Simultaneous Epicardial and Endocardial Activation Sequence Mapping in the Isolated Canine Right Atrium

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**Background.** Since the atria are thin-walled structures, most studies that have examined the spread of activation in the atria have assumed that they behave electrophysiologically as a two-dimensional surface. It was the objective of this study to determine whether or not this assumption is true by simultaneously mapping the epicardial and endocardial activation sequences in the right atrium.

**Methods and Results.** Identical precisely superimposed epicardial and endocardial electrode templates with 250 unipolar electrodes each were used to map the isolated canine right atrium (n=8) during continuous perfusion and superfusion with Krebs-Henseleit buffer. Data were recorded during control conditions (normal sinus rhythm), continuous pacing (S1S1=300 msec), and premature stimulation (S1S2=effective refractory period+5 msec). Pacing was performed at two sites, one located on the inferior crista terminalis and one lateral to the crista terminalis on a pectinate muscle. Tachyarrhythmias were induced by a single extrastimulus during the continuous perfusion of acetylcholine (10^{-5} mol/L). Individual electrode sites were correlated with the gross anatomy and histology. Activation time differences were calculated between each two corresponding epicardial and endocardial sites. There were differences in the activation times between the epicardium and endocardium during all experimental conditions. However, the average difference for each condition was <1 msec, suggesting that overall activation did not spread faster on either the epicardium or the endocardium, even though in certain regions one surface could lead the other. The dispersion of time differences was smallest during normal sinus rhythm and continuous pacing (SD=5.6–5.8 msec) and largest after premature stimulation (SD=6.3 msec for crista pacing, p<0.05; SD=8.1 msec for pacing lateral to the crista, p<0.001). Differences in the activation sequence correlated with the underlying anatomic architecture. The largest differences in activation times between the epicardium and endocardium were associated with those regions of the atrium where pectinate muscles ran below the epicardial surface. The pectinate muscles in those areas were often discontinuous with the epicardial surface and facilitated the discordant epicardial–endocardial activation. The discordant activation was also found in regions where the atrial wall thickness was <0.5 mm and correlated with transmural differences in fiber orientation. A tachyarrhythmia induced in the presence of acetylcholine, which demonstrated a focal activation pattern, was shown to have a reentrant loop that used free-running muscle bundles connecting the epicardial and endocardial surfaces, resulting in a three-dimensional pathway.

**Conclusions.** The findings of this study demonstrate that epicardial and endocardial activation can be discordant in specific regions and that discordance increases with abnormal activation sequences. Many of the differences in the epicardial and endocardial activation can be correlated with the heterogeneity of the anatomic architecture of the right atrium. The study also demonstrates that reentry can occur in a three-dimensional plane using the epicardial and endocardial surfaces connected by transmural muscle fibers. (*Circulation* 1993;88:250-263)

**Key Words** • mapping, endocardial/epicardial • electrophysiology • atrial anatomy

Ativation sequence mapping has been used to understand the mechanisms of various atrial rhythms and arrhythmias. An underlying assumption has been that the atrium acts electrophysiologically as a two-dimensional surface. Therefore, inves-tigators have recorded either from the epicardial1-5 or from the endocardial surface.6-8 Mapping of canine atrial flutter and atrial fibrillation induced by extrastimulation has occasionally demonstrated a focal activation pattern, even though the method of initiation would favor reentry.9-11 Studies involving the mapping of atrial fibrillation in humans have also shown similar focal

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patterns. These patterns have also been observed in dogs even in the presence of high concentrations of acetylcholine (ACh), which would suppress most focal mechanisms, such as automaticity or triggered activity. If these focal activation patterns are indeed reentrant, it is possible that this reentry occurs at a microscopic level below the resolution of the mapping electrode grid, or it may be that the reentry occurs outside the surface plane of the atrium. This has been suggested by Zaitsev et al., who mapped the isolated canine right atrium from both epicardium and endocardium during cholinergic atrial fibrillation. Medvinsky et al. have also suggested the possibility of intramural reentry in the left atrium.

The structure of the right atrial myocardium is complex. Some pectinate muscles are discontinuous with the epicardial surface, connected only at their ends and traversing cavitory structures. Within Bachmann’s bundle, fascicles are electrically insulated from adjacent fascicles by a perimysial septum of connective tissue. These anatomic complexities, coupled with the abrupt differences in fiber orientation over short distances, potentiate the likelihood of conduction block. When the fibers of the atrium run in the plane of the surface, nonuniform anisotropic conduction and conduction block, which occur transverse to the fibers, should occur not only in the surface plane but also transmurally. Similarly, block that occurs in the longitudinal direction of the fibers could produce a transmural difference in activation when the fiber orientation is transmurally anisotropic or when the fibers run perpendicular to the surface. The purpose of this study was 1) to determine whether the epicardial and endocardial activations of the isolated perfused canine right atrium are concordant, 2) to relate any differences between the activation of the epicardium and endocardium to the underlying anatomy, and 3) to determine whether these differences result in any reentrant pathways outside the epicardial or endocardial plane.

