Wave-Front Curvature as a Cause of Slow Conduction and Block in Isolated Cardiac Muscle

Cándido Cabo, Arkady M. Pertsov, William T. Baxter, Jorge M. Davidenko, Richard A. Gray, José Jalife

Abstract We have investigated the role of wave-front curvature on propagation by following the wave front that was diffracted through a narrow isthmus created in a two-dimensional ionic model (Luo-Rudy) of ventricular muscle and in a thin (0.5-mm) sheet of sheep ventricular epicardial muscle. The electrical activity in the experimental preparations was imaged by using a high-resolution video camera that monitored the changes in fluorescence of the potentiometric dye di-4-AEPFP on the surface of the tissue. Isthmuses were created both parallel and perpendicular to the fiber orientation. In both numerical and biological experiments, when a planar wave front reached the isthmus, it was diffracted to an elliptical wave front whose pronounced curvature was very similar to that of a wave front initiated by point stimulation. In addition, the velocity of propagation was reduced in relation to that of the original planar wave. Furthermore, as shown by the numerical results, wave-front curvature changed as a function of the distance from the isthmus. Such changes in local curvature were accompanied by corresponding changes in velocity of propagation. In the model, the critical isthmus width was 200 μm for longitudinal propagation and 600 μm for transverse propagation of a single planar wave initiated proximal to the isthmus. In the experiments, propagation depended on the width of the isthmus for a fixed stimulation frequency. Propagation through an isthmus of fixed width was rate dependent both along and across fibers. Thus, the critical isthmus width for propagation was estimated in both directions for different frequencies of stimulation. In the longitudinal direction, for cycle lengths between 200 and 500 milliseconds, the critical width was <1 mm; for 150 milliseconds, it was estimated to be between 1.3 and 2 mm; and for the maximum frequency of stimulation (117±15 milliseconds), it was >2.5 mm. In the transverse direction, critical width was between 1.78 and 2.32 mm for a basic cycle length of 200 milliseconds. It increased to values between 2.46 and 3.53 mm for a basic cycle length of 150 milliseconds. The overall results demonstrate that the curvature of the wave front plays an important role in propagation in two-dimensional cardiac muscle and that changes in curvature may cause slow conduction or block.

Key Words • isthmus • critical curvature • safety factor • video imaging • cardiac ionic model

In a cable of electrically coupled cells, slow conduction and block often result from a decreased transmembrane inward current and/or uncoupling between cells. In each case, the “safety factor,” defined as the ratio between the current available to excite cells downstream (the “source”) and the current needed to excite those cells (the “sink”), determines whether there will be conduction or block. If there is conduction, the safety factor determines the velocity of propagation. However, in normal cardiac muscle, certain structural factors may lead to an “impedance mismatch” between the sink and the source, with a consequent alteration of the propagation process. Such factors have been well studied in a number of experimental situations, eg, at branching sites, at the Purkinje-muscle junction, during conduction through a narrow isthmus of cardiac tissue, and in Purkinje fibers positioned asymmetrically in a sucrose gap. In addition, a number of theoretical studies have used computer models to investigate the effects of geometry on propagation in one-dimensional cables. Yet, to our knowledge, very few studies have aimed at determining the safety factor or estimating the “liminal area” (similar to liminal length) for propagation in two-dimensional cardiac tissue.

An analogue to the safety factor has been studied thoroughly in other excitable media that share some basic properties with cardiac tissue. However, the terminology that has been used in those studies differs from that commonly used in cardiac electrophysiology. In two-dimensional excitable media, other than cardiac muscle, the geometry of the wave front (ie, its curvature) is thought to be a major determinant of the success or failure of propagation, and numerical and analytical studies of those media have suggested that the more convexly curved a wave front is, the slower its velocity of propagation. Thus, thinking in terms of sink/source properties, one would predict that the more convexly curved a wave front is, the greater the imbalance between the source
and the sink and the lower the safety factor for propagation. In cardiac tissue, the effect of wave-front geometry has been demonstrated in studies in which the velocity of propagation of a planar wave front (zero curvature) is compared with that of an elliptical (convexly curved) wave front. The theory of wave propagation in excitatory media also predicts that there is a critical wave-front curvature for propagation to proceed. In addition, numerical experiments using the FitzHugh–Nagumo equations have measured the critical curvature during propagation through a narrow hole in an impermeable screen and shown that block occurs when the size of the hole is reduced to a certain critical width. To our knowledge, the critical curvature for propagation has not been estimated previously in two-dimensional cardiac muscle.

We present the results of a study on the effect of curvature on propagation in two-dimensional cardiac ventricular muscle. Wave fronts of pronounced curvature are expected during propagation of an action potential through a narrow isthmus. Such a situation was simulated in a two-dimensional computer model using the most updated mathematical description of the ventricular cardiac muscle membrane (the Luo-Rudy model) and was created in thin slices of sheep epicardial tissue. Our results (1) show that there is a critical width of the isthmus for propagation in two dimensions, (2) give estimates of the critical isthmus for different stimulation frequencies, and (3) show that the velocity of propagation decreases immediately after crossing the isthmus. The overall results suggest that the curvature of a wave front can be a cause of slow conduction and block in cardiac tissue.

