Anisotropic Reentry in a Perfused 2-Dimensional Layer of Rabbit Ventricular Myocardium

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Background—Anisotropy creates nonuniformity in electrical propagation and may contribute to the occurrence of unidirectional conduction block and reentry. We describe the characteristics of reentrant tachycardia in a 2D layer of anisotropic ventricular myocardium.

Methods and Results—A Langendorff-perfused epicardial sheet (1.0±0.4 mm, n=35) was created by freezing the intramural layers of the rabbit left ventricle. Epicardial activation maps were constructed by use of different high-resolution mapping arrays connected to a mapping system. In 5 experiments, monophasic action potentials were recorded. In the intact left ventricle, no arrhythmias except VF could be induced. After freezing, programmed electrical stimulation or rapid pacing led to the induction of sustained VT (cycle length 130±11 ms). VT was caused by reentry around a functional line of block oriented parallel to the epicardial fiber direction. Action potential recordings demonstrated that the central line of block was kept refractory by electrotonic currents generated by the depolarization waves propagating at either side of the line of block. At the pivot points of the line of block, the pronounced curvature of the turning wave and abrupt loading changes created an excitible gap of 30 ms in the reentrant pathway.

Conclusions—In uniform anisotropic myocardium, reentry around a functional Z-shaped line of block may occur. The core of the circuit is kept refractory by electrotonic currents. The pronounced wave-front curvature and abrupt loading changes at the pivot points cause local conduction delay and create a small excitible gap. (Circulation. 2000;102:2650-2658.)

Key Words: anisotropy ■ reentry ■ tachycardia ■ electrophysiology

Unidirectional conduction block and slow conduction, prerequisites for the induction of reentry, require some kind of spatial heterogeneity.1 Nonuniformity in electrical properties has been attributed to spatial dispersion in refractory period,1–3 local differences in excitability,4,5 nonuniformity in the amount of excitatory current generated by the cell membrane,6 or anisotropy.7–10 In anisotropic myocardium, local differences in electrical load exist because of resistive discontinuities resulting from the anisotropic distribution of the electrical connections between the cells.7 This may result in unidirectional conduction block and slow conduction.9,10 Another role of anisotropy is that it provides a slow return pathway to the site of block to allow reexcitation of the myocardium proximal to the line of block.9,10 The importance of anisotropy in the genesis of reentry is supported by the observation that ventricular tachycardia (VT) developing in the canine heart 3 to 4 days after myocardial infarction originates from a thin epicardial border zone overlying the infarcted area.11–13 Debate still continues about the role of anisotropy in the occurrence of functional block and the creation of an excitible gap in the reentrant pathway.12,13 Functional reentry in ventricular myocardium has also been described as spiral wave reentry.14 During spiral wave reentry, the cycle length (CL) and width of the excitible gap are determined by the curvature of the turning wave front.14–17 This study was performed to determine the influence of uniform anisotropy on functional reentry. To avoid confounding ischemic alterations, we used a 2D preparation of perfused left ventricular epicardium.5,11,18,19

Methods

Flemish rabbits (n=35, 4.5 to 6.5 kg) of both sexes were used. Animal care complied with the guidelines of the Governmental Veterinary Committee Netherlands. After euthanasia, the thorax was opened and the heart was rapidly removed and placed in cold perfusion fluid (10°C). The aorta was cannulated, and the heart was connected to a Langendorff perfusion system. The coronary arteries were perfused with a perfusion pressure of 50 mm Hg, resulting in a flow of 35 to 45 mL/min.9

Experimental Model

To create a 2D preparation, the endocardial and intramural layers of the left ventricle (LV) were cryoablated by freezing with liquid nitrogen (−192°C). Cryoablation resulted in a surviving epicardial layer 1.0±0.4 mm thick. Epicardial conduction velocity and refractory period were not affected by the freezing procedure.9 An extensive evaluation of this preparation has been given previously.9,19
Mapping System
Unipolar electrograms were recorded with the stainless steel aortic cannula used as indifferent electrode. After amplification and filtering (bandwidth 1 to 400 Hz), the signals were multiplexed (sampling rate 1000 Hz), digitized (8 bits), and stored on tape. Local activation times were detected automatically. In case of fragmented electrograms, the component with the steepest negative slope was taken as the actual local activation time.

