Effects of Gap Geometry on Conduction Through Discontinuous Radiofrequency Lesions

Francisco J. Pérez, MD; Mark A. Wood, MD; Christine M. Schubert, PhD

Background—Gaps of sufficient cross-sectional dimensions within linear radiofrequency (RF) lesions may allow conduction through the lesion. The purpose of this study was to examine the effects of different gap geometries on conduction through discontinuous RF lesions.

Methods and Results—Radiofrequency lesions were created in isolated, perfused rabbit right ventricular (RV) free wall preparations to produce gaps with 3 different lesion geometries: straight, bifurcated, and angled (n=10 each group). Angled preparations contained 2 right angles within the conduction path. Optical mapping was used to assess bidirectional conduction through the myocardium before and after gap formation during pacing at 1000-, 400-, and 200-ms cycle lengths. Histological analysis was performed on each preparation after optical mapping. After lesion formation, 9 of 10 straight gap preparations and 1 of 10 angled gap preparations demonstrated bidirectional conduction (P<0.001) at all cycle lengths. Nine of 10 bifurcated gap preparations demonstrated bidirectional conduction and 1 demonstrated unidirectional conduction at all cycle lengths. Two bifurcated gap preparations showed rate-dependent unidirectional 2:1 conduction. All unidirectional and rate-dependent block occurred during impulse propagation in the direction of diverging arms of the bifurcation. The occurrence of bidirectional conduction in the gaps was associated with the gap geometry (P<0.0001). Histological analysis confirmed the continuity of viable myocardium transmurally throughout the length of the gap in each preparation. The sites of conduction block were demonstrated to be just after the first angle in the conduction path for angled gaps and at the branch point of a bifurcated gap. The predominant myofiber orientation was changed relative to the conduction path at angulations of the gaps. Flecainide (0.1 μmol/L) produced bidirectional conduction block in straight and bifurcated gap preparations with bidirectional conduction at baseline.

Conclusions—Conduction through discontinuities in RF lesions is associated with gap geometry. Complex gap geometry may allow for unidirectional and/or rate-dependent block. Gaps within RF lesions are susceptible to pharmacological blockade. (Circulation. 2006;113:1723-1729.)

Key Words: ablation • conduction • lesion • radiofrequency

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In the treatment of atrial flutters and to the circumferential approach to pulmonary vein isolation for atrial fibrillation, linear radiofrequency (RF) lesions are necessary to the treatment of atrial flutters and to the circumferential approach to pulmonary vein isolation for atrial fibrillation.1–5 In practice, the creation of long continuous RF lesions is difficult to achieve, especially within the left atrium.5–7 The electrophysiological effects of discontinuous RF lesions appear to be complex. Discontinuities within linear ablation lesions are believed to be a common cause of procedural failure and may provide the substrate for new reentrant arrhythmias.6,8 In animal studies, however, the presence of anatomic gaps within linear ablation lesions does not preclude therapeutic effects.7 This suggests that anatomic gaps may not always conduct electrically. Previous studies have shown that the cross-sectional dimensions of linear gaps in ablation lesions is a determinant of conduction through the discontinuity.9–14 The effects of more complex gap geometries on conduction have not been described. Features such as tissue curvature and expansion of the conduction path may alter propagation through anisotropic conduction or impedance mismatch effects.15–18 The purpose of the present study was to examine the effects of gap geometry on conduction through discontinuities in RF lesions.

Methods

Tissue Preparation

The studies were performed with the use of a rabbit right ventricular (RV) free wall preparation, as previously described.12 New Zealand White rabbits (Robinson Services, Inc, Clemmons, NC) were anesthetized with xylazine (7 mg/kg) and ketamine (100 mg/kg), according to American Veterinary Association Panel on Euthanasia guidelines. The hearts were rapidly removed, and the aortic root was...
perfused under continuous pressure (80 cm H₂O) with oxygenated Krebs-Henseleit buffer (37°C). The atroventricular junction was crushed to create complete heart block. The RV free wall was cut free from the septum, leaving the right coronary artery intact. The RV flap was pinned (epicardial surface exposed) to the floor of a shallow tissue bath flooded with perfusate solution.