Materials and Methods

Computer Model

The two-dimensional computer model is that of a continuum anisotropic (3:1) piece of "cardiac muscle" in which the junctional and myoplasmic resistances are distributed uniformly in an effective intracellular resistance. The modeled tissue is assumed to be in an unbounded medium; hence, the extracellular resistance is set to zero. The membrane impedance consists of a single capacitance in parallel with the ionic currents described by the Luo-Rudy model. The governing equation in cartesian coordinates can be expressed as follows:

\[ I_m = \left(1/\lambda_i\right) \left((1/R_m)(V_m)_x + (1/R_m)(V_m)_y\right) = I_{ion} + C_m(V_m) \]

where \( I_m \) is the transmembrane current at a certain patch of the membrane (in microamperes per square centimeter), \( S_i \) is the surface-to-volume ratio of the preparation (2000 cm\(^{-3}\)), \( R_m \) is the intracellular resistance in the longitudinal direction (0.5 K\(\Omega \cdot \)cm), \( R_x \) is the intracellular resistance in the transverse direction (4.5 K\(\Omega \cdot \)cm), \( V_m \) is the transmembrane potential (in millivolts), \( I_{ion} \) is the total ionic current (in microamperes per square centimeter), and \( C_m \) is the specific membrane capacitance (1 \(\mu\)F/cm\(^2\)). The differential equation was discretized and advanced in time with an explicit forward Euler scheme. The spatial discretization step was 50 \(\mu\)m, and the time integration step was 2 microseconds. A reduction of the discretization step to 25 \(\mu\)m and the time integration step to 0.5 microsecond changed our computations of the conduction velocities in the longitudinal direction by <1% and in the transverse direction by <5%. The size of the medium was 10\(\times\)5 mm\(^2\) (=15 space constants in the longitudinal direction and =25 space constants in the transverse direction). The boundary nodes in the sheet were considered sealed (ie, with no intracellular current flow out of the domain), so the boundary conditions were \( \partial \rho_{in}/\partial x = 0 \) and \( \partial \rho_{in}/\partial y = 0 \). To reproduce the data obtained in the experiments regarding the planar conduction velocity, we used a maximum conductance for sodium of 11.5 milli-siemens (mS/cm\(^2\)) (half the standard in the Luo-Rudy model) as an equivalent to the condition produced by stimulating our preparations at a basic cycle length (BCL) of 500 milliseconds.

We simulated the isthmus by imposing an impermeable screen with a center hole, dividing the excitable medium in two parts (see Fig 2). The screen prevented electrotonic interactions between the two parts of the medium; interactions were only possible through the hole. The screen was implemented by disconnecting nodes at both sides of the screen. Therefore, the width of the screen coincided with the space discretization step, which was much smaller than the space constant in either the longitudinal (=600 \(\mu\)m) or transverse (=200 \(\mu\)m)] directions. We define the proximal side of the isthmus as the part of the excitable medium where the external stimulation was applied. The distal side of the isthmus is the part of the excitable medium that is beyond the hole.

Measurements of Curvature

The wave front at each time was defined as the locus of the nodes being activated at that time. The time of activation of a node was the time of occurrence of the maximum inward current. The curvature of the wave front was measured by fitting an ellipse to the wave front at each time (ie, the isochronal line at that time). Both axes of the ellipse were measured parallel (longitudinal direction, axis a) and perpendicular (transverse direction, axis b) to the isthmus orientation. From the definition of curvature and basic differential geometry, it can be shown that the curvature of the ellipse in the longitudinal direction is \( a/b^2 \) and the curvature in the transverse direction is \( b/a^2 \).

Experimental Model

Young sheep were anesthetized with sodium pentobarbital (35 mg/kg IV). The hearts were rapidly removed and placed in warm oxygenated Tyrode's solution. Square pieces of epicardial muscle (approximate size, 20 \(\times\)20 \(\times\)0.5 mm) were cut with a dermatome. Care was taken to avoid the regions of the main coronary arteries or any large bands of connective or fatty tissue. Suitable preparations were immediately transferred to a Plexiglas chamber and pinned to the wax floor of the chamber, which was mounted in an antivibration table. The preparations were continuously superfused (flow, 20 mL/min) with Tyrode's solution containing (mmol/L) NaCl 130, KCl 4, NaHCO\(_3\) 24, Na\(_2\)HPO\(_4\) 1.2, MgCl\(_2\) 1, CaCl\(_2\) 1.8, and glucose 5.6. Solutions were bubbled with 95% O\(_2\)/5% CO\(_2\) (pH 7.4; temperature, 37±1°C). The preparation was allowed to equilibrate for 1 hour by continuous superfusion of a recirculating volume (1000 mL) of Tyrode's solution. For the optical recording of the transmembrane potential, the preparations were stained with the voltage-sensitive dye di-4-ANEPPS (20 \(\mu\)mol/L, Molecular Probes, Inc). To avoid mechanical artifacts caused by the contractions of the preparations, diacetyl monoxide (DAM, 15 mmol/L) was added to the superfusate before the beginning of the optical recordings.

Optical Mapping System

The tissue fluorescence was excited with light from a tungsten-halogen lamp (24 V, 250 W), collimated, filtered (490 nm), reflected with a dichroic mirror (560 nm), and focused on the preparation. The transmitted light was filtered (645 nm) and recorded by a CCD video camera (Cohu series 6510). An A/D frame grabber (Epix, Inc), mounted in an IBM-compatible personal computer, acquired the video images at a rate of 60 frames per second (16.67 milliseconds per frame). In each frame, the shutter was open for 16.67 milliseconds. The
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as in panel A and stained with the
antibodies against the gap junction protein connexin43 is shown. Arrows indicate the localization of connexin43 in sheep heart tissue at the intercalated disks. Note clear distinction between damaged area near the cut end and viable tissue within the isthmus. C. Phase-contrast image of the same section as in panel B. Scale bars=100 μm.

lengths indicated above, with the stimulating electrodes being parallel to the cuts. The width of the isthmus was then narrowed by gradually extending one of the original cuts. For each width (ie, after each cut), the tissue was given half an hour to heal, and the stimulation protocol was again repeated. Typically, data from isthmuses of three or four different widths were recorded from each preparation. The number of isthmuses studied in each preparation was limited either after reaching an isthmus width for which there was no propagation or by loss of optical signal (dye bleaching), since the preparation was not restained during the protocol. At the end of the experiment, two of the preparations were fresh-frozen with liquid nitrogen for subsequent histological examination (see below).

