Anisotropic Conduction Properties in Canine Atria Analyzed by High-Resolution Optical Mapping
Preferential Direction of Conduction Block Changes From Longitudinal to Transverse With Increasing Age

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Background—Anisotropic conduction properties may provide a substrate for reentrant arrhythmias. We investigated the age-dependent changes of structural and functional anisotropy in isolated right atria from infant (1 to 2 months), young (6 to 12 months), and old (6 to 10 years) dogs.

Methods and Results—The histology of the mapped atrial tissues (a small subepicardial area, 2.8×4.2 mm) was characterized by an age-dependent increase of myofiber width and fat cell infiltration between myofibers. Connexin43 was distributed homogeneously over the entire cell surface in infant dogs, whereas it progressively polarized to the cell termini with increasing age. The activation sequences were analyzed by high-resolution optical mapping using a voltage-sensitive dye. Activation fronts from the pacing site proceeded more rapidly along fiber orientation (longitudinal) than across it (transverse). Infant dogs showed “elliptical” isochrones with a smooth transition between longitudinal and transverse propagation, whereas old dogs had a “square” pattern with a sharp transition. Conduction block occurred predominantly during longitudinal propagation in infant dogs but during transverse propagation in old dogs. The shape of the wave front and the degree of lateral uncoupling seemed to decide the preferential direction of block. A zigzag activation causing an extremely slow transverse conduction was observed only in old dogs.

Conclusions—Along with the age-dependent structural anisotropy, the preferential direction of block changed from longitudinal to transverse in association with a change in the wave front configuration. A zigzag propagation based on lateral uncoupling would predispose the elderly to multiple reentry and a higher incidence of atrial fibrillation. (Circulation. 2002;105:2092-2098.)

Key Words: atrium ■ anisotropy ■ conduction ■ gap junctions

The conduction of excitation in the heart is determined not only by active membrane properties but also by the passive electrical properties of cardiac muscle. Conduction velocity and safety, as well as the action potential configuration, are influenced by the direction of propagation in relation to the fiber orientation. This anisotropic characteristic of propagation, which is defined by cell shapes and cell packing architecture and by the expression and distribution of gap junctions, has been a matter of great concern to cardiologists, because it may set the stage for the reentry of excitation, leading to life-threatening tachyarrhythmias.

Unidirectional conduction block is known to occur in association with tissue anisotropy, but there are conflicting results about whether longitudinal or transverse block preferentially occurs. We hypothesized that the preferential direction of conduction block might be altered in association with age-dependent changes in the structural anisotropy. Regarding the age-dependent changes of the anisotropic conduction properties of atrial muscle, extensive studies were performed by Spach and coworkers in dogs and humans by mapping the extracellular potentials. Their observations suggest a basis of microscopic anisotropic reentry in an area as small as 1 to 2 mm. Because of the technical limitation of extracellular potential mapping in such a small area, however, this issue has not been substantiated by the direct demonstration of complex activation sequences.

In the present study, we investigated the age-dependent changes in structural and functional anisotropy in a small area (2.8×4.2 mm) of the right atria in infant, young, and old dogs by high-resolution optical mapping and immunohistochemis-
try. With increasing age, the activation pattern changed from “elliptical” in infants to “square” in old dogs. Conduction block occurred predominantly during longitudinal propagation in infants, whereas the block occurred during transverse propagation in old dogs. A zigzag trajectory of activation, causing an extremely slow effective transverse propagation, was demonstrated only in old dogs. These changes were associated with age-dependent alterations in the distribution of gap junctions and the cell-packing architecture.

**Methods**

**Animals**

Beagle dogs aged 1 to 2 months (infant), 6 to 12 months (young), and 6 to 10 years (old) were killed under anesthesia with pentobarbital sodium (30 mg/kg IV) to obtain right atrial free walls around the atrial branch of the right coronary artery (Figure 1). All procedures were conducted in accordance with the guidelines of the institutional scientific committee.

**Histology**

For standard microscopy, 2 atrial tissue sections along the fiber orientation and 2 sections across it were obtained from 12 dogs (4 infant, 4 young, and 4 old). The sections were stained with hematoxylin and eosin, Masson trichrome, or phosphotungstic acid hematoxylin.