**Methods**

Normal dogs (n = 8) weighing between 20 and 25 kg were anesthetized with pentobarbital (30 mg/kg i.v.). The animals were intubated and ventilated with a positive-pressure respirator. A median sternotomy was performed, and the azygous vein was ligated and divided. The fat pad on the atrioventricular groove was opened to expose the right coronary artery with its atrial and ventricular branches. The animals were heparinized with sodium heparin (100 units/kg), and the ventricular branches of the right coronary artery were ligated. The inferior and superior venae cavae were ligated and divided. The aorta was cross-clamped, and cold cardioplegia was infused into the aortic root. The heart was covered with iced saline, and the right atrium was rapidly excised. The ostium of the right coronary artery was cannulated with polyethylene tubing (PE-50, Intramedic; i.d., 0.58 mm; o.d., 0.965 mm). The ventricular and excess atrial tissue were removed from the preparation, and the atrium was unfolded (Figure 1A) and mounted epicardial side down (Figure 1B) on a flat platform template containing 250 unipolar silver electrodes (diameter, 200 μm) with an interelectrode distance of 3.5–5 mm. The tip of the right atrial appendage was inserted into a slot in the electrode template to allow the atrium to lie flat. The surface area of the preparation was approximately 38 cm². The preparation did not include the intra-atrial septum.

An identical electrode template containing 250 unipolar electrodes was placed over the endocardial surface. The two templates were aligned spatially, using common fixation rods at the four corners, which passed through corresponding common holes in the electrode template. The distribution and spacing of each two corresponding electrodes on the epicardial and endocardial templates were identical and superimposed over one another. The preparation was then placed in a temperature-controlled bath at 37°C and perfused at a rate of 8–10 mL/min with Krebs-Henseleit solution to approximate the normal flow in the canine right atrium. At the same time, the preparation was also continuously superfused. Within 5 minutes of establishment of perfusion, the preparation beat spontaneously. Further details of the technique and the normal electrophysiology of the preparation have been reported previously.

Before placement of the endocardial template, bipolar pacing electrodes were sutured to the endocardium on the lateral side of the posterior right atrial free wall and on the inferior portion of the crista terminals. A pacing threshold was determined, and the pacing stimulus was set at 1.5 times the threshold. Electrogams were recorded simultaneously from all 500 sites during control conditions (normal sinus rhythm), during continuous pacing at an S1,S2 interval of 300 msec, and at an S1,S2 interval 5 msec longer than the effective refractory period. ACh was infused for 2 minutes at a concentration of 10⁻³ to 10⁻⁴ mol/L. Then, without interrupting the continuous infusion of ACh, a single extrastimulus was used to induce tachyarrhythmias, and electrograms were recorded. This pacing protocol was performed from the pacing site on the crista terminals and from the site lateral to the crista. After the data were recorded, the infusion of ACh was terminated. The purpose of inducing tachyarrhythmias in the presence of high concentrations of ACh was to suppress all automaticity and induce intra-atrial reentry with a short wavelength. It was anticipated that these conditions would favor the possibility of reentry outside the surface plane.

Two 256-channel computerized data acquisition and analysis systems were used to collect, process, and display data. The data acquisition on the two systems was synchronized by use of a common timing signal that was recorded simultaneously on both systems, along with a common epicardial electrogram. Specifically, this was done by recording, on both systems, a 50-mV square wave (pulse width, 50 msec; pulse interval, 100 msec; rise time, <100 μsec) by use of a WPI series 1800 pulse generator driving a WPI Omnical 2010 voltage calibrator. Data recording was stopped simultaneously on both systems, and data were analyzed relative to leading or falling edge of the common timing pulse. In addition, in all but the recordings of sinus rhythm, the data also included pacing pulse artifacts that confirmed the alignment (Figure 4). All the hardware and software of both data acquisition systems were identical. This allowed the data collection on both systems to be time-aligned. Each 256-channel mapping system is based on a VAX Station III GPX graphics workstation connected to two 128-channel DPD 1123–based data acquisition subsystems. Each PDP system contains two data translation DT3362 64-channel analog-to-digital
Figure 1. Panel A: Schematic of the isolated right atrium with the remainder of the atria and ventricles removed. Large arrow shows how the atrium is unfolded. Panel B: Photograph of an isolated right atrium mounted on the epicardial electrode template. Arrow marks the ostium of the right coronary artery. View is of the endocardial surface. Asterisk marks sites of pacing. RAA, right atrial appendage; RCA, right coronary artery; SVC, superior vena cava; CT, crista terminalis; ICB, intercaval band; IVC, inferior vena cava; ANT, anterior; POST, posterior.