Data Analysis

Width of the Isthmus

The width of the isthmus was measured by the following procedures: (1) from the real image of the tissue on the video screen, by counting the number of pixels occupied by the tissue within the isthmus, (2) from the video images of dye fluorescence during propagation, by measuring the width of excited tissue at the isthmus (measurements were made after subtraction of background fluorescence and before spatial filtering [see below]), and (3) by histological analysis in two preparations after the optical mapping experiment (Fig 1). The technique uses 0.5% pinacyanol iodide stain (Sigma Chemical Co) dissolved in 70% ethanol for the sections of fresh-frozen unfixed tissue, which was cut and prepared as described previously.34 To determine the precise fiber orientation across the isthmus, identically prepared sections of fresh-frozen unfixed sheep myocardium were stained for gap junction protein (Fig 1B) according to the same protocol described previously.35

Isochrones and Conduction Velocity

Spatial low-pass filtering was applied to the optical recordings to improve the visualization of the signal. The filtered value for each pixel from each frame was calculated as a weighted average with its neighbors. The highest weight corresponded to the pixel being calculated, and the weights of the neighbors dropped linearly to zero at a distance of 0.4 to 0.5 mm from the pixel considered. Isochrones were calculated from the filtered optical recordings by analyzing the value of each pixel over time. For each pixel, activation times were defined as the times of fastest upstroke in the optical transmembrane potentials. In that way, it was possible to plot the pixels activated for every frame (16.67 milliseconds), which was equivalent to the distance traveled by the wave front during 16.67 milliseconds. The velocity was estimated as that
distance divided by 16.67 milliseconds. The values of the velocity of propagation estimated in that way differed by <5% at a BCL of 500 milliseconds, both in the longitudinal and transverse directions, from the values of the velocities in the same area estimated from a pair of microelectrode recordings in two test cases.

**Time-Space Plots**

The "frame-stack procedure" used to construct time-space plots has been described in detail previously. The time-space plots were used to show in a single picture the electrical activity from a selected region during a certain period of time. The time-space plots were constructed for the pixels contained in a rectangle centered at the isthmus. The plots included data from part of the proximal side of the isthmus, the isthmus, and part of the distal side of the isthmus. The optical transmembrane potentials at pixels in a given row in the rectangle were averaged to obtain a single value. As a result of repeating the process for all rows, the electrical activity in the rectangle was represented by a single vertical line at every time step. The time-space plots were obtained by stacking all those vertical lines. The ratio between space and time is the velocity. Therefore, quantitative information on the velocity of propagation was obtained from the time-space plots.

**Results**

**Computer Simulations**

**Critical Size of the Isthmus for Propagation**

An isthmus was simulated in a two-dimensional homogeneous medium with an anisotropic ratio of 3:1 by placing an impermeable screen with a hole at the center of the medium. Propagation was initiated by planar stimulation in the proximal side, with the resulting wave front being parallel to the screen. The velocity of propagation of the planar wave was 41 cm/s. The size of the isthmus was reduced gradually until the wave failed to propagate from the proximal to the distal side. The critical width of the isthmus was shown to be a function of the direction of propagation. In the longitudinal and in the transverse directions, the critical widths were 200 and 600 µm, respectively. It is interesting that the width of the critical isthmus is scaled in the same way as the anisotropic ratio (ie, the ratio of longitudinal to transverse propagation velocity is 3 in the present study). The critical size of the isthmus for propagation was strongly dependent on the excitability of the medium. We decreased the maximum value of the sodium conductance from 11.5 mS/cm² (the value we take as normal; see "Materials and Methods") to 6.9 mS/cm² (which simulated the conditions observed experimentally during rapid stimulation). Such a drop in excitability resulted in a decrease in the propagation velocity of a planar wave in the longitudinal direction to 31 cm/s and an increase in the size of the critical isthmus to 500 µm. Fig 2A shows data for longitudinal propagation through a 550-µm-wide isthmus, in the case of reduced excitability. A planar wave front (white) initiated in the proximal side was diffracted to an elliptical wave front after reaching the isthmus. After passing the isthmus, the wave front had a pronounced curvature that decreased as the wave propagated away from the isthmus. Fig 2B shows the case of block at a 450-µm-wide isthmus. The incoming wave front propagated a short distance away from the isthmus, but it eventually stopped.

**Local Curvature and Local Conduction Velocity**

**Longitudinal propagation.** We carried out additional simulations in which we measured both the local conduction velocity and the curvature. The case for longitudinal propagation in a matrix having a 250-µm-wide isthmus is presented in Fig 3A. Panel A1 shows an isochronal map with isochrones displayed every 5 milliseconds. Panel A2 depicts the local conduction velocity along a line in the direction of propagation through the center of the isthmus. Proximal to the isthmus, the velocity of the wave front increased just before reaching the isthmus. The velocity decreased to
a minimum (25 cm/s) after passing the isthmus and then progressively increased again toward the value of the velocity of a planar wave. Note that direct activation of the nodes close to the stimulus site (left side of panel A2) caused transient (and artifactual) high values of the conduction velocity at those nodes (see below). Also note that when the wave front was very close to the boundary of the medium (right side in panel A2), the velocity of propagation increased because the medium was sealed and there was no intracellular current flowing out of the domain. Panel A3 shows the changes in wave-front curvature as a function of space. Clearly, the changes in the curvature correlated very well with changes in the conduction velocity: because of the high resistivity at the barrier, the increase in the conduction velocity just before the isthmus corresponded to that of a wave front of negative curvature (concave). On the other hand, the decrease in the conduction velocity beyond the isthmus corresponded to wave fronts of positive curvature (convex). The minimum velocity occurred at a distance of 0.05 cm, where the propagated wave front had its more pronounced curvature. These results suggest that the curvature of the wave front and the velocity of propagation are indeed very much related to each other. An isochronal map for longitudinal propagation through a wider isthmus (950 μm) is presented in panel B1 of Fig 3. Panels B2 and B3 show local changes in conduction velocity and curvature of the wave front during propagation. Qualitatively, the results are similar to those shown in the case of propagation through a narrow isthmus (Fig 3A): velocity and curvature were again related to each other. However, quantitatively, there were some differences: the decrease in the conduction velocity (minimum conduction velocity, 39 cm/s) and the increase in the curvature of the wave front were not as dramatic as in the case of the narrow isthmus. The minimum value of velocity occurred further away from the isthmus and over a wider range of distances.