Optical Mapping
Optical mapping was chosen over contact electrode arrays to assess myocardial conduction for the following reasons: (1) Optical mapping rapidly confirmed conduction as occurring exclusively through the gaps, (2) the precise location of the entrance and exit points of the gap could not be predicted before lesion creation. After lesion creation, optical mapping allowed retrospective measurement of conduction between the same tissue points in the baseline state, (3) optical mapping is rapid and minimized time-dependent deterioration of the tissue, and (4) optical mapping might visualize the exact site of conduction block or slowing within the gap. The optical mapping techniques used have been described previously.14–16,19 A bolus of 150 μg of di-4-ANEPPS (aminonaphthylethenepyrindinium) was injected through the perfusion cannula into the coronary arteries and allowed to recirculate in the perfusate. The tissue was illuminated with a tungsten halogen lamp (6618, Oriel, Stratford, Conn) collimated and made quasimonochromatic by the use of a filter (520±30 nm, Omega Optical, Brattleboro, Vt). An objective lens (25 mm, 1.085, Fujinon, Saitama, Japan) collected the emitted light, which was then transmitted through an emission filter (645 nm, R60, Nikon, Melville, NY) and projected onto a CCD video camera (CA-D1–0256T-STDL, DALSA, Waterloo, Ontario, Canada). Video camera images with an acquisition rate of 257 frames per second (257 fps) were cropped to highlight the lesion gaps, and angled preparations are shown (top) with examples of actual preparations beneath. Dimensions of the optical mapping field are 40x40 mm. Still images were cropped to highlight lesion gaps.

Radiofrequency Lesion Formation
Radiofrequency lesions were created by using a 4-mm-tip, 8F ablation catheter (Medtronic Conductor, Minneapolis, Minn) and generator (Medtronic Atakr). RF energy (50°C, 30 seconds, 4 to 10 W) was delivered in a unipolar, temperature-controlled mode with the catheter tip perpendicular to the epicardial surface of the RV flap. Gaps within the RF lesions were created through the use of a flexible plastic cannula (0.95-mm internal diameter) in contact with the tissue to provide localized conductive tissue cooling during RF delivery. This technique has been previously demonstrated to produce electrically conductive gaps in RF lesions.20 The cannula was perfused with room temperature saline at 12 mL/min and shaped to produce the desired gap geometry. Three morphologies of gaps through the RF lesions were produced: straight, bifurcated, and angled (Figure 1). The bifurcated lesion comprised a “Y,” with the stem toward the base of the heart and the 2 arms toward the apex. The angled gap produced a zigzag path through the lesions with 2 right angle turns. Two, 3, and 4 RF lesions were delivered to create the straight, bifurcated, and angled gaps, respectively (Figure 1).

Protocol and Data Analysis
The heart was placed in the bath and perfused with optical dye. Pacing was performed from 2 pairs of bipolar electrodes at the base and apex of the RV flap. Baseline video (2-second sample) of myocardial activation was taken during pacing at 1000-, 400-, and 200-ms cycle lengths from both the base and apex. The RF lesions were created and incisions made from the lateral borders of the tissue to edges of the RF lesions to prevent conduction around the lesions. Video was again taken during pacing from both sites at the 3 cycle lengths. From the stored video, conduction times through the gap until exit from the opposite end. The spacial coordinates for these points were used to determine conduction through the same area of myocardium before the lesion delivers from the baseline video. Ten preparations were studied for each of the gap geometries.

After all recordings were made, the heart was perfused with nitroblue tetrazolium (0.5 mg/mL) to delineate viable from nonviable tissue.9,21 Still digital images of the epicardial and endocardial surfaces were taken. The path length of the gaps was measured electronically from the still images (ImageJ software, NIH, Bethesda, Md). The tissue was then sectioned perpendicular to the axis of the gap at 3 (straight gaps) or 5 points (bifurcated or angled gaps). For all hearts, sections were made at the entrance and exit from the gaps. For straight gaps, an additional section was taken in the midpoint of the gap. For the bifurcated lesions, sections were also taken just above the bifurcation and at the midpoint of each arm of the “Y.” For the angled gaps, sections were also taken at each right angle and at the midpoint of the horizontal section connecting the 2 angles. Still images of the cross sections of the gap were made for electronic measurement of the cross-sectional area by planimetry and dimension of preserved myocardium in the transmural (height) and the transverse (width) directions (Figure 2). The average values for these parameters were calculated as the mean of all measures for a given preparation. The minimal value is the smallest value for a parameter measured from any of the cross sections. The minimal cross-sectional dimension is the smallest height or width measure from any of the cross sections. After imaging, all pieces of the tissue were sectioned along the long axis of the gap to confirm the continuity of viable myocardium throughout the gap.

