, we attempted to simulate such heterogeneously uncoupled myocardium and to examine the effect of that uncoupling on dispersion of action potential duration as a function of pacing site. In these experiments, the overall ratio of longitudinal to transverse resistivity was 2,500:10,000 Ω-cm. In addition, the area from element locations 15,15 to 30,30 was programmed to have complete transverse uncoupling; that is, the transverse resistivity in that region was made infinite. Longitudinal resistivity was not altered. The only difference in the experiments depicted in Figures 8A and 8B is the pacing site. In Figure 8A, pacing was initiated from a 2×2 area at the midpoint of the y axis. We can see some slight changes in the activation isochrones resulting from the transverse uncoupling, along with some relatively mild "rippling" of the action potential duration surface corresponding to that region. In this case, dispersion of action potential duration (maximum minus minimum) over the uncoupled region is only 0.6 msec. But when pacing is initiated from the midpoint of the x axis (Figure 8B), more dramatic changes are noted. The activation isochrones in the uncoupled region are bunched together; this bunching indicates marked slowing of conduction. Because there is no transverse coupling in this slow region and the activation wavefront is moving essentially parallel to the direction of uncoupling, collision of wavefronts occurs as activation spreads inward from the relatively better-coupled proximal and distal lateral edges of the sheet toward the center.

Thus, in Figure 8B, two related electrophysiologic effects contribute to the marked dispersion of action potential duration, which equals 9.5 msec in this case. Collision of wavefronts is known to shorten action potential duration, but such marked spatial dispersion of action potential duration, such as along the line y=30 from x=15 to 30, could not occur if it were not for the complete transverse uncoupling in that region allowing closely spaced membrane patches to repolarize at such disparate times. Therefore, in nonuniformly anisotropic myocardium that provides the substrate for reentrant ventricular tachycardia, electrical uncoupling also increases dispersion of action potential duration.

**Figure 6.** Graph showing the effect of anisotropic resistivity on directional dispersion of action potential duration. Longitudinal resistivity was kept at 5,000 Ω-cm, and transverse resistivity was varied up to 20,000 Ω-cm. The root mean square of the standard deviation of APD for each of the 10 rows (i.e., longitudinal direction) and 10 columns (i.e., transverse direction) in the central 10×10-element area was calculated as longitudinal and transverse dispersion, respectively. As transverse resistivity was increased, there was a small increase in longitudinal dispersion and a larger increase in transverse dispersion of APD. Anisotropic axial resistivity unmask intrinsic differences in APD to a greater degree in the direction of relatively poorer coupling.
Figure 7. Bar graphs showing the effect of pacing site on overall and directional dispersion of action potential duration (APD) for uniform anisotropy for two anisotropic coupling ratios. Panel A: Longitudinal resistivity ($R_L$) is 5,000 ohm.cm, and transverse resistivity ($R_T$) is 20,000 ohm.cm (1-4). Panel B: $R_L$ is 2,500 ohm.cm, and $R_T$ is 20,000 ohm.cm (1-8). With the lower $R_L$ present in panel B, all measures of dispersion are lower than with the higher resistivity in panel A. However, for a given ratio of coupling, altering pacing site does not alter the greater dispersion of APD in the transverse direction.

Discussion

Although slow conduction is a requirement for the perpetuation of stable, sustained reentry, it alone is not sufficient for the initiation of reentry. Additionally, unidirectional block and recovery of excitability distal to the site of block must occur. Thus, any description of the electrophysiological determinants of reentry must explain both slow conduction and unidirectional block to be comprehensive. Recently researchers have examined the importance of increased axial resistivity and anisotropy on slowing of conduction. However, less
work has explored the relation of axial resistivity to dispersion of local refractoriness, a condition favorable to the development of unidirectional block. In this study we have shown, using a detailed ionic current–based model of cardiac electrical propagation, that changes in axial resistivity can modulate how spatial dispersion of intrinsic action potential duration is manifest. When cells are well coupled, an attempt by one cell to repolarize at a substantially different time than a neighboring cell will result in current flow between the cells. This electrotonic current flow will tend to delay repolarization in the cell attempting to repolarize early (i.e., the cell with a relatively short intrinsic action potential duration) and to accelerate repolarization in the cell attempting to repolarize late (i.e., the cell with a relatively long intrinsic action potential duration). By fundamental electric circuit theory, the magnitude of electrotonic current flow between cells during repolarization and, therefore, the degree to which intrinsic differences in action potential duration are masked are dependent on the coupling resistance: the higher the coupling resistance, the less the electrotonic interaction and the greater the unmasking of local differences in intrinsic action potential duration.