corresponding converter boards and a 4 MB memory board. The PDPs are diskless systems and are run by a control program that is downloaded from the VAX. The PDP memory is configured as a circular buffer that allows the most recent 16 seconds of data from the continuous-input stream to be saved. When the desired data are obtained, they are uploaded to the VAX via DMA direct memory access interface. The system uses an in-house designed and built 256-channel bipolar amplification system with selectable high- and low-pass filters and gains. The system is run with in-house developed software for data acquisition control, data management, raw data display, and data analysis. A selected electrogram was also continuously monitored on an oscilloscope throughout the study. Unipolar electrograms were recorded at a gain of 1000 with a frequency response of 0.05–500 Hz. A silver common unipolar reference electrode was placed in the bath approximately 8 cm below the preparation. Each channel was digitized at 1000 Hz with a 12-bit resolution. Local epicardial activation times were determined from the maximum negative derivative of the unipolar electrogram. All electrograms were edited visually to verify accuracy of the computer-picked activation times. These activation times were displayed on a schematic diagram of the atrium as an activation time map (Figures 2A and 2B). Concentric computer-generated isochronous contour lines were determined for 10-msec intervals arising from the earliest activation (time, 0 msec) on the map.

After each experiment, the atrium was fixed in formaldehyde and mounted on a clear plastic template at fixation points identical to the electrode template. The atrium was also marked with sutures at various sites to determine the amount of tissue shrinkage occurring from the fixation. The atrium was then photographed with and without backlighting from its epicardial and endocardial surfaces. This allowed correlation of the anatomy with the specific electrode positions. After analysis of the data, 2×0.5-cm strips of tissue were cut from two of the preparations for histology. Five evenly spaced longitudinal sections were made from the strips and stained with Masson’s trichrome. The center section was used to correlate with electrode position.

For all activation sequence maps, the differences between epicardial and endocardial activation time were calculated. A negative difference indicates that the epicardium activated before the endocardium. The differences in the epicardial activation times recorded from two successive sinus beats were calculated. Any discrepancy between the two time differences at a given location represented not only the effect of random electrical noise on the activation time algorithm but also the beat-to-beat variability in activation. The difference was $-0.5 \pm 0.6$ msec, with 98% of the times within 1 msec of each other. For each map, the mean, SD, and minimum and maximum activation time differences were calculated. The statistics for the activation time differences were also calculated for the combined data of all preparations for each experimental condition. SDs were compared by an $F$ test, with $p<0.05$ considered statistically significant. All data, unless otherwise noted, are expressed as mean±SD.

All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by
FIGURE 2. Computer display (dog 7) of the epicardial (panel A) and endocardial (panel B) activation maps during normal sinus rhythm shown from the epicardial perspective. Times are all adjusted to the earliest activation time that occurred on the endocardial surface, designated as zero. Computer-generated 10-msec isochrons are also shown. RA, right atrium; SVC, superior vena cava; CT, crista terminalis; RAA, right atrial appendage; SNA, sinus node artery; IVC, inferior vena cava; ICB, intercaval band.

the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985). In addition, the study protocol was approved by the Washington University Animal Studies Committee.

Results
A summary of the differences between the epicardial and endocardial activation times is shown in Table 1 for each dog during normal sinus rhythm, continuous pacing (S1S1=300 msec) from the crista and lateral to the crista, and premature stimulation (S1S1=effective refractory period+5 msec) from both those sites. The average difference for all the preparations was <1 msec for both the control and paced data. The SDs were all significantly greater (p<0.001) than the timing error, and the average SD for normal sinus rhythm was 5.7 msec. Overall, for the group of animals, this SD did not increase during continuous pacing (S1S1=300 msec) either from the crista or the lateral side of the crista.

<table>
<thead>
<tr>
<th>Dog</th>
<th>Control NSR</th>
<th>S1S1=300 msec</th>
<th>S1S1=ERP+5 msec</th>
<th>S1S1=300 msec</th>
<th>S1S1=ERP+5 msec</th>
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<tbody>
<tr>
<td>1</td>
<td>0.4±7.4</td>
<td>0.6±4.5</td>
<td>0.3±4.8</td>
<td>0.1±4.6</td>
<td>-0.1±6.4</td>
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<tr>
<td>2</td>
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<td>-0.1±4.0</td>
<td>-0.9±3.5</td>
<td>-0.2±6.9</td>
<td>-0.8±6.5</td>
</tr>
<tr>
<td>3</td>
<td>1.0±7.0</td>
<td>0.9±9.0</td>
<td>-0.2±9.9</td>
<td>-1.0±7.5</td>
<td>-2.1±13.0</td>
</tr>
<tr>
<td>4</td>
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<td>-0.9±6.2</td>
<td>-0.8±6.2</td>
<td>0.1±5.5</td>
<td>0.4±9.9</td>
</tr>
<tr>
<td>5</td>
<td>0.8±5.5</td>
<td>1.2±7.5</td>
<td>2.3±7.6</td>
<td>1.2±8.9</td>
<td>1.0±7.7</td>
</tr>
<tr>
<td>6</td>
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<td>1.3±7.1</td>
<td>0.9±4.2</td>
<td>0.8±6.5</td>
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<tr>
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<td>...</td>
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<td>1.6±8.3</td>
</tr>
<tr>
<td>Mean±SD</td>
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<td>0.6±5.8</td>
<td>0.4±6.6*</td>
<td>0.5±6.0</td>
<td>0.3±8.4†</td>
</tr>
</tbody>
</table>