Pharmacological Challenge to Conduction
In addition to the 30 hearts described above, flecainide (final concentration, 0.1 μmol/L) was added to the perfusate in 6 hearts (2...
bifurcated gaps, 2 straight gaps, and 2 control hearts with no RF lesions) to assess for the development of pharmacological block within the gaps.20,21

Microscopic Histology
In addition to all the experiments described above, 8 RV preparations underwent microscopic histological analysis to determine the transmural myofiber orientation in relation to the geometry of the gaps. Two hearts were without any RF lesions, 2 had straight gaps, 2 had bifurcated gaps, and 2 had angled gaps. All specimens were preserved in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 6 µm, and stained with phosphotungsic acid. All preparations were sectioned both parallel and perpendicular to the conduction path of the gaps.

Statistics
Each of the 3 gap geometry preparations were examined in 10 preparations, for a total of 30 experiments combined. For each preparation, measurements of gap length and cross-sectional dimensions were used to examine the differences in properties among the straight, bifurcated, and angled gap geometries. Five gap properties were determined for each experiment: length, average width, average cross-sectional area, minimum cross-sectional area, and minimal cross-sectional dimension (see Protocol and Data Analysis). Multiple representations of cross-sectional measures were included to consider the determinants of conduction through gaps documented in previous studies and the potential cumulative effects of gap length combined with indicators of overall (average) cross-sectional measures on conduction. Bidirectional conduction through the gap was treated as a dichotomous variable.

All continuous data are presented as mean±SD. Significant differences among the 5 gap physical dimensional properties were determined by ANOVA, using the Tukey procedure to adjust for multiple comparisons. The Fisher exact test was conducted on a 2×3 table to determine whether there was an association between gap geometry type and bidirectional conduction. Further examination of this association was conducted with the use of logistic regression. First, using only gap geometry in the model, significant differences in conduction ability between each pair of geometries, straight, bifurcated, and angled, were examined. Then, average cross-sectional area and length for each geometry were added separately to the logistic model as covariates to determine if the association between bidirectional conduction and gap geometry remained independent of cross-sectional area or length for each preparation. All analyses were conducted with the use of SAS v9.1.3 (SAS Institute, Cary, NC) and assumed a value of \( P<0.05 \) as significant. Multiple comparison procedures were conducted at an experiment-wise \( \alpha=0.05 \).

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results
Conduction Through Gaps
Bidirectional conduction through the RF lesions was present at all 3 cycle lengths in 9 of 10 straight gap preparations, 1 of 10 of angled gaps, and 9 of 10 bifurcated gaps (Table 1 and Figure 3). One of the 10 bifurcated gap preparations demonstrated unidirectional block at all cycle lengths with conduction intact from the apex to the base but block in the direction from base to apex. This corresponded to block in the direction of divergence of the gap arms but conduction in the direction of convergence of the arms. In 3 of the 9 bifurcated preparations with base-to-apex conduction, conduction occurred in only 1 arm distal to the bifurcation. Histological sectioning showed continuity of viable myocardium throughout the gaps in all preparations. The areas of viable myocardium were transmural in all preparations.

Regional properties of the 3 gap geometries were similar, with the exception of gap length (Table 1). The straight geometry had a significantly shorter mean length, bifurcated

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<th>TABLE 1. Properties of Gaps</th>
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*P<0.05 vs straight gap; †P<0.05 vs bifurcated gap.

Figure 3. Optical mapping images of conduction through straight and bifurcated gap preparations. Positions of the RF lesions are denoted by broken circular lines. Straight lines indicate the positions of incisions from the tissue edges to the RF lesions. Orientation of the preparations with respect to base and apex is labeled. In the top panels, the orange wavefronts of depolarization have propagated from the pacing sites (asterisks) to the entrances to the gaps. In the lower panels, the wavefronts have emerged from the straight and bifurcated gaps after 4 and 8 ms, respectively. Note the exit of 2 wavefronts from the 2 arms of the bifurcated gap.
geometry the next longest, and angled geometry the longest mean gap length ($P<0.001$). Despite these differences in gap length, there were no significant differences in average cross-sectional width or area or minimal cross-sectional area or dimension among the 3 gap geometries (all $P>0.067$).

