Interdependence of Modulated Dispersion and Tissue Structure in the Mechanism of Unidirectional Block

Kenneth R. Laurita, David S. Rosenbaum

Abstract—We previously showed that a premature stimulus can significantly alter vulnerability to arrhythmias by modulating spatial gradients of ventricular repolarization (ie, modulated dispersion). However, it is not clear if such changes in arrhythmia vulnerability can be attributed to the formation of an electrophysiological substrate for unidirectional block and what the potential role is of tissue structure in this process. Therefore, the main objective of the present study was to examine the concomitant effect repolarization gradients and tissue structure have on unidirectional block. Optical action potentials were recorded from 128 ventricular sites (1 cm²) in 8 Langendorff-perfused guinea pig hearts. Propagation was confined to the epicardial surface using an endocardial cryoablation procedure, and a 12-mm barrier with a 1.5-mm isthmus was etched with a laser onto the epicardium. A premature stimulus (S2) was delivered over a range of S1S2 coupling intervals to modulate repolarization gradients in a predictable fashion. When a second premature stimulus (S3) was delivered from the center of the isthmus, the occurrence and orientation of unidirectional block were highly dependent on repolarization gradients created by the S2 beat. In this model, a local repolarization gradient of 3.2 ms/mm was required for unidirectional block at this isthmus. In addition, the formation of unidirectional block was critically dependent on the presence of the source-sink mismatch imposed by the isthmus. These results may explain how the interplay between spatial heterogeneities of repolarization and tissue structure form a substrate for unidirectional block and reentry. (Circ Res. 2000;87:922-928.)

Key Words: optical mapping ■ source-sink mismatch ■ repolarization ■ reentry ■ premature stimulation

Received August 21, 2000; revision received September 7, 2000; accepted September 7, 2000.
From the Heart and Vascular Research Center, MetroHealth Campus, Case Western Reserve University, Cleveland, Ohio.
Correspondence to Kenneth R. Laurita, PhD, MetroHealth Campus, Case Western Reserve University, 2500 MetroHealth Dr, Rammelkamp, 6th floor, Cleveland, Ohio 44109-1998. E-mail klaurita@metrohealth.org © 2000 American Heart Association, Inc.
Circulation Research is available at http://www.circresaha.org

922
potential mapping with voltage-sensitive dye was used to measure spatial gradients of cellular repolarization and the propagation of an extrastimulus in the wake of such gradients. The mechanism of unidirectional block could not be explained by repolarization gradients or source-sink mismatch alone but was critically dependent on both influences.

Materials and Methods

Experimental Preparation
All experiments were carried out in accordance with Public Health Service guidelines for the care and use of laboratory animals. Guinea pigs (n=8) were anesthetized (30 mg/kg pentobarbital, IP), and their hearts were rapidly excised and perfused as Langendorff preparations (perfusion pressure 70 mm Hg) with oxygenated (95% O\textsubscript{2}, 5% CO\textsubscript{2}) Tyrode’s solution containing (mmol/L) NaCl130, NaHCO\textsubscript{3}25, MgSO\textsubscript{4}1.2, KCl1.25, dextrose 5, and CaCl\textsubscript{2}1.25 (pH 7.4, 31°C to 33°C). The right and left atria were excised to avoid competitive simulation from the SA node and to provide access to the ventricles for the cryoablation procedure (see below). Hearts were stained with voltage-sensitive dye di-4-ANEPPS (10 μmol/L) by direct coronary perfusion for 10 minutes.

Beating and perfused hearts were immersed in a Tyrode-filled custom-built Lexan chamber. Gentle pressure was applied with a movable piston to the posterior surface of the heart during action potential recordings, allowing the heart to contract freely except for within the mapping field. Because gentle pressure may cause transient ischemia, recordings were brief and action potentials were continually monitored for signs of ischemia (eg, triangulated action potentials). If ischemia was evident, the experiment was not included in the analysis. Cardiac rhythm was monitored using 3 silver disk electrodes fixed to the chamber in positions corresponding to ECG limb leads I, II, and III. The ECG signals were filtered (0.3 to 300 Hz), amplified (1000×), and displayed on a digital recorder (WIN-DOGRAF, Gould Inc). The optical mapping system used in this study has been described in detail elsewhere.\textsuperscript{13,22} In the present study, an optical magnification of 1.8× was used, corresponding to a mapping field of 1×1 cm and 0.08 cm spatial resolution between recording pixels.