The hearts were stimulated with a pulse width of 2 ms by a constant-current stimulator (Medtronic). The output of the stimulator could be connected to any pair of electrodes in the mapping array (regular pacing; stimulus strength, 2 times diastolic threshold; premature stimuli: 4 times diastolic threshold). During VT, reset curves of several recording sites were obtained by premature stimulation at decreasing coupling intervals (V1S). At the pacing site, the V2V3 interval represents the return cycle of the premature stimulus.

Total epicardial mapping was performed with a “spoon-shaped” electrode (384 silver electrodes, diameter 0.3 mm, resolution 2 mm) covering the apex and entire free wall of the LV. For high-resolution mapping of part of the epicardium, a rectangular (13×15-mm) array containing 192 silver electrodes (diameter 0.3 mm, interelectrode

Figure 1. Initiation of VT. The 192-electrode mapping array (13×15 mm) was positioned on LV free wall. Isochrones at 10-ms intervals.
distance 1 mm) was used. A double-row electrode of 2×48 electrodes (diameter 0.05 mm, interelectrode distance 0.185 mm) was used to map the central line of block.

**Action Potential Recordings**

Monophasic action potentials (MAPs) were recorded by use of standard microelectrode techniques (glass capillaries filled with 3 mol/L KCl and a tip resistance of 10 to 30 MΩ). The microelectrode was impaled in the core of the circuit at 0.5-mm intervals with an electrically driven micromanipulator. Because of mechanical movement of the 2D layer, the tip of the microelectrode frequently broke during the first impalement. However, MAPs of lower amplitude could still be recorded, allowing reliable evaluation of the shape and duration of the local action potentials. During MAP recordings, a bipolar electrode sutured to the LV served as time reference.

**Results**

In the intact LV, ventricular fibrillation (VF) or VT could not be initiated by premature stimuli. Incremental rapid pacing never produced VT but led exclusively to VF. In the 2D preparation, VF could no longer be induced. In 30 of 35 hearts, 2 to 3 early premature stimuli or rapid pacing resulted in VT (130±11 ms, Figure 1). Pacing with a CL of 350 ms (S1) at the center of the mapping array produced an ellipsoidal spread of activation with the long axis parallel to the fiber orientation. Premature stimuli (S2–S4) also resulted in an ellipsoidal spread of activation, but during S4, the longitudinal wave front propagating downward was blocked. Distal to the line of block, the myocardium was activated 90 ms after S4 by a wave front turning clockwise around the line of block. Reentry occurred (VT1) when the impulse reexcited the tissue proximal to the line of block. During VT1, short lines of transverse block were present. VT2 started as a small vortex in the lower right corner of the mapping electrode. However, this circuit shifted outside the mapping area. Usually, the first 3 to 4 beats of VT were polymorphic because the reentrant circuit shifted its position. Only after the circulating impulse became anchored at a fixed position, VT became monomorphic and long-lasting (Figure 2).

**Sustained VT**

During VT, single-loop reentry was present in 28 of 30 preparations. In 2 cases, a figure-8 reentry was found. In Figure 3, VT maps of 4 different hearts are shown. The upper panels show 2 examples of counterclockwise reentry. The lower left panel represents a clockwise VT, and the lower right panel shows a figure-8 reentry. The conduction velocity along the circuit varied with the fiber orientation. Longitudinal conduction velocity was 60 cm/s. Transverse conduction velocity at the pivot points was <20 cm/s (Figure 4). In 75%, the line of block (length 20±5 mm, CL 129±20 ms) was oriented parallel to the fiber direction (Figure 5). In 25%, the line of block was L-shaped (lower panel). The length of the L-shaped lines of block (19±16 mm) and the CL (123±4 ms) were not different from the longitudinally oriented lines.