The Fisher exact test of bidirectional conduction with gap geometry type confirmed a significant association between gap geometry and conduction ($P<0.0001$). Further, logistic regression using only these 3 gap types demonstrated a significant difference in the presence of bidirectional conduction between the straight and angled and the bifurcated and angled geometries (both $P=0.0032$) but not the straight and bifurcated geometries ($P=1.0$). In both cases, straight or bifurcated geometries were more likely to conduct than angled geometry.

Logistic regressions were repeated, adjusting separately for gap length, average cross-sectional width, minimal cross-sectional area, minimal cross-sectional area, and minimal cross-sectional dimension. The bifurcated geometry was significantly more likely to conduct than the angled geometry independent of gap length, average cross-sectional width, average cross-sectional area, or minimal cross-sectional dimension (all $P<0.04$). There were no significant differences in the occurrence of bidirectional conduction between bifurcated and straight geometries or between straight and angled geometries once average cross-sectional width, minimal cross-sectional area, or gap length were included in the model. The straight geometry remained significantly more likely to conduct than the angled geometry, independent of average cross-sectional area or minimal cross-sectional dimension (both $P<0.011$). There were no significant differences in conduction between any gap geometry independent of minimal cross-sectional area. These findings are interpreted to indicate that conductance through the gap is associated with gap geometry and that gap cross-sectional dimensions can modify this association.

For all straight and angled preparations, conduction was concordant (either present or absent) at all 3 cycle lengths. Two of the 10 bifurcated preparations demonstrated unidirectional 2:1 conduction at cycle lengths of 200 ms with pacing from the base to apex (conduction in the direction of divergence of the arms). In both of these preparations, conduction was intact through both arms of the bifurcation. In both of these preparations, 1:1 conduction occurred at 200-ms cycle length when pacing from apex to base (conduction in direction of convergence of the 2 arms). At 400- and 1000-ms cycle lengths, conduction was concordant in both directions for the remaining bifurcated gap preparations.

The conduction times through the preparations from base to apex before and after gap formation are shown in Table 2. In each case, conduction times were shorter after gap formation (all $P<0.001$). The conduction times from apex to base were virtually identical to those in the base to apex direction in every case (data not shown). These conduction times are near the temporal resolution of the recording system, and these findings should be interpreted with caution.

The point of block in the gaps could not be directly visualized by optical mapping despite efforts to increase resolution, including repeated dye applications, demonstration of heat stability of the dye, shielding highly reflective areas, and increased light intensity. In 1 of the 3 bifurcated preparations with block in 1 arm, the wavefront was seen emerging from 1 arm and conducting retrogradely into the other arm back to the point of bifurcation. This was interpreted as bidirectional block occurring at the branch point of 1 arm. The impulse did not propagate between the 2 arms of the other 2 preparations. To assess the point of block in the angled gaps, 4 additional angled gap preparations underwent mapping with a bipolar electrode probe (1-mm spacing). In 3 preparations, electrograms were recorded <1 mm past the first angle encountered in the conduction path during pacing in both the base to apex and apex to base directions. Block therefore occurred on entry into the horizontal section of the gap connecting the 2 angulations. In the remaining preparation, block occurred within the first angulation encountered in both directions.

### Response to Flecainide

In 2 preparations with no RF lesions, flecainide (0.1 μmol/L) did not block conduction across the RV flap in either direction. In 2 bifurcated gap and 2 straight gap preparations, each with bidirectional conduction before flecainide, flecainide produced bidirectional block through gaps in every case.