**Immunohistochemistry**

Cryosections of the atrial tissue were prepared from 2% paraformaldehyde-fixed samples. For the immunodetection of gap junctions, a mouse monoclonal anti-connexin43 (Cx43) antibody (Chemicon) was used. After permeabilization (0.3% Triton X-100), quenching (0.1 mol/L lysine), and blocking (3% goat serum/5% bovine serum albumin), the samples were incubated with the antibody overnight. Primary antibody-bound Cx43 was visualized by FITC-conjugated anti-mouse IgG. The labeled samples were examined using a confocal microscope (Zeiss LSM-510).

The proportion of Cx43 immunolabeling at the intercalated disks relative to overall Cx43 staining was quantified according to a procedure described previously.

**Optical Mapping of Excitation**

The right atrial free walls (30×40 mm) were placed in a tissue bath with the epicardial surface up for both superfusion and perfusion through the atrial branches with Tyrode’s solution (in mmol/L: NaCl 131, KCl 4, NaHCO3 18, NaH2PO4 1.8, CaCl2 2.7, MgCl2 0.5, and glucose 5.5) at 36.5°C. The preparations were stained with a voltage-sensitive dye, di-4-ANEPPS (10 μmol/L, 20 mL), and their contraction was prevented by cytochalasin D (25 μmol/L). The optical signals of the action potentials were recorded from a small square (2.8×4.2 mm) of the epicardial area by a fluorescence microscope equipped with a high-speed, high-resolution CCD video camera (MCAM01, BrainVision Inc, RIKEN). The epicardial surface was illuminated by an excitation light passing through a filter (λ=520±20 nm), and the emitted fluorescence was detected through a long-pass filter (λ>610 nm). The video images (60×90 pixels) were analyzed by a computer with a time and spatial resolution of 2 ms and 45 μm, respectively. The resolution of depolarization was 4096 levels. The tissue was stimulated from a point in the signal recording area (Figure 1) via a small unipolar tungsten electrode at a cycle length (CL) ranging from 150 to 500 ms (1.0-ms square wave pulses 1.5 times the threshold). Rapid pacing and short-coupled extra stimuli (S1-S2) were applied to induce conduction block. The local activation time at the respective pixel site was determined by 50% depolarization in the upstream of the action potential signal. The conduction velocities longitudinal and transverse to the fiber orientation were calculated from the slope of a linear least-square fit of the activation times plotted against the distance. A 3×3 (for the image plane) ×3 (for time) Gaussian 3D-matrix filter was used for the activation times plotted against the distance. A 3×3 (for the image plane) ×3 (for time) Gaussian 3D-matrix filter was used for the activation times plotted against the distance.

**Statistics**

Descriptive statistics are expressed as the mean±SEM. Data were analyzed using ANOVA followed by Bonferroni’s test, unless otherwise noted. Differences were considered significant at P<0.05.

**Results**

**Standard Light Microscopy**

Age-dependent changes in histologic features were examined using standard light microscopy. The right atrial tissue sections exhibited a parallel myofiber orientation (Figure 1). The myofiber width increased from infant to young and old dogs. The cross-sectional area of the myofibers was 32.6±0.8 μm² in infant, 118.3±2.9 μm² in young, and 190.0±3.5 μm² in old dogs (n=155 to 157 myofibers; P<0.05 infant versus...
young versus old). The atrial tissues of the old dogs were characterized by a large amount of fat cell infiltration, causing a wide separation of the unit bundles. The area occupied by fat cells in the field of view was 0.4±0.1% in infant, 1.3±0.2% in young, and 17.9±2.4% in old dogs (n=8 fields; P<0.05 old versus infant and young).

**Distribution of Immunolabeled Gap Junctions**

Figure 2 shows single confocal optical slices sectioned longitudinally (parallel to the fiber orientation). In infant dogs, the punctate Cx43 immunolabeling was distributed uniformly over the entire surface of the myocytes (Figure 2A). In old dogs, the Cx43 labeling formed clusters of punctate immunofluorescence confined to the cell termini at the intercalated disks running across the longitudinal axis (Figure 2C). Young dogs (Figure 2B) had an intermediate pattern; some of the Cx43-labeled spots had polarized to the cell termini, but others were at the lateral cell abutments (Figure 2B). We estimated the proportion of Cx43 labeling in the transverse array (at the position of intercalated disks) over the total labeling. The results obtained from 12 dogs (4 infant, 4 young, and 4 old) are summarized in Figure 2D. The proportion of labeling at the intercalated disks increased with age.