Experimental Protocol
To confine propagation to the epicardial surface and avoid the confounding influence of subepicardial breakthrough from the His-Purkinje system, the endocardial muscle layers were eliminated using a cryoablation procedure described previously.\textsuperscript{22} To create a source-sink mismatch, a linear barrier containing a 1.5-mm isthmus was etched precisely (6 m) onto the epicardial surface (n=5) using a 5W argon ion laser guided by computer controlled micropositioners (Figure 1).\textsuperscript{22} The width of the isthmus was based on findings using a 5W argon ion laser guided by computer controlled micropositioners. The barrier was perpendicular to the orientation of repolarization typically found in guinea pig.\textsuperscript{1} In 3 additional control hearts, no barrier was created.

Baseline pacing (S1S1=600 ms) and a single premature stimulus (S2) were delivered at 2× diastolic threshold using a Teflon-coated silver bipolar electrode (DTU 101, Bloom Associates LTD) from the same site near the base of the left ventricle corresponding to the basal end of the laser barrier, when present (Figure 1). The location of S1S2 stimulation was carefully chosen with respect to the barrier to reduce the possibility of unequal conduction delays on either side of the isthmus. Four different S1S2 coupling intervals were tested in each experiment, decrementing from a coupling interval equal to the baseline pacing cycle length down to a short coupling interval just above the refractory period of the S1 beat. For each S1S2 coupling interval tested, a second premature stimulus (S3) was delivered at 2× diastolic threshold from the center of the isthmus (or from the center of the mapping field in the absence of a barrier) using a Teflon-coated silver unipolar electrode (0.1 mm diameter) connected to a second stimulator (DCI-1114, Digital Cardiovascular Instruments Inc). The S3 stimulus was always delivered just above the effective refractory period (<2 ms) of the S2 beat.

Data Analysis
In all experiments and for each S1S2 coupling interval tested, automated algorithms were used to determine depolarization time and repolarization time relative to a single fiducial point (ie, the stimulus).\textsuperscript{2} Repolarization time was defined as the maximum positive curvature (maximum positive second derivative) during repolarization\textsuperscript{23} and corresponds to ∼95% repolarization\textsuperscript{24} (ie, APD\textsubscript{95}). To quantify the local gradient of repolarization surrounding the isthmus, the average repolarization times from 2 regions (3×3 pixels), each immediately adjacent to either side of the isthmus without overlapping the barrier, were subtracted and divided by the center-to-center distance between each region (∼2 to 3 mm). Shown in Figure 1 are the 2 regions chosen (white squares) from a representative experiment. The direction of the local repolarization gradient was defined as either positive (left ventricle apex to right ventricle base repolarization sequence) or negative (right ventricle base to left ventricle apex repolarization sequence). Local repolarization gradients were determined for all coupling intervals tested. Successful propagation was defined as cell-to-cell impulse propagation occurring across at least 3 recording sites.

Results
Modulated Dispersion After a Premature Stimulus
Shown in Figure 2 is a representative example of depolarization and repolarization during premature stimulation in the
Modulated Dispersion and Unidirectional Block

To determine if coupling interval–dependent changes in repolarization determine the occurrence of unidirectional block, we examined the characteristics of propagation of an S3 stimulus delivered from the center of an isthmus in the wake of repolarization gradients established by an S2 beat. Shown in Figure 3 are data from a representative experiment where S1S2 coupling interval was shortened from 600 ms to 225 ms. The contour maps across the top show the pattern of repolarization surrounding the isthmus after each S2 beat. Shown below each repolarization map is a contour map demonstrating depolarization of an S3 beat in the wake of repolarization gradients established by each S2 beat. For an S1S2 coupling interval equal to the baseline cycle length (Figure 3A), a gradient of repolarization is present that delays repolarization on the left side of the isthmus. An S3 impulse delivered in the wake of this repolarization pattern failed to propagate to the left side of the isthmus in the direction of the repolarization gradient. However, propagation (velocity = 0.44 ± 0.11 m/sec) was successful to the right (ie, unidirectional block) and continued around both ends of the barrier in a pattern like figure-of-eight reentry. This is also reflected in the action potentials shown at the bottom of Figure 3 that were recorded from equally spaced sites perpendicular to the barrier. Propagation failed in the direction of site a but was successful in the direction of site b, continuing around, much later, to site a. Thus, during baseline pacing, the electrophysiological requirements for unidirectional block were present.