### Myofiber Orientation

The histological analysis demonstrated a consistent pattern of myocyte orientation in the rabbit RV free wall. The RV wall thickness was 2 to 3 mm in all specimens. Narrow subepicardial ($\approx$0.1 mm thick) and subendocardial ($\approx$0.5 mm thick) layers had myocytes predominantly oriented longitudinally from the base to the apex (Figure 4). The midmyocardial layer of the RV free wall has myocytes predominantly oriented transversely to the base-apex axis. The basic fiber orientation for the subepicardial, midmyocardial, and subendocardial layers in relation to the 3 gap geometries is shown in Figure 5. For the straight gaps, the fiber orientation was parallel to the conduction path in the subepicardial and subendocardial layers but generally transverse in the midmyocardial layer (Figure 5). The bifurcated preparations demonstrated subepicardial and subendocardial myofiber ori-
Presentations parallel to the stem of the Y-shaped gap but a change to an oblique subendocardial and subepicardial myofiber orientation in each of the arms (Figure 5). In the midmyocardial layer, the myofiber orientation in the arms of the Y lesion tended to be oblique in all portions of the gap. In the angled preparations, the subendocardial and subepicardial myofiber orientation was parallel to the conduction path in the sections leading up to the angulations (Figures 4 and 5).

The myofiber orientation in the subepicardial and subendocardial layers was predominantly transverse to the conduction path in the horizontal section of the gap connecting the angulations (Figures 4 and 5). In the midmyocardial layer, the fiber orientation was predominantly oblique to the conduction axis in the entrance and exit sections of the gap but more parallel to the conduction axis in the horizontal section (Figures 4 and 5).

Discussion

The major findings of this study are (1) conduction through gaps in RF lesions is associated with gap geometry, and this association may be further influenced by gap cross-sectional dimensions, (2) the geometry of the gaps may allow for complex conduction behaviors, specifically, unidirectional block and/or rate-dependent block, (3) conduction through gaps in RF lesions is responsive to pharmacological blockade.

Previous studies have shown that for myocardial tissue, there exist critically small gap or isthmus cross-sectional dimensions below which conduction will fail.9–14 These studies have examined surgically or laser-induced isthmuses in myocardial tissue or simple interruptions in linear RF lesions. Mitchell et al9 found gaps present in each of 7 dogs with pacing induced atrial fibrillation that underwent left atrial linear RF ablation. Despite discontinuities in the lesions at necropsy, no dog had atrial fibrillation after ablation despite recurrent atrial fibrillation in all control animals. The dimensions of the lesion gaps were not reported. Thomas et al10 found isthmuses created by laser lesions in dog atria to fail conduction if \( H < 0.8 \text{ mm}^2 \) in cross-sectional area. Our laboratory has shown that cross-sectional area is a determinant of conduction through gaps in RF lesions in the rabbit RV flap preparation.12 The gap geometry in all of these studies was linear, comparable to the straight gap in our current study.

The new finding in this study is that the geometry of the gap is also associated with conduction through discontinuities in RF lesions. The effects of cardiac tissue geometry on impulse propagation have been described in patterned cell cultures and myocardial sheets but not for whole-tissue...
preparations. Angulations in a gap may pose obstacles to conduction through changes in gap cross-sectional dimensions and/or through changes in the orientation of the myofibers relative to the conduction path. The effects of gap width and fiber orientation on conduction are interrelated. Cabo et al examined conduction through isthmuses in sheets of sheep ventricular myocardium by using optical mapping. The isthmuses were created by surgical incisions that oriented the conduction path either parallel or perpendicular to the myofiber orientation. For isthmus conduction parallel to the longitudinal fiber orientation, the critical isthmus width for block (<1 mm) was significantly smaller than for isthmuses oriented perpendicular to the fibers (1.78 to 2.32 mm). In addition, block occurred distal to the isthmus at the sites of maximal curvature of the wavefront. Block was attributed to a current source-sink mismatch imposed by the expanding wavefront and exaggerated by anisotropic conduction properties when propagation was transverse to the fiber orientation. Although our study was not designed to define the precise mechanism of block resulting from gap geometry, our findings are consistent with these established principles governing myocardial conduction. In our study, conduction failed at the site of wavefront curvature in the angled preparations. Because the myofiber orientations within the different layers of the RV wall does not change from base to apex, angulation of the conduction path necessitates changes in the direction of wavefront propagation relative to the myofiber orientation at all layers within the myocardium (Figure 5). Conceivably, the gap dimensions that supported conduction along the longitudinal fiber orientation were insufficient for conduction orthogonal to this fiber orientation. In our study, the association of gap geometry with conduction through the gap could be modified by its cross-sectional dimensions. This finding is consistent with the previous demonstrations of the critical role of cross-sectional dimensions in determining conduction through lesion discontinuities. It is likely that gap geometry, physical dimensions, and relation to myofiber orientation are all interdependent in determining conduction through a lesion discontinuity.