NSR, normal sinus rhythm; ERP, effective refractory period. All data are expressed as mean±SD.

*p<0.05 compared with control (differences in SD).

†p<0.001 compared with control and crista S1S1 (differences in SD).
However, the SD did increase with premature pacing from the crista compared with normal sinus rhythm ($p<0.05$) and also increased with premature pacing lateral to the crista ($p<0.001$). In addition, the SD of the premature beat from the lateral pacing site was greater ($p<0.001$) than the SD for the premature beat from the pacing site on the crista. An example of the distribution of the time differences is illustrated in Figure 3. This figure shows a histogram of the differences during control normal sinus rhythm conditions (panel A), during continuous pacing ($S_1S_1=300$ msec) from the crista (panel B), and a premature stimulation ($S_1S_2=170$ msec) from the same site (panel C). The time differences during one cycle of an induced tachyarrhythmia (panel D) are also shown. In this example, the control SD was 3.6 msec, with a maximum difference between the epicardial and endocardial time of 13 msec. During continuous pacing, the SD increased to 4.6 msec ($p<0.05$), with a maximum difference of 22 msec. With premature stimulation, the SD increased to 7.1 msec ($p<0.001$), with a maximum difference of 30 msec. For the cycle of the tachyarrhythmia shown, the SD was 6.9 msec, which was not significantly different from the $S_1S_2$ SD; however, the distribution became more peaked and narrow (leptokurtic) and skewed. In general, the distributions of the control $S_1$ pacing and premature stimulation differences all showed a relatively normal distribution. With pacing, however, there was an increase in the number of outliers.

Figure 4 illustrates the relation of the epicardial and endocardial recordings to the gross anatomy. Electrograms recorded from two sites during normal sinus rhythm, the last continuously paced beat ($S_1$), and the premature beat ($S_2$) are shown. The electrograms illustrated were recorded from two sites in the lateral atrium over pectinate muscles. Site 194 shows close concordance of the epicardial and endocardial activation during control conditions. At site 195, there was a difference of 5 msec, with the epicardial surface activating before the endocardial surface. The difference shown in channel 195 was representative of the type of differences seen during normal sinus rhythm. When a difference of 5 msec or more occurred, it was usually in an area over a thicker section of the atrium associated with an underlying pectinate muscle. During sinus rhythm, the earliest site of activation occurred on the epicardium in four preparations, on the endocardium in two preparations, and on the epicardium and endocardium.
simultaneously in two preparations. The maximum time difference in the initiation of activation between the two surfaces was 6 msec. The sites of earliest activation were concordant in four preparations. In three preparations, the earliest activation occurred at an adjacent site on the opposite surface. In one preparation, the location of the earliest activation on the epicardial surface (time, 0 msec) was separated by 10.2 mm from the location of the earliest endocardial activation (time, 0 msec). During continuous pacing at 300 msec, the differences at site 194 (Figure 4) were not any greater than the control values; however, note that the A, paced waveform is much lower in amplitude, and even though the computer picked the maximum negative derivative, the low
amplitude and slowness of the derivative suggest the possibility that this site actually blocked. At site 195, the time difference is now increased by 4 msec to 9 msec, with the epicardial activation ahead of the endocardial activation on the pectinate muscle. The premature beat at site 194 again shows only a small activation time difference but a decreased amplitude and derivative of the endocardial waveform compared with the control values. At site 195, which activates later than 194, the epicardial–endocardial difference is even greater than the difference for continuous pacing, with the epicardium leading the endocardium by 19 msec.