Spach and Kootsey proposed that the anisotropic properties of cardiac muscle may cause block of a premature impulse because of a reduced safety factor for conduction that is longitudinal to fiber orientation, even though conduction is faster in that direction. They showed such block to occur as a wavefront progressed along fiber axis, despite no apparent increase in the refractory period at the site of block. In their study, refactoriness at a given site was measured by pacing and introduction of premature beats at that site. In our study, we have hoped to show a distinction between intrinsic action potential duration and that which is manifest during a propagated impulse. The “traditional” method of measuring the effective refractory period at a given test site by programmed stimulation at that site may present the tissue with a very different spatial and temporal distribution of electrical current than is obtained when a propagating action potential reaches that site, especially when heterogeneous cellular coupling is present, and may be an insensitive indicator of true dispersion of local action potential duration and refactoriness. Recent evidence suggests there may in fact be an interaction between anisotropy and intrinsic differences in action potential duration determined with microelectrodes in canine atrial muscle bundles. One advantage of our computer model is the ability to “noninvasively” examine the action potential duration of a large number (2,500 in this case) of very small regions of ventricular myocardium during a propagated impulse and to do so under varying axial resistivity. Clearly, such detailed mapping of action potential characteristics would not be possible in any experimental preparation in vivo or in vitro.

There is an alternative to reduced safety factor secondary to anisotropy as a mechanism for block: anisotropic tissue structure, and cellular coupling in general, can electrotonically modulate action potential duration and, thus, dynamically affect the local dispersion of refractoriness needed for block. That myocardial refractoriness is not independent of the pattern and timing of activation has been shown in a variety of preparations. Sucrose gap experiments showed that, with high coupling resistance in the sucrose gap, propagation between the proximal and distal segments failed and electrotonic influences shortened the action potential of the proximal segment. As coupling resistance was lowered to a critical value, propagation from the region proximal to the region distal to the gap occurred. At the same time, the proximal segment action potential was electrotonically prolonged. Sasyuniuk and Mendez examined the critical role of coupling in modulation of action potential duration. Alterations in passive coupling resistance allowed for unidirectional block in their canine Purkinje fiber–papillary muscle preparation.

In a numerical model, Joyner examined the interaction between the spatial distribution of “intrinsic” cellular properties and coupling resistance. He modeled two cellular aggregates connected with a single resistor, the value of which could be varied from simulation to simulation. He showed that, when the cellular aggregates were well coupled (low resistance), intrinsic differences in action potential duration were obscured. As coupling resistance was increased, the intrinsic differences of action potential duration became increasingly manifest. Our numerical model is similar to that of Joyner, though we have examined dispersion of action potential duration in a more complex two-dimensional system.

Venstra and DeHaan provided an experimental example of electrotonic coupling of repolarization in a preparation consisting of cultured chick embryo ventricular cells of different ages. In isolation, aggregates of 7-day embryo myocytes have longer intrinsic action potential durations than those of 4-day embryos. When the aggregates were brought into contact, new gap junctions formed over a period of several hours. As coupling improved, the manifest action potential durations of the two aggregates became nearly identical to one another as a result of electrotonic modulation.

In vivo experiments have shown that the direction of an activation wavefront influences refractory periods via alterations in the electrotonic environment. Abildskov found that the time to recovery of excitability at a given test site in the canine ventricle was shorter when collision of activation wavefronts occurred near the test site than when activation was moving steadily away from the site. Toyoshima and Burgess confirmed this finding and showed that in canine ventricular myocardium, because of electrotonic interaction during repolarization, refractory periods decreased as the distance between measurement and pacing site...
increased. Tsuboi et al.\textsuperscript{48} found in canine ventricle that action potential duration was longer during longitudinal than during transverse propagation, though their purpose was not to evaluate dispersion of action potential duration as we did in our model. Delmar et al.\textsuperscript{49} have shown that recovery of excitability is a function of propagation direction with longer refractory periods during transverse propagation. Again, they did not propose to evaluate local dispersion of action potential duration. Electrotonic interactions have also been implicated in local reflection of impulses leading to reentry on that basis.\textsuperscript{8,11,50,51}

Recently,\textsuperscript{38} the relation between nonuniform cellular coupling, nonuniform activation, and repolarization was examined in the canine pulmonary conus with supportive evidence provided by numerical simulation. By varying the direction of the activation wavefront relative to the direction of lowest axial resistivity, the uniformity of activation at a given test site was varied. These workers concluded that nonuniform activation resulting from abnormal coupling resistance modulates repolarization electrotonically. Our modeling results corroborate these findings and suggest a possible additional factor for the correlation between nonuniform activation and repolarization found in their study. Since in their study nonuniform activation was the result of transverse uncoupling, unmasking of intrinsic differences in action potential duration may also have played a role in the dispersion of repolarization noted. We found that when nonuniform anisotropy is present, such as depicted in Figure 8, marked pacing-site dependency in dispersion of action potential duration occurred (Figure 8A vs. 8B).