During VT, it was possible to capture the myocardium with single stimuli, elucidating an excitable gap of 30±7 ms. However, although premature stimuli (Figure 6) captured the myocardium, they did not reset VT, indicating that an excitable gap was present only in parts of the circuit. MAP recordings (see below) showed that the excitable gap was absent at the pivot points of the circuit.

Extracellular electrograms were recorded across the line of block (n=10) with the double electrode. Figure 7 shows a clockwise circuit. The electrograms in 7B were recorded from the center of the line of block. At the line of block, double potentials were recorded, reflecting the 2 wave fronts propagating in opposite directions at either side. An isoelectric segment was present between the 2 potentials; the absence of fragmented potentials indicates that slow conduction across the line of block did not occur. Because multiple fragmented potentials are considered to be the hallmark of nonuniform anisotropy, the absence of multiphasic deflections in the extracellular waveforms recorded across the line of block demonstrates the uniformly anisotropic nature of the tissue. Near the pivot point (7A), the interval between the double potentials shortened. At the pivot point (7C), single potentials were recorded together with a low-amplitude electrotonic potential. Proximal to the pivot points, the local deflection preceded the electrotonic potential, whereas distal to the pivot points, local activation occurred after the electrotonic potential.

**Monophasic Action Potentials**

MAPs were recorded in 5 hearts. A detailed map showing reentry around a Z-shaped line of block could be constructed...
in 3 experiments; in the other 2 experiments, only incomplete maps could be constructed.

In Figure 8, clockwise reentry around a Z-shaped line of block (thick line) caused VT. Normal MAPs, without steps in the depolarization phase or electronic humps during the repolarization phase, were recorded along the circuit. A short (30-ms) isoelectric segment was present between the action potentials, indicating complete repolarization and a gap of full excitability. Recordings obtained across the center of the line of block (Figure 9) showed a phase shift of 180° between the opposite limbs. Low-amplitude electrotonic potentials were recorded at the line of block with a total width of 1.5 mm, showing that the central line of block was kept nonexcitable by electrotonic current flowing between the opposed limbs of the circuit. This electrotonic current kept the cells depolarized, although sometimes the nonexcitable core was excited (Figure 10, electrodes 3 and 4). Because of electrotonic prolongation of the action potentials, the cells at the central line of block thus responded in a 2:1 or 3:2 manner to the circulating impulse. At the pivot points, the potentials were clearly prolonged but still responded in a 1:1 manner (Figure 10, electrodes 1, 2, 7, and 8). In contrast to other parts of the circuit, no isoelectric segment was present, and the next depolarization occurred immediately after the cells were repolarized (no phase 4). Approximately 2 mm distal to the turning points (Figure 11, electrodes 6 and 12), the depolarization showed no steps, and the action potentials exhibited a clear excitable gap. The short limbs of the Z-shaped line of block thus were caused by discontinuous propagation due to the interplay between the anisotropic tissue properties and the curvature of the turning wave front.

Discussion

In anisotropic myocardium, the heterogeneous distribution of cell-to-cell connections and directional differences in current load may result in unidirectional block and slow conduction. In the 3D ventricle, the effect of anisotropy is diminished by a gradual change in the angle of the long axis of the myocardial fibers from endocardium to epicardium. This explains why VT cannot be induced in a normal heart. In the 2D preparation, deeper layers were no longer present, and epicardial breakthroughs no longer occurred. Effective conduction perpendicular to the fiber axis now slowed to <20 cm/s. In this 2D preparation, sustained VT could be induced. After the initial 3 to 4 beats, VT anchored at a fixed position. In addition to microanatomic obstacles, which might have served as anchors, the anisotropic characteristics of the myocardium provide an alternative explanation for this anchoring behavior. During sustained VT, the refractory periods...
at the pivot points were prolonged, thereby preventing the impulse from short-circuiting (Figure 11); this resulted in a stable VT and a fixed position of the circuit. Because action potential prolongation at the pivot points was caused by delayed activation of the area distal to the pivot points, this could occur only after the initial beats (the impulse had to turn around a pivot point first). Thus, during these first beats, the action potentials will not be prolonged, which results in “meandering” of the pivot points. After stabilization, the electrotonic interaction between the opposing longitudinal limbs of the circuit created a line of block with a width of 1.5 mm.