Selected electrograms recorded from this same preparation during the induced tachyarrhythmia are shown in Figure 5. In channel 195, the maximum difference at this site is 19 msec and occurs during the A2 cycle. For cycles A3–A6, it is only 7 to 9 msec. In channel 194, for cycles A2–A4, the amplitude and derivative of the signal increase with minimal time differences. Electrograms from sites 164 and 165 are also recorded from a pectinate muscle just cranial to 194 and 195. At site 164, time differences during the fibrillation were 23 to 26 msec, with the epicardium again leading the activation. Just adjacent to that at site 165, however, the maximum difference is only 5 msec. Signals recorded from the area medial to the crista terminalis are shown from sites 106 and 107. The time differences from site 106 range from 7 to 9 msec, with the endocardial activation occurring before the epicardial activation. At site 107, the differences are smaller, but the endocardium still leads the epicardium. To relate the time differences to the architecture of the muscle bundles, the positions of the electrograms were correlated with the histology. This is illustrated in Figures 6 and 7. In Figure 6, an approximately 2-cm strip of tissue is shown from sites 163–167 in the atrium shown in Figure 4. Epicardial and endocardial electrograms recorded from one cycle during the induced tachyarrhythmia are shown below. The black dots on the surfaces represent the epicardial and endocardial electrode positions. The size of the dots correlates with the actual size of the silver electrode (200-μm diameter) used in this study. The histology (Figure 6) demonstrates a very complex architecture, with the pectinate muscle fibers running oblique to the epicardial fibers. In addition, muscle bundles are separated from each other by connective-tissue septa or open space. The pectinate muscle, which originates approximately at site 165 and extends past site 164, is discontinuous with the epicardial surface, and an instrument could be inserted between the pectinate muscle and the epicardial surface without disrupting any tissue. The time differences (Figure 5) that occurred at sites 195 and 164 were in areas where the pectinate muscle was separate from the epicardial surface. The average total thickness of the atrial wall, including the pectinate muscle, was 3 to 4 mm. Also notice the low amplitude of the epicardial signal in channel 163. The dotted line on the lateral epicardial surface shows the extension of the fat pad in the epicardial region. During preparation of the tissue, the fat pad separated from the tissue, and the line represents the approximation of the fat pad. This fat pad can be seen in Figure 4 on the epicardial view of the tissue. The thickness of the wall at this point was approximately 7 mm, with 3 mm of that tissue being fat.

A second example of the correlation from the same preparation as shown in Figure 4 of the histology with the electrophysiology is shown in Figure 7. The strip of tissue was taken from the medial side of the crista terminalis just cranial and medial to the sinus node between sites 107 and 119. The atrial wall thickness was 0.5 to 1 mm thick in this region. During this recorded run of the tachyarrhythmia, the wave front was moving in the cranial medial direction. The sinus node was
adjacent to site 119. Near the sinus node, the time difference was 13 msec, with the epicardium activating before the endocardium. As the wave front moved from the right to the left, the endocardium then led the epicardial activation. Note between electrodes 118 and 107 that in the tissue immediately under the endocardium, the fibers are running parallel to the direction of the wave front and the epicardial and midwall fibers are running transverse to the spread of the wave front.

Electrograms recorded from another preparation during an induced tachyarrhythmia are shown in Figure 8. Also shown is a photograph of the endocardial surface. The three electrograms shown from three sites are recorded from a region near the cavoappendicular junction. The epicardial–endocardial time differences range from 10 to 28 msec. Also note the gross difference in the morphology of the waveforms, particularly in the electrogram from site 59, which shows multiple deflections on the endocardial recording but a smooth deflection on the epicardial recording. To a lesser degree, the electrogram recorded from site 41 shows the same pattern. The muscle bundle on which site 43 is located is not continuous with the epicardial surface under site 43. The bundle originates on the superior portion of the crista terminalis just medial to site 59 and inserts just below the upper edge of the area of tissue outlined by the rectangle. It is also continuous along portions of its medial edge. Below it is another muscle bundle on which sites 41 and 59 are located. The muscle bundle originates from several bundles on its inferior aspect and inserts under the bundle at site 43. As a result, conduction cannot traverse from sites 41 and 59 to 43 directly, but would have to go superior, inferior, or up under the muscle to get to site 43. Similarly, endocardial conduction cannot traverse directly laterally from site 41.

Epicardial and endocardial activation sequence maps from the region outlined by the rectangle on the endocardial surface in Figure 8 are shown in Figure 9. The maps are shown in the same orientation as the photograph in Figure 8, with the endocardial surface on top and the epicardial surface below. The epicardial and endocardial activation patterns both demonstrated a focal pattern of activation. However, the two focal sites of origin of the wave fronts were separated by 1.5 cm, with the wave fronts moving in opposite directions between the epicardial and endocardial sites of earliest activation. The initial activation first emerges in the A, beat at site A at 115 msec. It moves in a caudal direction to site D. The thickness of the tissue at this point is
approximately 1 mm. The corresponding electrograms are shown in the left part of the figure. At 129 msec, the activation breaks out onto the epicardial surface at site E, which is opposite to site D on the endocardial surface, and the wave front moves out radially and asymmetrically from this site. The wave front moves from site E to site J on the epicardial surface, which opposes site A on the endocardial map. The thickness of the tissue between A and J is approximately 5 mm. At 165 msec, the wave front emerges again on the endocardial surface in A, and moves caudally to site D. At the corresponding epicardial site E, at 182 msec, activation breaks out onto the epicardial surface and the pattern repeats. The remainder of the atrium is activated from this region during the course of the tachycardia.