Bifurcation of a conduction path may prevent propagation by curvature in the conduction path and by exaggeration of the current source-sink mismatch. In bifurcated gaps, the current available to depolarize myocardium in advance of the wavefront may be insufficient to activate the enlarging current sink imposed by the bifurcation. Fast and Kleber demonstrated unidirectional block in the direction of expanding tissue geometry in patterned myocyte cell cultures. The pattern of conduction through the bifurcated gaps in our study is consistent with the source-sink mismatch model and the findings of Fast and Kleber. As expected, rate-dependent and/or unidirectional block only occurred in our preparations in the direction of diverging arms of the gap, which would result in a smaller current source feeding a larger current sink. In low concentration, the sodium channel blocker flecaïnide produced block within straight and bifurcated gap preparations. Sodium channel blockade causes lower maximal dV/dt of the action potential and reduced cellular excitability. In the setting of a current source-sink mismatch, these actions have the combined effects of reducing the amplitude of the current source and increasing the current needed for tissue excitation of the sink. Similarly, anisotropic conduction may be critically dependent on action potential dV/dt and subject to inhibition by sodium channel blockade.

The apparent shortening of conduction times through the gap after ablation is consistent with previous reports. The mechanism for this is unknown. Alterations in cell-to-cell coupling or changes in electrotropic forces may be potential causes. The measures themselves are near the temporal resolution of the recording system and should be interpreted with caution.

Limitations
The relevance of animal models to clinical phenomenon is always uncertain. The tissue architecture of the rabbit right ventricle differs from that of human atrium. Histological staining may underestimate the true lesion sizes. The findings are described for acute RF lesions that may be associated with reversible electrophysiological effects. The conduction properties of gaps within chronic RF lesions may be different. The temporal resolution of the recording system limits the accuracy of the conduction times through the gaps.

Clinical Implications
The findings of this study may explain some aspects of outcomes after extensive linear ablation procedures such as pulmonary vein isolation procedures for atrial fibrillation. After this procedure, long-term success may be achieved despite anatomic discontinuities in the lesions. This may result from complete conduction failure or rate-dependent block that precludes rapid activation of the atria from the pulmonary veins. The ability to block conduction within these gaps pharmacologically is also consistent with the response of some patients to antiarrhythmic therapy after ablation. The development of unidirectional block contributes to the substrate for reentry. Reentry is a mechanism for some left atrial flutters after ablation procedures. Current ablation technology rarely produces uninterrupted long linear lesions in the left atrium, therefore gaps are to be expected. Complex gap geometries have the potential to block conduction or facilitate reentry.

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Disclosures
None.

References


Inoue H, Zipes DP. Conduction over an isthmus of atrial myocardium in CLINICAL PERSPECTIVE

The creation of linear ablation lesions is necessary for some catheter-based approaches to atrial fibrillation. Complete linear lesions are rarely achieved, and both therapeutic and proarrhythmic responses may result from such ablation procedures. Because discontinuities are common in linear ablation lesions, it is possible that features of the gaps influence the clinical outcomes. Previous work into the electrophysiological properties of gaps within ablation lesions has shown that a minimal cross-sectional dimension is necessary to support conduction. In this work, we demonstrate a highly significant effect of gap geometry on conduction through the discontinuity and the ability of a bifurcating gap geometry to produce complex conduction patterns of unidirectional and rate-dependent block. This work may elucidate the variety of clinical responses to linear ablation procedures for atrial fibrillation. Although discontinuities exist in the ablation lines in this procedure, gaps with small cross-sectional dimensions or with highly angulated geometries may fail to conduct. In this instance, a therapeutic effect may still occur. Our work also demonstrates the ability of gap geometry to provide the substrate for new reentrant arrhythmias through creation of unidirectional block. Proarrhythmia in the form of new reentrant atrial arrhythmias is known to occur after extensive linear ablation. Finally, our model demonstrates the susceptibility of the gaps to pharmacological blockade. This finding may explain the response to previously ineffective antiarrhythmic drug therapy after failure of ablation alone in the clinical setting.
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