Transverse propagation. Data obtained during transverse propagation with an isthmus wider than critical (width, 950 μm) are depicted in Fig 4A. The width of the isthmus was the same as for the case in Fig 3B. However, the changes in velocity (minimum conduction velocity, 8 cm/s; Fig 4B) were similar to those observed during propagation through a narrow isthmus in the longitudinal direction (Fig 3A). Again, changes in the velocity of propagation paralleled changes in the curvature of the wave front (Fig 4C). Note that even though local changes in velocity and curvature had similar shapes in the cases presented in Figs 3A and 4, the absolute values of those changes were very different, particularly for the curvature.

It is important to indicate at this point that the above results would be difficult to explain on the basis of the unidimensional concept of impedance mismatch. It is, in fact, unlikely that the impedance mismatch at the isthmus had any influence on the velocity of propagation far from the isthmus. If an impedance mismatch had any effect at the isthmus, then it should be expected that changes in conduction velocity should have occurred right at the isthmus and not far away from it. However, in Fig 3B, the velocity of propagation through the isthmus was normal because the wave front was flat;
the minimum velocity of propagation happened far away from the isthmus, where the curvature of the wave front was maximal. At a further distance away from the isthmus, the velocity was still slower than that of a planar wave, because the wave front was curved. Thus, according to the model results, the most important effect of the isthmus was to diffract the planar wave front into an elliptical curvature. The local changes in curvature themselves were responsible for the corresponding changes in velocity.

In light of the above results, the case of block at the isthmus shown in Fig 2B above can also be understood in terms of the curvature of the wave front. The wave (planar at the isthmus) invaded the distal side, and its curvature increased until propagation was blocked. Since a high curvature of the wave front causes a reduction in the velocity of propagation (see Fig 3A and 3B), it is reasonable to propose that propagation will be possible only for wave fronts whose curvature is less than a certain critical value. This would mean that as predicted by the theory of excitable media, there should be a critical curvature for propagation also in cardiac tissue.

**Relation Between Velocity of Propagation, Curvature, and Isthmus Size**

We have shown in the previous section that in the presence of an isthmus, the minimum velocity of propagation occurred after passing the isthmus, when the curvature of the wave front was maximal. In Fig 5A, we have plotted the velocity of the wave front as a function of the local curvature for the case of propagation through a 250-μm-wide isthmus (same as in Fig 3A). The data were well fitted by a straight line, whose slope of 0.102 cm²/s (95% confidence interval was between 0.096 and 0.108 cm²/s) is equivalent to the so-called “diffusion coefficient.” In the mathematical model used here (Equation 1), the diffusion coefficient is as follows: 1/[S,R,Cm(AR)²] = 0.11 cm²/s, where AR is the anisotropic ratio. From the graph, it is clear that the velocity of propagation decreases linearly with the curvature of the wave front. The three points above the fitted line, corresponding to a curvature of zero (planar wave), were caused by the direct (and almost simultaneous) activation of the nodes close to the stimulus site (see above).

We have also shown that the minimum velocity of propagation through the narrow isthmus (25 cm/s, Fig 3,
Propagation Through zero 2/w from the distance travelled isthmus B2). This was a consequence of the fact that the narrow isthmus imposed a more pronounced curvature on the propagating wave. When we calculated the average velocity of propagation after the isthmus for isthmuses of different sizes, we obtained the plot presented in Fig 5B. The average velocity was calculated from the distance travelled by the wave in the 5 milliseconds after the time at which the wave reached the isthmus (the velocity was that distance divided by 5 milliseconds). The maximum curvature of the wave front imposed by a certain isthmus is given by AR(2/w), where 2/w is twice the reciprocal of the width of the isthmus. Therefore, given the linear relation between curvature and propagation velocity (Fig 5A), it is not surprising that the points in 5B could be well fitted by a straight line.

The value of the curvature at which the velocity is zero is an estimate of the critical curvature for propagation. From the line in Fig 5A, the critical curvature in the longitudinal direction is 414.5 cm⁻¹ (=intercept/slope=41.45/0.102). When the error in the estimation of the intercept and the slope was taken into consideration, the 95% confidence interval for the critical curvature was estimated to be between 381 and 435 cm⁻¹. The value expected from the parameters of the model is 372.7 cm⁻¹ (=velocity of a planar wave/diffusion=41/0.11). The value of the critical curvature for propagation can also be estimated from the value of the critical isthmus as AR(2/w). In that case, the critical curvature is estimated to be 300 cm⁻¹ [=3(2/0.02)]. Differences in the estimations might be due to the spatial discretization step used in the model (50 μm).

Isolated Tissue Experiments
Propagation Through an Isthmus in Longitudinal and Transverse Directions
An example of longitudinal propagation through an isthmus in a thin sheet of epicardial muscle is presented in Fig 6. The leftmost frame on the top row shows the real image of the tissue to illustrate the two cuts produced horizontally across the fibers in the middle of the preparation. The additional five frames show images of the dye fluorescence (white) taken once every 16 milliseconds during propagation of a planar wave initiated at the top border of the preparation (proximal side of the isthmus) at a BCL of 500 milliseconds. On reaching the isthmus, the planar wave front was diffracted to an elliptical wave front that evidenced the fiber orientation: the velocity was much faster in the original direction of propagation than orthogonally (propagation in the direction of the cuts). The electrical activity emerging from the isthmus was similar to that which would be initiated by a point source.