During clinical electrophysiological studies, pacing-site dependency in arrhythmia initiation is often noted.\textsuperscript{32-34} One possible explanation for this phenomenon, based on the results of our study, is that in the setting of a nonuniformly anisotropic substrate there may be pacing-site-dependent changes of manifest action potential duration. For example, one could hypothesize that in Figure 8, unidirectional block in response to a premature beat is more likely to occur when pacing from along the transverse axis edge (Figure 8B) than from along the longitudinal axis edge (Figure 8A). However, when the anisotropy present was purely uniform as depicted in Figure 7, no such pacing-site-dependent changes in dispersion were noted. The potential arrhythmogenicity of a nonuniform as opposed to uniform anisotropic substrate has recently been emphasized,\textsuperscript{23} and our model supports this.

It remains unresolved whether block of premature beats that initiates reentry occurs purely as a result of reduced safety factor,\textsuperscript{34} by spatial disparity of refractory period as found by Gough\textsuperscript{55} in the canine infarct epicardial border zone, by geometric factors such as fiber branching or changes in fiber diameter,\textsuperscript{56} or by a combination of mechanisms. In a preliminary report using optical mapping of action potential duration,\textsuperscript{57} it was found that ischemia produced greater dispersion of action potential duration than anoxia. Since ischemia is probably a more potent stimulus for cellular uncoupling than anoxia,\textsuperscript{58} these findings are consistent with our results that abnormal coupling unmasks dispersion of action potential duration. In intact cardiac muscle, there is complex interaction between active membrane properties and passive cable properties.\textsuperscript{13-15} A propagating action potential is the result of this complex interaction. In our model, we have shown that passive cellular coupling interacts with membrane ionic currents during repolarization, the slow inward current in this case, to modulate how action potential duration is manifest. These results are not particularly dependent on the specific ionic current whose variation from cell to cell results in spatial variation in intrinsic action potential duration. No matter which ionic current or currents are involved, if a cell with a shorter intrinsic action potential duration is tending to repolarize while its well-coupled neighbor is still depolarized, electrotonic current flow between them will result in synchronized repolarization.

Limitations

One limitation of our study is that we have focused on dispersion of action potential duration rather than on recovery of excitability, and these are not strictly equivalent. However, in the BR model, the membrane patches exhibit normal ventricular myocardial ionic kinetics with a well-polarized resting potential of $-84.35$ mV. In such cells, one would not expect any significant loss of proportionality between action potential duration and refactoriness or recovery of excitability, though this might occur in the setting of ischemically injured cells with a depolarized resting potential.

The values for dispersion of action potential duration we obtained seem relatively modest (up to 2 msec for high-resistance isotropy and uniform anisotropy; less than 10 msec for nonuniform anisotropy). Part of the reason for these relatively low numbers relates to our method of calculation. Overall dispersion was based on the standard deviation of 100 elements at the center of the sheet, an area only 1 mm$^2$. If we had instead used the difference between the maximum and minimum values in this region as a measure, much larger values for dispersion would have resulted. For example, in the experiment in which isotropic coupling was simulated with resistivity equal 20,000 $\Omega$-cm, the overall dispersion by the standard deviation technique was 2.1 msec, but the maximum minus the minimum action potential duration in the center 1-mm$^2$ region was 10.1 msec. The other metrics we used (maximum and average local difference in action potential duration) quantitate dispersion over a distance of only 100 $\mu$m. A dispersion of, say, 2 msec over this distance represents a not insignificant
spatial dispersion gradient of 20 msec/mm. In any case, it is not known what degree of dispersion of action potential at the microscopic size scale used in our model would be required for unidirectional block and reentry initiation in intact myocardium, nor did we attempt to address this in the present study. However, even with a relatively small amount of dispersion, a critically timed and located premature beat might still be expected to block locally where action potential duration was longer than the premature coupling interval.