**Line of Block**

Activation maps obtained during initiation of VT did not reveal large anatomic obstacles. Only after application of shortly coupled premature stimuli did local conduction block develop. During VT (75%), the impulse circulated around a line of block oriented parallel to the epicardial fiber direction. In 25%, the line of block was oriented in a different direction or related to an epicardial blood vessel.

Microelectrode recordings revealed that the longitudinal block lines were in fact Z-shaped. When the central line of block was oriented parallel to the fiber orientation, the extracellular waveforms recorded across the line of block showed double potentials without the characteristic multiphasic components as found in the central line of block of VT in infarcted tissue. Together with the transverse electrotonic interaction over 1.5 mm, this rules out a longitudinal side-to-side uncoupling of fibers occurring in nonuniform anisotropic preparations. In our preparation, the effective transverse space constant was much higher than the space constant in nonuniform anisotropic tissue. This implies a major difference between the central line of block in uniform anisotropic tissue and the very slow transverse conduction zones identified during VT in infarcted tissue.

Several mechanisms were involved in the creation of the Z-shaped line of block. As in the computer simulations by van Capelle and Durrer and reentry within small pieces of atrial myocardium, the core of the circuit was kept nonexcitable by electrotonic current from the longitudinal limbs of the circuit at either side of the central part of the Z line. The low-amplitude
electrotonic potentials visible in the recordings from these sites prevented activation of the center of the circuit.

Depending on the amount and timing of these currents, some sites within the core of the circuit were activated in a 2:1 or 3:2 manner. At the pivot points, the curvature of the circulating wave front is high. Because a convex wave front must excite more tissue ahead than a planar wave front, the excitatory current is dissipated, and consequently, a curved wave front propagates at reduced speed. Above a certain critical curvature, the current drain becomes so high that conduction block occurs despite full excitability of the tissue ahead. At the pivot points, the impulse made a sharp U turn, first changing its direction from longitudinal to transverse propagation and then back to longitudinal. The current drain during the transition from transverse to longitudinal propagation resulted in delayed activation and created the short limbs of the Z-shaped line of block.

Excitable Gap
Originally, reentry involving a functional block was thought to have only a partially excitable gap because of the tight fit

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**Figure 7.** High-density mapping during VT (CL = 135 ms). Line of block was oriented parallel to fiber direction. A, B, and C, Electrograms recorded across different levels of central line of block. Isochrones at 10-ms intervals. Arrows in A, B, and C point to electrotonic interaction between 2 longitudinal limbs of circuit. LAD indicates left anterior descending coronary artery.

**Figure 8.** VT (CL = 140 ms) around a Z-shaped line of block. MAPs recorded did not show either steps in depolarization phase or electrotonic humps during plateau or repolarization phase. A phase 4 of 30 ms was present between action potentials.
between the crest and the tail of the reentrant wave. Later, it was recognized that during functional reentry, an excitable gap might exist. In this study, an excitable gap of 30 ms was present during functional reentry. Several mechanisms contributed to the creation of this excitable gap. First, microscopic barriers may anchor the functional circuit at a fixed position. Such barriers may arise when adjacent fibers become separated by interposition of collagenous septa. Although during pacing, no indication for fixed conduction barriers were found, it cannot be excluded that small barriers provide stable pivot points for the anisotropic circuit. Also, during functional reentry, local conduction delay at the pivot points may create an excitable gap. As shown, at the pivot points, propagation temporarily stopped, resulting in a step in the depolarization phase of the action potentials. Activation of the longitudinal limbs occurred only after the wave had made a wider turn. The conduction delay of 30 ms at the pivot points resulted in an excitable gap in other parts of the circuit and caused a clear difference in activation at either side of the pivot point. This resulted in a prolongation of the action potentials proximal to the pivot points by current flowing during the repolarization phase from cells distal to the pivot points (which are still depolarized because they were activated later). The electrotonic prolongation of the action potentials proximal to the pivot points closed the excitable gap at the beginning of the U turn and contributed to the stability of the pivot points. The excitable gap during anisotropic reentry thus is caused primarily by local conduction delay at the pivot points due to the curvature of the turning wave front and the low longitudinal resistance (resulting in a high current drain) at the exit of the pivot points. Because the action potentials at the entrance of the pivot points were prolonged, premature activation of the pivot points was not possible. This explains why it was not possible to reset VT with single stimuli. Although it was possible to capture the myocardium, the reentrant wave was forced to turn around the pivot points because no excitable gap was present at these sites.