Fig 7 shows data from another experiment in which the cuts were produced along the fibers in the middle of the preparation. In this case also, a planar wave front was initiated at the top of the preparation at a BCL of 500 milliseconds. On crossing the isthmus, the shape of the wave front changed from planar to elliptical. The velocity in this case was faster in the direction of the cuts than along the original direction of propagation. Because of the size and the high anisotropy of the preparation, the elliptical wave front in the distal side of the isthmus soon became almost planar. As in the previous case, the electrical activity emerging from the isthmus was similar to that which would be initiated by a point source.

Conduction Block at the Isthmus: Liminal Area and Curvature
As predicted by the model results, the presence of an isthmus may result in propagation block. In the experiment presented in Fig 8A, three different frames are shown during successful propagation of a single wave front initiated on the top border of the tissue at a BCL of 130 milliseconds. Propagation was longitudinal, and cuts were made across the fibers for an isthmus 2.04 mm wide. In Fig 8B, the immediately succeeding wave front was stopped even though the side distal to the isthmus was partially activated. It is unlikely that the block was caused by spatial differences in the refractory period because, before the isthmus was created, all planar waves initiated in the same manner at a BCL of 130 milliseconds were propagated without delay throughout the tissue. It may be argued that damage caused by the cuts caused the block in the distal side. However, from the optical recordings in the area of the isthmus and
from histological and immunocytochemical analysis of two preparations (Fig 1), it was clear that most of the tissue was viable there. In addition, the block did not occur at the isthmus itself, where the largest degree of damage would be expected, but approximately in the center of the distal side. Moreover, the data are similar to those obtained in computer simulations (see Fig 2B), which predict that block should occur after partial invasion of the distal side, when the width of the isthmus is close to critical. In both cases, the isthmus reduced the safety factor for propagation to such an extent that the amount of tissue invaded by the wave front in the distal end was insufficient to activate tissue downstream. Hence, the data suggest that in two-dimensional cardiac muscle, a minimal area of tissue must be activated for propagation to proceed. In other words, similar to the idea of liminal length in one-dimensional cables,\textsuperscript{1,2} it may be postulated that whether the result of an electrical stimulus or of propagation through a narrow isthmus, the initiation of a propagated wave front in two-dimensional cardiac muscle requires activation of a liminal area of tissue. Moreover, since the electrical activity in an anisotropic medium spreads in an elliptical pattern, the concept of liminal area is equivalent to the concept of critical curvature for propagation; ie, the liminal area must have a certain curvature, which is imposed by the anisotropy of the medium.

**Frequency Dependence of Propagation Through an Isthmus**

The ability of a wave front to propagate through an isthmus of a given width depended on the frequency of stimulation. An example is presented in Fig 9 in the form of time-space plots constructed using a framessack procedure.\textsuperscript{32} A 1.48-mm isthmus was cut perpendicular to the fiber direction; thus, propagation was in the longitudinal direction. Fig 9A shows an image of the preparation with a superimposed rectangle that delineates the area where the time-space plots (time in the horizontal and space in the vertical axes) in Fig 9B through 9D were calculated. The preparation was stimulated at the top with the long electrodes (not shown in the image) to create a planar wave front. In Fig 9B, when the BCL was 300 milliseconds, propagation through the isthmus was 1:1, as shown by the interrupted vertical bands (each band represents one propagating wave). In Fig 9C, the BCL was reduced to 160

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**Fig 7.** Video frames of experiment in a sheep epicardial preparation showing transverse propagation through a 2.60-mm isthmus. A planar wave front, initiated at the top of the preparation at a basic cycle length of 500 milliseconds, propagated downward through the isthmus. The first frame (top left) is an image of the preparation. Subsequent frames show the electrical activity every 16 milliseconds. Depolarized tissue is shown in white, and resting tissue is shown in black.

**Fig 8.** Video frames demonstrating a liminal area for activation of tissue through a narrow isthmus in an isolated sheep epicardial preparation. The electrical activity was initiated at the top of the preparation by using point stimulation. The cycle length of stimulation was 130 milliseconds, and propagation through the 2.04-mm isthmus was 2:1. A, Propagation of a single wave. B, The next wave, blocked as it was diffracted at the isthmus. Note that, in this case, the distal side (bottom) was partially activated. However, the amount of tissue activated was insufficient to activate tissue downstream (compare this figure with the computer simulations in Fig 2).
milliseconds, and propagation through the isthmus became 3:2 (note that there are three bands on the upper part of the plot for each two in the lower part). In Fig 9D, a further reduction of the BCL to 140 milliseconds yielded a 2:1 pattern of propagation through the isthmus. For this preparation, the shortest BCLs that sustained 1:1 propagation in the absence and in the presence of an isthmus were 110 and 200 milliseconds, respectively. Therefore, the various patterns of intermittent propagation found at BCLs of 160 and 140 milliseconds were the result of the presence of the isthmus.