Another example of an induced tachyarrhythmia is illustrated in Figure 10. This run was recorded from the same preparation as shown in Figure 4. The tachyarrhythmia was induced by a single extrastimulus placed lateral to the crista (asterisk) during continuous ACh infusion. The wave front slowed in the lateral right atrium in the endocardium during the A; beat, and in the epicardium, it slowed at the junction of the crista terminalis and on the medial side of the atrium. Figures 5, 6, and 7 illustrate electrograms recorded from these regions. The first spontaneous beat, A₁, is initiated multifocally on the epicardial surface at 94 msec and unifocally on the endocardial surface at 92 msec. The location of the earliest times on the epicardial surface are sites 134 and 119, shown in Figure 4. The endocardial location of earliest activation of the A₁ beat occurred at site 156. Site 119 lies just medial to the sinus node (see Figures 4 and 7). Site 156 is approximately 3 mm lateral to the inferior portion of the sinus node (see Figure 4). The tissue thickness varies from 0.5 mm in the nodal region (Figure 7) to 4 to 5 mm in the crista terminalis. On the epicardial surface, notice that the 110-msec isochron extends 45 mm from its most cranial to its most caudal boundary. On the endocardial surface, the 110-msec isochron extends over 65 mm. On the epicardial surface, the multifocal pattern in A₁ is repeated throughout the tachycardia, as illustrated in cycles A₁-A₄. Similarly, on the endocardial surface, the pattern shown in the A₁ beat repeats in cycles A₁-A₄. There were no obvious reentrant circuits on either the epicardial or endocardial surface. In the regions of the epicardial and endocardial earliest activations in each cycle, the opposing surface is always within 5 to 10 msec of the one on the opposite surface, with no adjacent times from late in the previous cycle.

The electrograms in Figures 4 through 8 show a variety of morphologies. Both the epicardial and endocardial electrograms recorded from site 194 (Figure 4) during control rhythm show a simple biphasic waveform with close concordance. At site 195, the epicardial waveform during control conditions is biphasic, but the endocardial recording has a negative deflection after the initial positive deflection that corresponded to the rapid deflection (maximum negative derivative indicated by arrowheads on electrogram) in the epicardial surface. However, the rapid deflection on the endocardial recording is not reflected on the epicardial waveform. Correspondences can also be seen between sites on the same surface, as well as between the epicardial and endocardial surfaces. The endocardial waveform at site 195 initiated by the premature stimulus shows three positive deflections before the rapid negative deflection. The first positive deflection correlates with the activation at site 194 and is also seen as a positive deflection in the epicardial recording at site 195. The second positive deflection on the endocardial electrogram at site 195 correlates with the activation of the epicardial site. On the epicardial electrogram, there is a small triphasic deflection after the major negative deflection.
that correlates with the endocardial activations. Differences between epicardial and endocardial activation are reflected in the waveform morphology during the tachyarrhythmia as illustrated in the recordings made from sites 194 and 195 in Figure 5. On the other hand, dramatic differences in waveform morphologies are not necessarily reflected on the opposite surface, as shown in Figure 8. In these recordings, the epicardial waveforms are regular with small inflections, whereas the endocardial waveforms are polyphasic.

The amplitudes of the electrograms also correlated with the anatomy. The epicardial recording showed a lower amplitude at electrode sites overlying the epicardial fat pad (Figure 6, site 163). The amplitude was generally lower in thinner tissue than in thicker regions in the same preparation. As an example, the average peak-to-peak amplitude of the electrograms in Figure 6 is 4.5±1.6 mV, with an average thickness of 3 mm. The average amplitude of the electrograms in Figure 7 was 2.1±0.7 mV, and the thickness of tissue was 0.6 mm.

**Discussion**

The data in this study demonstrate that differences exist between epicardial and endocardial activation in the right atrium. The differences are smallest during normal sinus rhythm and continuous pacing and increase with premature stimulation (Table 1 and Figure 3). These differences are physiologically significant during induced tachyarrhythmias when, as illustrated in Figure 9, different portions of the putative reentrant circuit appear to involve both the epicardial and endocardial surfaces. These differences occur even though the wall thickness of the canine right atrium is <5 mm in most regions. The fact that the average differences (Table 1) are close to zero suggests that neither surface facilitates faster propagation of the wave fronts. In some
regions, the epicardium activates before the endocardium (Figure 4), and in other regions the reverse occurs. (Figure 8). The differences are dependent not only on the prematurity of a wave front but also on the direction of spread. The premature beats initiated from a site lateral to the crista produce a larger difference than those resulting from a premature beat initiated on the crista.