Functional Reentry

Three types of functional reentry have been distinguished so far. During leading-circle reentry, the core of the circuit is...
formed by centripetal wavelets colliding in the center of the circuit. The reentrant pathway is determined by the smallest circuit in which the wave front can circulate. Because the head of the impulse propagates in just recovered myocardium, only a small partially excitable gap exists, and the CL is determined mainly by the refractory period of the tissue. Only in case of an extreme shortening of the action potential may the circuit become so small that the high curvature of the wave front limits a further shortening of the circuit, thus creating an excitable gap. Similar to leading-circle reentry, during anisotropic reentry, the 2 opposed longitudinal limbs of the circuit cannot reexcite each other, because they are mutually refractory. Instead, the central line of block is continuously depolarized by electrotonic current flowing between the depolarizing waves passing at either side. During anisotropic reentry, a clear excitable gap exists, caused by conduction delay at the pivot points. Because of the existence of an excitable gap, anisotropic reentry is anchored at a fixed position and long-lasting. The stability of anisotropic reentry is enhanced by the electrotonic prolongation of the action potentials at the entrance of the pivot points, thereby acting as functional anchoring points. At the pivot points, no excitable gap is present, and the circulating impulse is unable to short-circuit the reentrant pathway. In nonuniform anisotropic myocardium, the transverse conduction velocity may be $<5$ cm/s, and the conduction delay at the transverse pivot points may be larger than in uniform anisotropic myocardium. Consequently, the line of block shortens, and the impulse rotates around a small functional fulcrum.

Self-sustained rotating spiral waves can occur in any excitable medium. Because of the pronounced curvature of the wave front, at the tip of the spiral, the current load is high and the safety factor for conduction may become $<1$. Therefore, the core of a spiral wave, although fully excitable, is not excited. However, it is questionable whether the core of a spiral wave in cardiac tissue is fully excitable. As in leading-circle and anisotropic reentry, the center of a spiral wave will be depolarized electrotonically by the cells around the core. In fact, recordings obtained from the core of a spiral wave clearly show multiple low-amplitude potentials. This suggests that the core is kept depolarized above its threshold for excitation by the depolarization wave wrapped around it. As in anisotropic reentry, an excitable gap is present, caused by the curvature of the turning wave preventing it from approaching closer to its tail of refractoriness.

**Clinical Implications**
Reentry is the mechanism of most VT after myocardial infarction in patients. Surviving nonuniform anisotropic cell layers within the infarcted myocardium play a key role in the induction and perpetuation of reentry. Although the structure of these surviving cell layers will be more complex, the model of anisotropy presented in this study may be useful to study the mechanism of VT in anisotropic myocardium.

**Limitations of the Study**
Although activation maps during pacing did not reveal anatomic obstacles, histological studies were not performed to confirm the electrophysiological data. Therefore, it could not be excluded that microanatomic lesions served to anchor the pivot points and determined the length of the central line of block during VT.

**Conclusions**
In uniform anisotropic myocardium, reentry around a functional Z-shaped line of block may occur. The central line of
block was kept depolarized by electrotonic currents flowing between the depolarization waves at either side of the line of block. At the pivot points, the pronounced wave-front curvature and the high current drain along the longitudinal axis caused local conduction delay, thus creating an excitable gap. The mechanisms involved are not fully understood and warrant further study, however, and detailed descriptors of anisotropic resistive properties at each size scale are needed.

References

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