**Effect of Width of the Isthmus on Propagation**

For a fixed frequency of stimulation, the degree of propagation (or block) through the isthmus depended on the width of the isthmus. The data in Fig 10 were taken from the same experiment as in Fig 9. We present data in which the proximal end was driven at a constant BCL of 160 milliseconds for isthmuses of three different widths. In all panels, the top frame is the real image of the tissue showing the width of the isthmus. The time-space plots (time is in the horizontal axis; space, in the vertical axis) shown in the bottom frames were taken from the same region as in Fig 9. In Fig 10A, an isthmus of 2.11 mm allowed 1:1 propagation. In Fig 10B, the width of the isthmus was reduced to 1.48 mm, and propagation became 3:2 (same time-space plot as in Fig 9C). In Fig 10C, when the width of the isthmus was further narrowed to 1.09 mm, propagation became 2:1. Again, the refractory period in the absence of the cuts...
Table 1. Critical Size of the Isthmus

<table>
<thead>
<tr>
<th>BCL, ms</th>
<th>Lower Boundary, mm</th>
<th>Mean±SD</th>
<th>n</th>
<th>Upper Boundary, mm</th>
<th>Mean±SD</th>
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<tr>
<td>500</td>
<td></td>
<td>0.48±0.15</td>
<td>6</td>
<td></td>
<td>1.06±0.59</td>
<td>9</td>
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<tr>
<td>200</td>
<td></td>
<td>0.60±0.14</td>
<td>5</td>
<td></td>
<td>1.09±0.28</td>
<td>7</td>
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<tr>
<td>150</td>
<td></td>
<td>1.29±0.63</td>
<td>7</td>
<td></td>
<td>1.96±0.85</td>
<td>7</td>
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<tr>
<td>117±15  (max)</td>
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<td>2.64±0.83</td>
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BCL indicates basic cycle length; max, maximum frequency of stimulation.
conduction was caused by the pronounced curvature caused by the isthmus.

**Effect of Anisotropy on the Critical Isthmus**

Some of the isthmuses were created by cutting in a direction parallel to the fiber orientation to study propagation through the isthmus in the transverse direction (Fig 7). The critical isthmus (estimated in a way similar to that for propagation in the longitudinal direction) for a BCL of 200 milliseconds was between 1.78±0.47 and 2.32±0.50 mm (n=4); for a BCL of 150 milliseconds, it increased to a value between 2.46±0.68 and 3.53±1.11 mm (n=6). We also studied the effect of the width of the isthmus on the velocity of propagation. As for the longitudinal case, the velocity at the isthmus decreased with decreasing width of the isthmus (and increasing 2/w). The equation used to model the changes in velocity with the width of the isthmus was as before: \( v = v_c - D(2/w) \). Table 3 summarizes the results of six experiments during propagation through the isthmus in the transverse direction with a stimulating frequency of 500 milliseconds. However, as in Table 2, the estimates in Table 3 for each experiment are based on very few points (three to five isthmuses plus the value of the planar velocity). To obtain a better estimate of the slope, we normalized the values of the velocities and 2/w with respect to the planar velocity of propagation for each experiment. Fig 13B summarizes the results of six experiments for transverse propagation at a BCL of 500 milliseconds. The values were fitted by a straight line (correlation coefficient, 0.7) having a slope of 0.27 cm²/s, with the 95% confidence interval being between 0.16 and 0.38 cm²/s.

The ratio between the critical width in the transverse and longitudinal directions was 1.89 (2.46/1.3) at a BCL of 150 milliseconds and 2.96 (1.78/0.6) at a BCL of 200 milliseconds. In a continuous homogeneous medium where anisotropy is a mere rescaling of the spatial dimensions, the ratio between the width of the isthmuses should be the same as the anisotropy ratio (see results from computer simulations). In our preparations, that ratio between spatial dimensions was less than the anisotropy ratio, which was \( \approx 4:1 \) (37.72/9.48=3.97; see first column in Tables 2 and 3). Possible explanations for this difference are discussed below.

**TABLE 2. Longitudinal Propagation Through the Isthmus**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Planar Velocity, cm/s</th>
<th>No. of Isthmus</th>
<th>Slope, cm²/s</th>
<th>Intercept, cm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>99</td>
<td>37.27</td>
<td>4</td>
<td>0.56</td>
<td>39.54</td>
</tr>
<tr>
<td>100</td>
<td>38.65</td>
<td>4</td>
<td>0.52</td>
<td>38.70</td>
</tr>
<tr>
<td>109</td>
<td>41.96</td>
<td>3</td>
<td>0.52</td>
<td>43.06</td>
</tr>
<tr>
<td>111</td>
<td>50.54</td>
<td>5</td>
<td>0.49</td>
<td>48.62</td>
</tr>
<tr>
<td>116</td>
<td>37.36</td>
<td>3</td>
<td>0.51</td>
<td>39.64</td>
</tr>
<tr>
<td>118</td>
<td>25.79</td>
<td>3</td>
<td>0.28</td>
<td>26.60</td>
</tr>
<tr>
<td>122</td>
<td>32.51</td>
<td>5</td>
<td>0.41</td>
<td>32.94</td>
</tr>
</tbody>
</table>

Total 37.72±7.10 ... 0.47±0.08 38.44±6.52

*Values are for basic cycle length of 500 milliseconds.*
Discussion

The role of the curvature of the wave front on propagation was studied by determining the characteristics of propagation through isthmuses of varying widths by use of a computer model of two-dimensional cardiac muscle and thin slices of sheep epicardial tissue. From the numerical results, it became clear that despite the impedance mismatch caused by the isthmus, changes in the velocity of propagation were more related to the curvature of the wave front. Moreover, in both numerical and experimental approaches, the critical curvature for propagation was estimated through measurements of the critical isthmus size for propagation. In the experiments, the critical size depends on the frequency of stimulation and ranges from hundreds of micrometers at slow frequencies to several millimeters at fast frequencies. Most important, in both computer and biological results, there was a good negative correlation between the velocity of propagation after the isthmus and 2/w. Also, in both, spatial changes in curvature of the wave front resulted in spatial changes in velocity. In addition, our previous results have shown that after crossing an isthmus, the morphology of the leading edge of the wave front (ie, phase 0 of the action potential) also changes. Overall, the data argue in favor of the idea that the curvature of the wave front is an important determinant of propagation velocity in cardiac muscle and that changes in curvature may be a cause of slow conduction and block.