**Wave-Front Configuration and Conduction Velocity**

Figure 3 shows the representative action potentials and activation wave fronts during centrifugal propagation from a stimulating point at a CL of 500 ms. Sequential images of the propagation in a small square (2.8×4.2 mm) visualized by our optical mapping system are presented in 2-ms steps. In infant dogs, all 10 preparations showed “teardrop” or “elliptical” activation patterns, with a smooth transition between the faster longitudinal propagation and the slower transverse propagation. In contrast, all 10 preparations from old dogs produced “square” wave fronts, with an abrupt transition from the very fast longitudinal propagation to the slow transverse propagation; the activation front in either direction was almost flat. Intermediate patterns were observed in the young dogs; 5 of 11 preparations exhibited an elongated ellipse pattern, whereas the remaining 6 showed an almost square pattern.

We estimated the conduction velocities during longitudinal and transverse propagation (θL and θT) at CLs ranging from 150 to 500 ms. The results obtained from 31 dogs are summarized in Figure 4A. The young and old dogs had a significantly higher θT than the infant dogs. The average values of θL in the old dogs were greater than those in the infant dogs.
young dogs, although the difference was not significant. The age-dependence of the conduction velocity was less striking for $\theta_L$. Although the values were largest in the young dogs, smallest in the infant dogs, and intermediate in the old dogs, the differences did not reach statistical significance. The anisotropic ratio of the conduction velocity ($\theta_{L/T}$) increased with increasing age. $\theta_{L/T}$ was highest in the old dogs, and lowest in the infant dogs throughout the entire range of CL tested.

To highlight the aging effects on CL dependence, $\theta_L$, $\theta_T$, and $\theta_{L/T}$ were normalized to the values at a CL of 500 ms (Figure 4B). The reduction in $\theta_L$ at shorter CLs was most prominent in the infant dogs and less remarkable in the old dogs: $\theta_L$ decreased by 33.0±5.0% in the infant dogs when CL was reduced from 500 to 150 ms, whereas it decreased by 20.0±3.7% in the old dogs ($P<0.05$, infant versus old). The reduction in $\theta_T$ at shorter CLs was nearly identical among the 3 groups. As a result of this directional difference in rate

Figure 3. Age-dependent changes of the activation patterns. The atrial tissue was stimulated constantly at CL=500 ms from a point in the right middle. Fiber orientation was in a right-to-left direction. An area at the diastolic (resting) potential is shown black, and the color turns red on depolarization. The activation pattern changes from elliptical to square with increasing age. Right panels illustrate the schematic diagram of the activation wave fronts (T indicates transverse; and L, longitudinal). Representative action potentials are shown on the left.

Figure 4. CL dependence of conduction velocity and its anisotropic ratio. A, Longitudinal conduction velocity ($\theta_L$), transverse conduction velocity ($\theta_T$), and their ratio ($\theta_{L/T}$) were plotted against the CL of constant stimulation. B, $\theta_L$, $\theta_T$, and $\theta_{L/T}$ normalized to the values at CL 500 ms. Values are the mean±SEM of 10 infant, 11 young, and 10 old dogs. *Significantly different from the value at CL 500 ms; †significantly different from young and old dogs; ‡significantly different from the old dogs ($P<0.05$).
responses, old dogs exhibited the greatest increase in $\theta_{LT}$ at shorter CLs, whereas the change was minimal in infant dogs.