In our model, we have used axial resistivities that are higher than those which have generally been reported for normal ventricular muscle.\textsuperscript{16,18,59–61} Though in a finite element model such as ours,\textsuperscript{x and y discretization are not necessarily to be considered equivalent to cellular dimensions, the assumption that membrane patches were isopotential over the somewhat "coarse" resolution of 100 \(\mu\)m\(\times\)100 \(\mu\)m is probably not valid for real tissue. One reason these values of resistivity and grid discretization where chosen relates to an implementation trade-off between grid resolution, total size of the sheet, and the amount of computer time and memory needed for a reasonable simulation. Given the same total number of elements (50x50), lowering axial resistivity or making element size smaller yields several sources of potential artifact. First, the lower the axial resistivity and the smaller the element size, the farther toward the center of the grid will edge conditions be felt. Even under the circumstance of a completely uniform distribution of intrinsic action potential durations, because no current is allowed to flow off the edges, shorter action potentials are manifest at the edges. This effect can be seen as a slight folding-down of the action potential duration surface plots in Figures 3 and 8. In control experiments, the values of resistivity and grid discretization that we used produced less than 0.1 msec of dispersion of manifest action potential duration, measured at the central 10x10 region, when there was no dispersion of intrinsic action potential durations (\(g_m\) was constant throughout the sheet). We could have increased the total number of elements allowing smaller size per element for the same total sheet dimensions. However, despite the efficiencies introduced in \"Methods,\" a large computational load is present even with a 50x50 grid: in addition to integrating all of the BR ionic current and gating equations for each patch, solution of a 6,250,000-element matrix (2,500x2,500) is accomplished at each ADI time-step. Another reason we did not use lower axial resistivity is related to temporal resolution of the model. Since time is numerically discretized, we estimate the measurement accuracy of repolarization time and, therefore, of action potential duration to be on the order of 0.1 msec. Lower resistivity would produce faster conduction, and measurement of dispersion of action potential duration would impinge on the noise level of our model. In any case, our purpose was to study the effect of abnormally high axial resistivity on dispersion, since decoupling is present in the potentially arrhythmogenic substrate of chronic myocardial infarction.\textsuperscript{23–25}

Another limitation of the model is the use of a simplified geometry of a flat, thin sheet of muscle. Clearly, the complex three-dimensional geometry seen at the border zone of healed infarction, for example, where there is a distorted heterogeneous mixture of collagen and normal muscle is not accounted for by our approach.\textsuperscript{23–25} One would expect the effect of wavefront direction on dispersion of action potential duration, seen even in the case of the relatively simple nonuniform anisotropy shown in Figure 8, to be magnified under the conditions of complex three-dimensional heterogeneous coupling in the diseased heart.

Because of these limitations, our results should be treated as qualitative rather than strictly quantitative. We have attempted to show that, under the controlled conditions of our model, dispersion of action potential duration can be modulated by alterations in coupling. The results of our experiments may be important in understanding the mechanism of dispersion of action potential duration when potentially arrhythmogenic abnormalities of cellular coupling are present. Further work is needed that involves a combination of computer modeling and experiments in vivo and in vitro to understand the importance of the interaction between active and passive electrophysiological properties to both the initiation and perpetuation of sustained reentry.

\textbf{Conclusion}

Whether by abnormal gap junctions themselves or by physical disruption of normal fiber appositions, cellular uncoupling occurs in a number of pathological states. Examples include acute ischemia and chronic myocardial infarction. Our study supports a hypothesis that these disorders may be arrhythmogenic on several counts: high axial resistivity leads to slow conduction, one requirement for reentry; and poor coupling leads to unmasking of intrinsic differences in action potential duration, a condition favorable to the development of unidirectional block and tachycardia initiation. These results have potential therapeutic implications. Antiarrhythmic agents have generally been classified by their ability to alter active membrane ionic properties. Less is known about their direct and indirect actions on cellular coupling and anisotropy, although such effects may be as important if not more important to their mechanism of antiarrhythmic action. For example, Kadir et al.\textsuperscript{22} have shown that procainamide, in addition to its well-known ability to decrease the fast sodium current, normalizes anisotropy to some extent, which decreases the ratio of longitudinal to transverse conduction velocity. Therefore, one might speculate that the antiarrhythmic action of a drug such as procainamide could be related to masking of intrinsic differences in action potential duration.
brought about by relative normalization of coupling, especially in chronically infarcted myocardium where abnormal coupling is the predominant derangement.

At the other extreme, pharmacological uncoupling agents, which have been shown to have differential effects on longitudinal and transverse conduction, could be antiarrhythmic if they were able to isolate regions already affected by poor coupling and which manifest the most extreme dispersion of action potential duration. In this regard, (Spear et al, submitted manuscript) recently examined the effect of 0.2–1.0 mM heptanol, a potent cellular uncoupler, on conduction in isolated perfused strips of chronically infarcted canine myocardium. They found that regions of tissue with the most severely abnormal baseline coupling become electrically inactive after heptanol treatment. Heptanol is toxic in vivo; thus, its use as an antiarrhythmic agent is precluded. However, in the future, the design of new antiarrhythmic drugs may include agents whose primary mode of action is to alter cellular coupling. In this way, the interaction between passive and active properties of a poten-

tially arrhythmogenic substrate might be changed so as to mask intrinsic differences in action potential duration; thus, unidirectional block and initiation of reentry would be prevented.

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