Curvature and Liminal Area

The idea of critical curvature is indeed very much related to the concept of liminal area for propagation. Consider a two-dimensional sheet of anisotropic cardiac muscle being stimulated at its center by a point source. Because of anisotropy, the current supplied by the source would spread like an ellipse. Under these conditions, it is clear that the amount of muscle that needs to be excited directly by the stimulus to initiate propagation could be determined by measuring either the area (liminal area) or the curvature (critical curvature) of the ellipse. Thus, it is easy to see that if the anisotropy ratio is known, the liminal area of a two-dimensional piece of muscle could be calculated from the critical curvature and vice versa. On the other hand, discontinuities and/or differences in the intracellular and extracellular anisotropic ratios in two-dimensional cardiac tissue and/or twisted anisotropy in three-dimensional tissue would make matters much more complicated.

Presumably, in two dimensions, the ratio between sources and sinks also determines whether there will be propagation or block and whether reentry will occur. In addition, it is obvious that geometric factors must play a crucial role in this case. Since an increased curvature may reduce the safety factor for propagation and be a cause of slow conduction and block, the study of the role of curvature in two-dimensional propagation of action potentials may be of great importance for the understanding of the mechanisms of reentrant arrhythmias. Moreover, the recent introduction of the relation

TABLE 3. Transverse Propagation Through the Isthmus

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Planar Velocity, cm/s</th>
<th>No. of Isthmus</th>
<th>Slope, cm²/s</th>
<th>Intercept, cm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>6.18</td>
<td>3</td>
<td>0.29</td>
<td>5.83</td>
</tr>
<tr>
<td>107</td>
<td>8.75</td>
<td>3</td>
<td>0.34</td>
<td>9.72</td>
</tr>
<tr>
<td>108</td>
<td>7.77</td>
<td>4</td>
<td>0.24</td>
<td>6.87</td>
</tr>
<tr>
<td>116</td>
<td>13.55</td>
<td>3</td>
<td>0.24</td>
<td>14.43</td>
</tr>
<tr>
<td>118</td>
<td>8.27</td>
<td>3</td>
<td>0.14</td>
<td>7.83</td>
</tr>
<tr>
<td>122</td>
<td>12.38</td>
<td>5</td>
<td>0.47</td>
<td>10.41</td>
</tr>
</tbody>
</table>

Total 9.48±2.6 ... 0.28±0.10 9.18±2.82

Values are for basic cycle length of 500 milliseconds.
between velocity and curvature in a cellular automata model has made it possible to simulate realistic situations in cardiac tissue, in which the curvature of the wave front might approach a critical value and result in slow conduction and block. Three situations in cardiac tissue in which wave fronts of pronounced curvature are expected include (1) initiation of propagation by the discharge of a pacemaker cell or group of cells, (2) external stimulation using a small electrode, and (3) propagation through a narrow isthmus. It is important to note that previous computer simulation studies in two-dimensional media have reported a progressive increase in the velocity of propagation toward a steady-state value as the wave propagates away from a point source. We have related those changes in velocity with the changes in the curvature of the wave front after initiation of electrical activity with a point source. It has also been suggested that the pronounced curvature at the center of a vortex of functional reentry determines the functional characteristics of that center as well as the size of the excitable gap. Hence, the curvature of the wave front may be an important factor in the dynamics of reentrant activity as well as in the effects of external electrical stimulation on reentry. It is interesting that in the situation studied here (propagation through a narrow isthmus), the propagating wave front has a high curvature only transiently (see Fig 3). However, it has been shown that either steady-state propagation with constant curvature or propagation with changing curvature could be used to characterize the effect of curvature on propagation in a given medium. Therefore, the characterization of the effects of curvature on propagation described in the present study (slow conduction and block) should be applicable to other situations, such as during vortexlike reentry, where the wave front might have a permanently large curvature near the center of rotation.

Critical Isthmus and Critical Curvature

de la Fuente et al were first to study propagation through a narrow isthmus in canine atrial tissue. Although they did not precisely measure the critical isthmus size and used large isthmuses (~5 mm), the anatomic width at which they demonstrated conduction block at slow frequency was similar to ours, 0.5 to 1 mm. More recently, Fast and Kleber studied propagation through a narrowing in patterned rat myocyte cultures and found that an action potential emerging from a strand five to eight cells in width always propagates to a large growth area, albeit at a reduced velocity. The latter would mean that in that particular case the critical isthmus was ~65 to 130 μm (presumably for slow stimulation frequencies), which is much smaller than our estimate. Even though it is difficult to compare the results of both models, it is useful to consider facts that can account for the different data. The value of the critical isthmus depends on the excitability (sodium current) of the tissue. Therefore, differences in rat and sheep sodium currents might explain differences in the estimates: the more excitable, the smaller the critical isthmus. Furthermore, for a given membrane (fixed excitability) and a given longitudinal intracellular resistance, the critical isthmus (and any other spatial quantity for that matter) depends on the anisotropic ratio. In addition, the transition between the isthmus and the distal area is crucial in determining the critical isthmus: the more abrupt the change, the more precise the estimation. Another factor that could affect the critical isthmus is the length of tissue occupied by the isthmus. Previous studies have used long isthmuses (ie, one-dimensional propagation within the isthmus), whereas our results were based on isthmuses created by two lines of discontinuity (ie, two-dimensional propagation proximal to the isthmus), which imply short isthmuses. However, when propagation proximal to the isthmus is planar (ie, the two-dimensional case is equivalent to the one-dimensional case), both cases are essentially equivalent.