Most of the differences in the epicardial and endocardial activations correlate with the heterogeneous architecture of the right atrium. Studies by Dobler and Spach have shown that at the microscopic level (1 to 3 mm), extended fascicles are insulated by a connective-tissue septum from adjacent fascicles. In the present study, insulated muscle bundles were also seen, as illustrated in Figure 6 in the region above the pectinate muscles. These muscle bundles are more loosely juxtaposed than those seen in more compact structures such as Bachmann’s bundle. Fiber orientation may also have contributed to transmural activation difference. This is suggested in Figure 7, where during an induced tachyarrhythmia in a region in which the wall thickness was <0.5 mm, the endocardial activation leads the epicardial activation once it spreads past the sinus node. On the endocardium medial to the node, the fibers are running longitudinal to the spread of activation, whereas in the epicardium, the wave front is moving transverse to the fibers. This suggests that, because conduction velocity is faster in the longitudinal direction of fibers, the wave front may be propagating more rapidly along the endocardium.

There are other anatomic features of the atrium that did not result in any differences in epicardial–endocardial activation in this study but that potentially could, particularly in certain pathologies. In the thin-walled section of the atrium, as illustrated in Figure 7, the sinus node artery accounts for almost 50% of the thickness of the wall. In other regions, such as between sites 118 and 106 in Figure 7, an invagination reduces the wall thickness to 0.1 mm. This also occurs in thicker tissue, as shown in Figure 6, in which the transmural muscle in the wall is reduced to 0.1 mm. These regions can be seen grossly in the transillumination in Figure 4 as light areas between the darker muscle bundles. These thinner regions of tissue may be more susceptible to pericarditis, whether natural or induced, which may transmurally disrupt the thin muscle. There are even small regions between pectinate muscle in which there are no transmural muscle fibers and the wall thickness is composed entirely of connective tissue. These discontinuities, similar to the small invagination in Figure 7, extend over only a few millimeters and do not disrupt the macroscopic spread of activation. It is not clear whether such small discontinuities as these are a potential arrhythmogenic substrate, but some studies have suggested that small discontinuities, such as vessels, serve as organizing centers for vortical-type reentries.
At a more macroscopic level, activation time differences could be correlated with gross anatomic heterogeneity. The pectinate muscles, which extend from the crista to the lateral edge of the atrium, were often associated with differences in epicardial and endocardial activation times. Some of these muscles were not continuous with the epicardial wall. An example of the distribution of these muscles is shown in Figure 4, and a transmural cross section through a pectinate muscle is shown in Figure 6. These figures illustrate the degree of epicardial and endocardial dissociation that can occur in such a region. The pectinate muscle oriented longitudinally on the endocardial surface, which starts near site 165 and extends past site 164, is discontinuous with the epicardial surface. At site 164, there is a 25-msec difference in activation between the two surfaces, suggesting that the wave front slowed or even blocked, moving in the lateral direction. If the wave front were to block unidirectionally in either the epicardial or the endocardial plane, then a transmural circuit could be initiated that could rotate around the “hole” created by the space between the pectinate muscle and the epicardial surface.

Figures 8 and 9 illustrate an example where reentry appears to be occurring outside the surface plane. On the endocardial surface, the activation is initiated in a focal pattern. This repeats throughout the course of the tachyarrhythmia. Since it was induced by a single extrastimulus in the presence of a high concentration of ACh in normal atrial tissue, it is unlikely that the focal pattern results from automatic or triggered activity, because ACh would suppress these mechanisms. Notice, however, that the activation pattern on the epicardial surface also shows a focal pattern from a different site. Similar focal activation patterns have also been reported during induced flutter and fibrillation in both canine models and human studies. In a study of induced cholinergically mediated tachyarrhythmias in the same experimental model, the epicardial activation demonstrated a focal activation pattern in one third of the mapped cycles similar to the pattern in Figure 9. On the endocardial surface (Figure 9), the spread of activation is more irregular, with the irregularities in activation corresponding to the edges of the complex muscle bundles (Figure 8). Despite the irregularities, the pattern is still focal. The significant difference in the epicardial and endocardial pattern is that the sites of earliest activation on each surface are spatially discordant, with a separation of 15 mm between the earliest time on the endocardial surface (electrogram A) and the earliest time on the epicardial surface (electrogram E). In addition, the spread in activation between these two sites is caudal on the endocardial surface and cranial on the epicardial surface. This example demonstrates data that suggest that reentry can occur outside the epicardial plane.