An S3 stimulus was again introduced from the same location in the same heart, but in this case after a shorter (ie, during baseline pacing became earliest during the short premature coupling interval and vice versa. Shown in the Table are summary data of the local repolarization gradient at baseline pacing and for premature stimuli delivered at an intermediate and short coupling interval for every experiment with a barrier. In hearts without a barrier (ie, control), repolarization gradients were modulated in a similar fashion, as previously reported.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Coupling Interval, ms</th>
<th>Local Repolarization Gradient, ms/mm</th>
<th>Unidirectional Block</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BASE</td>
<td>INTER</td>
<td>SHORT</td>
</tr>
<tr>
<td>1</td>
<td>600</td>
<td>270</td>
<td>160</td>
</tr>
<tr>
<td>2</td>
<td>600</td>
<td>300</td>
<td>225</td>
</tr>
<tr>
<td>3</td>
<td>600</td>
<td>270</td>
<td>230</td>
</tr>
<tr>
<td>4</td>
<td>600</td>
<td>290</td>
<td>220</td>
</tr>
<tr>
<td>5</td>
<td>600</td>
<td>230</td>
<td>200</td>
</tr>
</tbody>
</table>

Mean ± SD

8.7 ± 3.2, 1.8 ± 1.3, 6.7 ± 3.7
intermediate) S1S2 coupling interval (Figure 3B). It is evident that after this premature stimulus, gradients of repolarization were greatly attenuated, and repolarization was nearly simultaneous on both sides of the isthmus. Under these circumstances, propagation after the S3 stimulus never blocked unidirectionally, but either propagated successfully (velocity=0.39±0.12 m/sec) in both directions (Figure 3B) or failed to capture the tissue at all (not shown). Thus, at an intermediate coupling interval, the electrophysiological requirements for unidirectional block were eliminated because of eradication of repolarization gradients. Finally, at a short S1S2 coupling interval (Figure 3C), the repolarization gradient is restored to a similar magnitude as that seen during baseline pacing; however, the orientation of the gradient is reversed. In this case, the S3 impulse failed to propagate to the right side of the barrier but successfully propagated (0.45±0.10 m/sec) to the left and continued around both ends of the barrier, meeting on the other side of the isthmus. Thus, at this coupling interval, unidirectional block occurred on the opposite side of the isthmus, following the direction of the repolarization gradient. Shown in the Table are the occurrence and direction of block as a function of S1S2 coupling interval and local repolarization gradient. In the absence of a barrier, unidirectional block was observed only once for all coupling intervals tested (n=12, not shown).

Reentrant beats after the S3 stimulus were observed in 3 of 5 experiments performed with a barrier and in no experiment when an isthmus was absent (ie, control). Shown in Figure 4 are examples of ECGs and action potentials recorded during premature stimulation, the formation of unidirectional block, and during multiple reentrant beats in 3 separate experiments. The activation sequence, determined from all 128 action potential recordings made during each episodes (not shown), indicates that unidirectional block of the S3 impulse initiated reentrant excitation.