Critical Isthmus and Stimulation Frequency

We have shown that the critical isthmus for propagation increased at high stimulation frequencies. On the other hand, in our computer simulations a reduction in the excitability of the medium also increased the critical isthmus. Therefore, it may be suggested that the increase in critical isthmus at high stimulating frequencies may result from a frequency-induced reduction of the excitability. This is in contradiction with previously reported experimental results in which an increase in stimulation frequency led to an increase in excitability. However, it should be considered that the BCLs in which those phenomena were observed were >300 milliseconds, whereas the dramatic changes in critical isthmus occurred at BCLs <200 milliseconds. Therefore, it may be possible that changes of excitability with frequency are different in those different ranges of BCLs.

Effect of Anisotropy on the Critical Isthmus

In estimating the critical isthmus in the longitudinal and transverse directions of our preparations, we found that the ratio between spatial dimensions was less than the anisotropic ratio. The latter results contrast with what is known from continuous homogeneous media, in which such ratios should be equal. Histological examination of preparations in which transverse propagation through the isthmus was studied suggests that indeed the fibers were not exactly parallel to the isthmus but crossed it at a relatively small angle (Fig 1). This would explain our results, since in that case propagation through the isthmus would be neither transverse nor longitudinal but somewhat diagonal. If this were the case, it would mean that the critical isthmus in the transverse direction is in fact narrower than its real size. Other factors that may account for the difference between the ratios of spatial dimension and anisotropy are inhomogeneities and differences in the anisotropic ratio of intracellular and extracellular resistivities.

Critical Isthmus and Spiral-Wave Activity

The theory of excitable media predicts that both the functional characteristics of the center of rotation of spiral-wave activity and the size of the excitable gap are determined by the curvature of the wave front close to the tip of the spiral. On the other hand, estimates of critical curvature from data available in the literature result in values of a few hundred micrometers, suggesting that perhaps the role of curvature in propagation in cardiac tissue is not that important. Although our results showing a critical isthmus <1 mm confirms those estimates for slow frequencies (500 and 200 milliseconds), they differ radically for fast frequencies (<200
milliseconds), where the critical isthmus may be of the order of one to several millimeters. Spiral waves obtained in preparations similar to those used in the present study to estimate the critical isthmus had a rotating period of <200 milliseconds.\textsuperscript{31,32} Since at the frequency of the spiral the critical isthmus is of the order of one to several millimeters, our results suggest that (as predicted by excitable media theory) curvature may in fact play a role in determining the dynamics of spiral waves.

Assumptions of the Computer Model

The concept of the curvature was first developed for the isotropic case.\textsuperscript{20} The validity of its applicability to an anisotropic medium depends on the assumptions made on the nature of the anisotropy. We have chosen the simplest model of anisotropy, i.e., monodomain anisotropy. In that case, if the medium is continuous, anisotropy is just a rescaling\textsuperscript{32} of the isotropic case. However, in a continuous medium with different anisotropies in the intracellular and extracellular spaces\textsuperscript{35,37} (or in the case of a discontinuous medium, the extension of the concept of curvature from the isotropic to the anisotropic case would be more complicated, and anisotropy would possibly be more than a simple rescaling.\textsuperscript{37}

In the simulations carried out using our computer model, we were interested in patterns of propagation through isthmuses. In selecting a computer model, we have surmised that the core-conductor model assumptions (extended to two-dimensional media) are accurate enough to represent our experimental preparation.\textsuperscript{49,50} As a consequence, the simulation of the two-dimensional sheet with a grounded extracellular medium (monodomain) seemed to be a good compromise between fidelity and simplicity. In that situation, the anisotropic monodomain model was equivalent to an anisotropic bidomain having the same anisotropic ratio in the intracellular and extracellular spaces.\textsuperscript{40} Under core-conductor model assumptions, the anisotropic monodomain can be used to represent accurately either a very thin preparation in an unbounded medium or a “typical” slice of tissue lying deep in a thicker preparation with a restricted extracellular space.\textsuperscript{50} In the neighborhood of the leading edge of the wave front (i.e., the phase of the action potential of interest in the present study), the core-conductor model rapidly becomes a good approximation at only very small depths.\textsuperscript{50}

Limitations of the Experimental Model

The Preparation

The preparations used in the present study were found to be electrophysiologically normal in terms of resting membrane potential and action potential duration. The conduction velocities obtained in the longitudinal and transverse directions were very close to those previously reported by our laboratory\textsuperscript{31,32} for sheep epicardial muscle and similar to those reported in the literature for other species.\textsuperscript{51-53} In our experiments, to avoid mechanical artifacts in the optical recordings, we chose to add DAM to the perfusate at a concentration of 15 mmol/L. DAM totally suppresses contractility without significant alterations in the electrical activity. Indeed, it has been shown\textsuperscript{54} that DAM produces a small decrease in the action potential duration, but it does not significantly alter upstroke velocity, action potential amplitude, or conduction velocity. Therefore, it is not likely that the presence or absence of DAM would significantly change our conclusions regarding critical isthmus or conduction velocities.

Measurements of Width of the Isthmus

From the optical recordings of potentiometric dye fluorescence during propagation, the width of the isthmus appeared only slightly smaller (100 to 200 μm) than that measured by direct counting of the number of pixels occupied by the isthmus in the video image of the preparation, suggesting that damage beyond the cuts was relatively small. This was confirmed by microelectrode recordings in the area of the isthmus as well as by histological examination (Fig 1). On the other hand, the spatial resolution in our measurements depended on the size of the image and was typically between 50 and 100 μm per pixel. Since our spatial resolution was between 50 and 100 μm, it is clear that the precision of a measurement decreases with the size of the object being measured. In particular, when measuring an isthmus of a size close to critical for slow frequencies (=500 μm at 500 milliseconds), our error could be as large as 50%. Again, it is important to note that estimations from direct optical measurement, visual inspection, and histology all yielded similar values.

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