**Preferential Direction of Conduction Block**

Conduction block in the mapped area was observed during pacing at CLs shorter than 150 ms, and the block pattern was different in the different age groups. Representative results in an infant and an old dog are shown in Figure 5. In the infant (Figure 5A), a shortening of the pacing CL from 500 to 200 ms resulted in more crowded elliptical isochrones reflecting slowing of both the longitudinal and transverse propagation. At a CL of 140 ms, longitudinal propagation from right to left was terminated near the center of the mapped area with a U-shaped block line with a sharp convexity toward the left. The atrial muscle on the left was excited by slow wave fronts circumventing the block line by transverse propagation from the bottom. In the old dog (Figure 5B), the reduction of conduction velocity (crowding of isochrones) was greater in the transverse compared with the longitudinal propagation, giving rise to a more elongated square-pattern of the activation sequence. At a CL of 140 ms, transverse propagation was terminated at 2 straight block lines running parallel to the fiber orientation, whereas longitudinal propagation was preserved. The activation distal to the upper block line came from the left.

In either case of longitudinal block in the infant or transverse block in the old dog, the action potentials proximal to the block line decreased progressively in their amplitude. The action potentials recorded from the block line showed low-amplitude double potentials reflecting electrotonic interaction of the proximal and distal sites.

The block line during longitudinal propagation was unstable (it shifted easily in the same preparation when the pacing rate, premature interval, or stimulating current was changed), suggesting a functional basis for the conduction failure. In contrast, the block line during transverse propagation was usually fixed, suggesting a morphological basis. Microscopic anisotropic reentry did not occur in our experiments when either longitudinal or transverse block was induced.

The Table summarizes the direction of the conduction block induced by rapid pacing or premature stimulation in 31 preparations (10 infant, 11 young, and 10 old dogs). The incidence of longitudinal block decreased, whereas the incidence of transverse block increased with aging.

**Zigzag Trajectory of Propagation**

In 2 of the 5 old canine preparations showing preferential block during transverse propagation, a zigzag trajectory of propagation was observed. The activation sequences of atrial tissue (optical images) of an infant and an old dog under stimulation at CL 500 ms, 200 ms, and 140 ms are presented by 1-ms isochrones. Conduction block at short (140 ms) CL occurred in the longitudinal (L) direction in the infant (A), whereas it was observed in the transverse (T) direction in the old (B). Eight action potentials recorded across the block line (a to h in the infant tissue, and 1 to 8 in the old tissue) are shown on the right.

![Figure 5](http://circ.ahajournals.org/)

**Incidence of Longitudinal and Transverse Block in Infant, Young, and Old Dogs**

<table>
<thead>
<tr>
<th></th>
<th>Longitudinal Block</th>
<th>Transverse Block</th>
<th>Whole Refractoriness*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant (n=10)</td>
<td>40% (4/10)†</td>
<td>20% (2/10)†</td>
<td>40% (4/10)</td>
</tr>
<tr>
<td>Young (n=11)</td>
<td>27% (3/11)</td>
<td>36% (4/11)</td>
<td>36% (4/11)</td>
</tr>
<tr>
<td>Old (n=10)</td>
<td>0% (0/10)</td>
<td>50% (5/10)</td>
<td>50% (5/10)</td>
</tr>
</tbody>
</table>

*Activation ceased simultaneously in all directions because of refractoriness. †$P<0.05$ for infant vs old ($\chi^2$ test).
(frame rate 2 msec)

propagation was recognized in the optical mapping images. Representative results are shown in Figure 6. In this case, the first excitation event in response to a premature stimulus (S2) with a short (140 ms) coupling interval was a narrow wave front of longitudinal propagation from right to left in the middle of the mapped area. This was followed by a second narrow wave front from left to right below the first one. The second wave front was again followed by a third one from right to left. The effective conduction velocity of the S2 excitation toward the bottom (across the apparent block lines) was calculated to be 3.5 cm/s.

Discussion

Using high-resolution optical mapping of excitations in dog atria, this study has demonstrated that the activation fronts from a single pacing site change from an “elliptical” to a “square” pattern in association with a progressive increase of the anisotropic ratio of the conduction velocity with aging and also that the preferential direction of conduction block changes from longitudinal to transverse with aging. These changes in the anisotropic conduction properties are accompanied by age-dependent alterations of cell-packing architecture and gap junction distribution.