**Requirements for Unidirectional Block**

When pacing from the center of the isthmus, the likelihood of unidirectional block was highly dependent on the magnitude and direction of the local repolarization gradient surrounding the isthmus. Shown in Figure 5 are summary data for all
experiments indicating the local repolarization gradient (ms/mm) that was associated with the formation (right) or lack of formation (left) of unidirectional block on either side of the isthmus. Local repolarization gradients for all S1S2 coupling intervals tested are shown. When pacing from the center of the isthmus, unidirectional block was induced 14 times out of 20 coupling intervals tested. In each of the 14 cases of unidirectional block, a local repolarization gradient $>3.2$ ms/mm was present. However, block did not occur when the repolarization gradient was $<3.7$ ms/mm ($n=5$). Thus, in this model there seems to be a distinct repolarization gradient threshold that is required for unidirectional block. In contrast, in the absence of a barrier over the same range of S1S2 coupling intervals tested (ie, control hearts), unidirectional block of the S3 beat was observed only once for 12 coupling intervals tested despite the presence of repolarization gradients ($3.8\pm1.3$ m/sec) $>3.2$ ms/mm. Therefore, the source-sink mismatch created by the isthmus seems to play a significantly important role in the formation of unidirectional block ($\chi^2,P<0.005$).

To test the relative importance of tissue structure and repolarization gradients on the occurrence of unidirectional block, in 2 experiments the S3 stimulus electrode was moved from the center of the isthmus to one side, at the basal entrance to the isthmus. In this configuration, the repolarization gradient is unchanged; however, the source-sink mismatch is no longer equal on both sides of the isthmus. On the basal side of the isthmus, the source-sink mismatch is absent, and on the apical side, it is present as the impulse propagates through the isthmus. Shown in Figure 6 is a representative example where a sufficiently large repolarization gradient (panel A) resulted in unidirectional block of the S3 beat when pacing from the center of the isthmus (panel B). The contour map demonstrates successful propagation to the apical side of the barrier, where repolarization was earliest (arrows), and propagation failure to the basal side of the barrier, where repolarization was latest. When the S3 stimulus was moved from the center of the isthmus to the basal entrance of the isthmus (panel C), propagation was successful toward the base of the heart, against the repolarization gradient (arrows). However, propagation failed toward the apex of the heart, in the direction of the source-sink mismatch. Therefore, in this configuration unidirectional block formed as a result of the source-sink mismatch imposed by the isthmus, not the local gradient of repolarization. These results additionally demonstrate that repolarization gradients, even in the presence of a barrier, are not sufficient to create block unless a source-sink mismatch is present.

**Discussion**

In this study, we report a potentially important mechanism for the initiation of reentrant excitation on the basis of the direct effect a premature stimulus has on modulating the electrophysiological substrate for reentry. We found that the electrophysiological requirements for unidirectional block were modulated in a coupling interval–dependent manner by pacing-induced modulation of repolarization gradients. Such changes in the formation of unidirectional block may underlie the mechanism of arrhythmia vulnerability associated with modulated dispersion, as we have shown previously.$^9$ In addition, our results suggest that the source-sink mismatch imposed by tissue structure also plays a critically important role in the occurrence of unidirectional block.

**Modulated Dispersion and Arrhythmia Vulnerability**

We previously showed that a premature stimulus can systematically modulate spatial gradients of action potential duration and repolarization in a coupling interval–dependent manner, which was explained on the basis of heterogeneities of repolarization kinetics between ventricular cells.$^1$ In the present study we have shown a similar response in the presence of a barrier, where repolarization gradients were modulated in a systematic fashion perpendicular to the barrier (Figure 2). Even though the modulated dispersion response,
in general, was not altered in the presence of a barrier, we did observe repolarization gradients that were greater in magnitude compared with control. This might be attributable to an insulating effect of the barrier that reveals intrinsic regional ionic properties. Nevertheless, in control hearts the local repolarization gradient (3.8±1.3 ms/mm) generally failed to produce unidirectional block, whereas comparable gradients (6.4±2.4 ms/mm) did cause unidirectional block in the presence of an isthmus. The results of the pullback experiment (Figure 6) provide additional evidence that the source-sink mismatch created by the isthmus is critically important, even if larger repolarization gradients are present because of the barrier. In this experiment, the insulating effect of the barrier is present, but the source-sink mismatch is absent on one side of the isthmus. In this case, despite the larger repolarization gradient in the presence of the barrier, block only occurred in the direction of the source-sink mismatch (Figure 6C). Therefore, even though the barrier creates slightly larger gradients of repolarization, it is the source-sink mismatch that seems to play a more important role. The fact that large repolarization gradients could not be achieved in the absence of a barrier is expected, because hearts are not normally prone to arrhythmias. However, it is possible to attain very large repolarization gradients and unidirectional block without a barrier, such as those seen during discordant repolarization alternans.25