Another example of an induced tachyarrhythmia in which there was a focal pattern of activation is illustrated in Figure 10. Again, the arrhythmia was induced by a single extrastimulus in the presence of a high concentration of ACh to suppress automaticity and trigger activity. In this example, the epicardial activation...
is multifocal, with two sites initiating the wave front consistently during each cycle. Two to three milliseconds before the epicardial initiation, a unifocal site initiates activation on the endocardial surface. Notice how widespread the 110-msec isochron is in A3, as well as the 170-msec, 230-msec, and 300-msec isochrons in A2–A6. If a single site of initiation were responsible for this pattern and were located at the midpoint of the isochrons, then the wave front would have to conduct at approximately 1.8 m/sec, which is faster than the maximum conduction velocity reported for canine atrial tissue. The widespread initiation of activation, the consistency of the pattern on repeated cycles, and the conditions of initiation suggest that this represents a breakthrough pattern of activation caused by an intramural reentrant event. The anatomic locations of the sites of earliest epicardial and endocardial activation (sites 119, 134, 156) are shown in Figure 4. These sites are adjacent to the sinus node region (Figure 4). Since ACh slows conduction and produces unidirectional block (entrance or exit block) in the sinus node, this particular example may represent the surface activation pattern during sinus node reentry. The sinus node in the dog is an intramural structure, and selective block into and out of the node could result in intramural reentry. Although it cannot be conclusively demonstrated that this is occurring in this example, the above arguments suggest that this is a reentrant event, and the surface activation pattern suggests that the reentry is occurring outside the surface planes.

The heterogeneity of architecture of the right atrial wall is also reflected in the morphology of the waveforms. Polyphasic deflections can reflect differences in activation between adjacent sites on the surface or between the surfaces, as illustrated at site 195 during pacing in Figure 4. However, because of the anisotropy of extracellular current flow, large deflections in the electrogram may not be detectable on the opposite surface. This is seen in Figure 8, in which the endocardial electrograms have multiple deflections but the adjacent epicardial electrogram is relatively smooth. Also significant is the amplitude of the signals. Epicardial fat diminishes the amplitude of the signal. The epicardial electrogram at site 163 is one eighth the amplitude of the endocardial site. This is because of the large fat pad in the atrioventricular groove on the lateral right edge of the atrium, which can extend 1 to 2 cm onto the atrial free wall and has a thickness of 2 to 3 mm (Figure 4). In addition, because the electrode is distant from the surface of the muscle, it no longer necessarily reflects the underlying activation but now is an average signal reflecting the activation in a larger segment of tissue. This region is a critical region of slow conduction in the sterile pericarditis model of flutter. Because of the overlying fat pad and the underlying pectinate muscles, it would be difficult to define the pathway of the flutter in this region from epicardial recordings alone.

This study is limited in that the tachyarrhythmias were induced in the presence of nonphysiological concentrations of ACh and had very short cycle lengths. Still, the study does demonstrate that significant differences can occur between epicardial and endocardial activation and that there is an anatomic basis for these differences. It is more likely that such differences would be present in the human atrium because it is much thicker. In addition, pathological features that selectively affect the epicardial or endocardial surface may promote epicardial–endocardial discordance. The study also demonstrates the importance of high-density mapping of these arrhythmias and may explain why other studies have failed to detect differences. The majority of the epicardial and endocardial sites activate within 10 msec of each other even in an induced tachyarrhythmia. If, for instance, the spatial resolution were cut in half, it would have been difficult to detect the reentry in Figure 9. Even the resolution used in this study was not adequate to define the pathways in Figure 10.

Another limitation involves an underlying assumption of all activation sequence mapping: that of assigning a single activation time to a specific electrode site. In theory, the maximum negative derivative of an electrogram corresponds to the upstroke of the action potential. However, an electrode covers many cells. The closest cells contribute the most to the signal. In the electrogram in Figure 4, the S2 waveforms from channel 195 clearly show two separate events peculiar to the epicardial and endocardial electrograms. In Figure 7, however, the electrograms from site 119 show multiple deflections, with three negative deflections in the epicardial electrogram and two in the endocardial. In this study, the maximum derivative was always used to assign an activation time. To minimize the averaging effect, the electrode was kept small (200 μm).

Lewis et al22 proposed that atrial activation spread out radially from the sinus node. Eyster and Meek26 and Bachmann24 demonstrated that activation spread was preferential and that the asymmetry in the spread of activation was related to the underlying anatomy. Subsequently, others have confirmed that the preferential spread of the activation wave front is related to the underlying architecture of the muscle bundles. Other studies have demonstrated that conduction is also affected by the microscopic anatomy and that during abnormal impulse formation, these features can become arrhythmogenic.11,20 These studies have looked at the effects of the anatomy on the spread of activation in the surface plane. The present study provides evidence that the architecture of the atrium is responsible not only for the asymmetric spread of activation in the surface plane of the atrium but also for transmural asymmetry. Furthermore, the asymmetry between the epicardial and endocardial activations can become important in the genesis of reentry outside the surface plane. Future studies of atrial arrhythmias will need to consider the possibility that the atrium is a three-dimensional electrophysiological structure and not simply a two-dimensional surface. These data are likely to apply to even thicker ventricular tissue when the potential for three-dimensional microreentry is even greater. Perhaps ventricular tachycardias induced by extrastimulation that exhibit focal activation are also a result of three-dimensional microreentry not resolved by the electrode density being used.

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