Different Curvature of Wave Fronts

Impulse propagation in cardiac tissue is influenced by the geometry of excitation wave fronts.15,16 When the excitation front curves outward (convex), the conduction velocity is lower than that with a flat wave front.17 This is because the local excitatory current supplied by the cells at the front of a convex wave distributes over a larger membrane area downstream. The degree of wave front bending is characterized by the local curvature (ρ), which is defined as the negative reciprocal of the local radius (r), and the convex wave front has a negative ρ. Because the conduction velocity decreases as the wave front curvature becomes more negative, it follows that the velocity will become zero at some critical level of ρ (ρc). In 2D normal mammalian cardiac tissues, Fast and Kleber15 estimated a critical curvature ρc = 66 cm⁻¹, which corresponds to a critical radius of rc = 152 μm.

In infant canine atria, the atrial cells of cylindrical shape were coupled by gap junctions distributed homogeneously. The tissue may, therefore, behave as continuous media, and the wave fronts had an elliptical configuration. Longitudinal propagation with a much larger (more negative) local curvature than transverse propagation would be expected to be blocked more easily at a shorter CL, where the excitatory sodium current is reduced to a critical level for propagation. This has been confirmed in the present study (Figures 4 and 5 and Table). Longitudinal block occurred most frequently in the infant atria with an elliptical activation pattern. The block line was unstable in the same tissue preparation. The local wave front radius at a short CL that caused longitudinal block in the infant atria (Figure 5A) was roughly 300 to 500 μm. This value is comparable to rc estimated by Fast and Kleber.15

With aging, the activation pattern changed to square and the incidence of longitudinal block decreased, whereas the incidence of transverse block increased. Longitudinal block was never observed in the old dogs (Table). The higher safety for longitudinal propagation in older dogs compared with younger dogs is probably due to the almost flat wave front geometry (minimal curvature) of longitudinal propagation in the older animals.

Lateral Uncoupling With Aging

The gap junction distribution became progressively more polarized with increasing age and localized to the cell termini, giving rise to a decrease of lateral cell coupling. The lateral uncoupling in older dogs was further enhanced by widening of interstitial spaces to allow fat cell infiltration. In the older dogs, transverse propagation was more susceptible to block at a shorter CL. Transverse block in the older dogs, unlike longitudinal block, occurred at a fixed line along the fiber orientation. This feature suggests a prominent microscopic structural barrier for a local current circuit.

Our optical mapping images first visualized a zigzag propagation producing a very slow effective θT (3.5 cm/s) in old dog atria. Such extremely slow conduction via a mechanism of microscopic zigzag conduction may set the stage for reentrant arrhythmias, as suggested by de Bakker et al18 in patients with myocardial infarction.
Preferential Direction of Conduction Block

In old canine and human atrial preparations showing discontinuous conduction properties, Spach et al. demonstrated that longitudinal propagation is more susceptible to blocking than is transverse propagation with short-coupled premature stimuli, while the propagation in young atrial muscle, which possesses continuous conduction properties, ceased simultaneously in all directions. These observations are contrary to our findings. Spach et al. analyzed the activation pattern by the sequential mapping of extracellular potentials. It may be difficult to recognize conduction failure at a microscopic level by such potential mapping experiments, especially when the block is unstable and can change on a beat-to-beat basis.

As to the preferential direction of conduction block in cardiac tissue, conflicting reports exist. Some studies showed that longitudinal propagation was more vulnerable to block under conditions to reduce the excitability of cardiac cells, while others showed that transverse block preferentially occurred. The safety of conduction depends on the curvature of wave fronts and on the electrical coupling between the cells. The conflicting reports could be due to the different contribution of these 2 factors in the respective experimental condition. Our results suggest that the curvature effect may play a pivotal role in the well-coupled continuous tissue, whereas its contribution may be less important in the discontinuous tissue with substantial lateral uncoupling.

Study Limitations

We examined anisotropic conduction properties in a small-restricted epicardial area of right atrial free wall. Because the atria have highly complicated structures, our results cannot be directly extrapolated to other sites, such as appendages and Bachmann’s bundles.

Acknowledgments

This study was carried out as a part of “Ground Research Announcement for Space Utilization” promoted by the Japan Space Forum. The authors express their gratitude to Drs Takashi Tominaga and Yoko Tominaga for their technical assistance.

References

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Circulation. 2002;105:2092-2098; originally published online April 22, 2002;
doi: 10.1161/01.CIR.000015506.36371.0D

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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