We found that stimulus-induced changes in repolarization gradients directly influenced the electrophysiological requirements for unidirectional block. As S1S2 coupling interval was shortened to an intermediate value, dispersion of repolarization decreased such that unidirectional block of a second premature beat was much less likely to occur. Additional shortening of S1S2 coupling interval to a value just longer than the effective refractory period markedly increased repolarization gradients, which, in-turn, restored the conditions necessary for the development of unidirectional block. We previously found that vulnerability to ventricular fibrillation is also influenced in a coupling interval–dependent fashion,9 where vulnerability decreased at intermediate coupling intervals when repolarization gradients were minimal. It is possible that such changes in vulnerability to fibrillation were a direct result of changes in the requirements for unidirectional block. When the site of S3 stimulation was moved from the center of the isthmus to the entrance of the isthmus, an unequal source-sink mismatch was created (Figure 6). In this situation, we found that unidirectional block occurred in the direction of the source-sink mismatch, opposite to the direction of the repolarization gradient. Therefore, moving the S3 stimulus location must have increased the source-sink mismatch such that a repolarization gradient was not required to obtain block. It is also possible that the lack of a source-sink mismatch on the side of the pacing site made propagation safer, thereby increasing the repolarization gradient required for block. Indeed, this may be the case, because in the absence of a source-sink mismatch (ie, in hearts without an isthmus), block was rarely observed despite the presence of significant repolarization gradients. In either case, these data suggest that a source-sink mismatch can be an overriding determinant of unidirectional block. These findings highlight the important interplay between tissue structure and repolarization heterogeneities that, in conjunction, define the electrophysiological substrate for reentrant arrhythmias. On the basis of these and other15 results, one would predict that in situations where very large repolarization gradients are present, such as in long-QT syndrome,26 only minimal or no structural elements are required to form a substrate for block, whereas in the presence of marked structures discontinuities in tissue, the development of even small gradients of repolarization can contribute significantly to the substrate for reentry. Other studies have shown that both repolarization heterogeneities and tissue structure can play an important role in the initiation of reentrant excitation.17,27,28 In particular, Spach et al27 showed in atrial tissue how repolarization heterogeneities interact with anisotropic conduction and discontinuities of axial resistance at muscle bundle junctions to produce delayed conduction and unidirectional block, respectively.

**Implications**

The geometrical characteristics of this experimental model may be analogous to several clinical situations where structural discontinuities in myocardial tissue exist, such as those imposed by a healing myocardial infarct,29 surgical suture lines,30 accessory pathways,31 and the complex structure of atrial endocardial tissue.10,18,32 In particular, the isthmus lo-
cated between the tricuspid annulus and the eustachian ridge, anterior to the inferior vena cava, has been shown to play a critically important role in the initiation and maintenance of atrial flutter. The occurrence of slow conduction and block at the isthmus may be explained by the interplay between tissue structure and heterogeneities of refractoriness. Such source-sink mismatches imposed by propagation through an isthmus may not necessarily result from distinct anatomical structures but may also form during reentry. For example, during figure-of-eight reentry, a central common pathway is formed where propagation through an isthmus is associated with spontaneous termination. Our results are not necessarily limited to source-sink mismatches imposed by an isthmus but can be extrapolated to other forms of source-sink mismatch, such as that associated with wave front curvature. Additional studies are required to determine how such source-sink mismatches interact with heterogeneities of repolarization and influence the initiation and maintenance of reentrant excitation.

Acknowledgments

This work was supported by the Medical Research Service of the Department of Veterans Affairs, National Institutes of Health (grant HL54807), Whitaker Foundation, and American Heart Association.

References

Interdependence of Modulated Dispersion and Tissue Structure in the Mechanism of Unidirectional Block
Kenneth R. Laurita and David S. Rosenbaum

Circ Res. 2000;87:922-928
doi: 10.1161/01.RES.87.10.922

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/87/10/922

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/