Effects of activation sequence and anisotropic cellular geometry on the repolarization phase of action potential of dog ventricular muscles

TOSHIYUKI OSAKA, M.D., ITSUO KODAMA, M.D., NAOYA TSUBOI, M.D., JUNJI TOYAMA, M.D., AND KAZUO YAMADA, M.D.

ABSTRACT  The influence of activation sequences on action potential configuration, especially in the repolarization phase, was examined in isolated canine ventricular muscles. Action potentials were recorded from the epicardial surface in the center of a preparation having nearly uniform fiber orientation (25 × 25 mm). Stimuli applied just adjacent to the recording site produced nearly centrifugal propagation. An activation sequence either parallel (longitudinal) or perpendicular (transverse) to the long axis of the muscle fibers was produced by peripheral stimulation. Action potential duration at -60 mV (APD₂₀₀₅₅) during centrifugal propagation was significantly longer than that during longitudinal propagation. Further shortening of APD₂₀₀₅₅ was observed during transverse propagation. When a collision of longitudinal or transverse wavefronts (longitudinal or transverse collision) was produced at the action potential recording site, the shortest APD was recorded. During centrifugal propagation, action potential mapping around the stimulating electrodes revealed that APD₂₀₀₅₅ shortened gradually as the recording site was moved further from the stimulation site. The spatial gradient of APD was steeper in the transverse than in the longitudinal direction, causing a distortion in the repolarization sequence and the recovery of excitability near the center of the tissue. Premature stimuli applied to an area near the central stimulation site induced one-way block and circus movement of the wavefront, indicating reentry of excitation. We concluded that the activation sequence and anisotropic cellular geometry substantially affect APD, and that such a change contributes to the spatial inhomogeneity of refactoriness leading to reentrant arrhythmias.


THE PROPAGATION of excitation in cardiac muscle has generally been treated as though it occurred in a continuous structure. According to the continuous-medium theory, the time courses of transmembrane action potentials in cardiac tissue with homogenous membrane properties should be the same everywhere when the resting or take-off potential is the same. Contrary to this prediction, recent experiments by Spach et al.1-5 demonstrated that the rate of rise of action potentials in atrial or ventricular muscles was greatly affected by the activation sequence. They explained these facts by assuming an electrotonic interaction between neighboring cells in tissue having anisotropic cellular geometry, and proposed a concept of “discontinuous conduction.” If the repolarization phase of an action potential were affected by similar electrotonic interaction during the propagation of excitation, a substantial change in action potential duration (APD) and refactoriness would be induced. However, little information is available on this issue.6-11

In the present study the effects of activation sequence on action potential configuration, especially in the repolarization phase, were examined in isolated canine ventricular muscle preparations. The influence of the resultant change in APD on the recovery of excitability from region to region was also investigated to evaluate possible roles for the genesis of reentrant arrhythmias.

Methods

Tissue preparation and equipment. Twenty-four mongrel dogs weighing 7 to 12 kg were anesthetized with sodium pentobarbital (30 mg/kg iv). The hearts were removed quickly and
dissected in aerated Krebs-Ringer solution at 4° C. A 25 mm square section of myocardium was isolated from the right ventricular free wall. The endocardial side of the preparation was sliced off by gentle dissection with scissors to limit its thickness to less than 2.0 mm. The epicardial surface of the preparation showed nearly uniform fiber orientation (figure 1). The tissue was mounted in a tissue bath, epicardial side up, and superfused with the solution gassed with 95% O₂ and 5% CO₂ at a rate of 50 ml/min. The composition of the solution was as follows (mM): NaCl, 120.0; KCl, 4.0; MgSO₄, 1.3; CaCl₂, 1.8; NaH₂PO₄, 1.2; NaHCO₃, 25.2; and glucose, 5.5. The temperature was maintained at either 30° or 35° C.

Figure 2 illustrates the electrode array used for recording and stimulation. Extracellular recording electrodes were made of Teflon-coated stainless wire with a diameter of 100 µm. In most cases, we used modified bipolar electrodes to record monopolarly from the surface of the muscle. One electrode was placed on the surface of the tissue and the other was clipped short so that it would remain 1 to 2 mm above the surface as a reference. These electrodes were aligned in a Plexiglas plate having small holes (less than 1.0 mm in diameter) spaced 3 mm apart in five rows. The rows of holes were adjusted to be parallel to the long axis of the muscle fibers under the microscope. Each electrode tip was passed through the hole and positioned gently on the epicardial surface of the preparation. At the center of the Plexiglas plate was a hole with a diameter of 5.0 mm for recording the transmembrane action potentials and/or extracellular potentials. A pair of contiguous bipolar electrodes with an interpolar distance of 100 µm was used for the central extracellular recording. These electrodes were also used as stimulating electrodes for centrifugal propagation of excitation. Sixteen pairs of contiguous bipolar electrodes made of silver wire with an interpolar distance of 500 µm were mounted in four groups on the margins of the Plexiglas plate. They were placed perpendicular to the long axis of the muscle fibers on the right (ST-L1) and left (ST-L2), and parallel to that axis on the top (ST-T1) and bottom (ST-T2).

Electrograms from the 25 extracellular electrodes were recorded simultaneously with the use of a mapping system for body surface and epicardial potentials (HPM6500, Chunichi Electric Co.). The alternating-current amplifier in each channel had a gain of 2000. The electrograms were digitized with a 12-bit analog-to-digital converter at a sampling rate of 1000/sec and stored in memory as well as on a floppy disk. The digitized data were redisplayed as waveforms. The arrival time of activation at each lead point was determined by the measurement of the interval from the stimulus artifact to the peak negative deflection of each electrogram. The system carried out the measurements automatically and constructed an isochronal map, with accuracy checked by the operator. Conduction velocity was calculated from the distance traveled by an isochrone per unit time.

Transmembrane action potentials were recorded from a surface cell using two standard glass microelectrodes filled with 3M KCl and placed closely together, one intracellularly and the other extracellularly. These microelectrodes were each connect-
ed by Ag-AgCl wire to a unity gain, high-input impedance differential amplifier. The upstrokes of action potentials were electrically differentiated to measure the maximum upstroke velocity (Vmax).

**Protocols.** First, we examined the influence of activation sequences on action potential configuration at the center of the tissue using the multiple electrode grid (figure 2). The tissue was constantly driven at 0.5 Hz through the central or peripheral electrodes. Pulses used for stimulation were 2 msec in duration and 1.2 times the diastolic threshold.

Second, the APD and the refractory time (RT) were mapped within an area of about 100 mm² around the basic stimulation electrodes at the center of the preparation. During the mapping of APD the tissue was stimulated constantly at 0.5 Hz, and action potentials were recorded sequentially from 20 to 40 sites. Pulses for stimulation were 2 msec in duration and 1.2 times the threshold. When RT was measured, a premature test stimulus (S2) was applied through another pair of contiguous bipolar electrodes after the eighth basic stimulus (S1) at 0.5 Hz. S1 electrodes were fixed at the center of the tissue, while S2 electrodes (exploring electrodes) were moved in sequence from 20 to 30 points within an area of about 25 mm² surrounding the S1 electrodes. Pulses for S2 stimuli were 2 msec in duration and twice the diastolic threshold. The S1-S2 interval was shortened in 2 msec steps up to the instant when S2 was no longer captured by the overall excitation of the preparation. This S1-S2 interval was defined as RT.

Finally, we attempted to induce the "reentry" of excitation by applying premature stimuli. As in the case of RT measurement, two pairs of stimulation electrodes were used, one for basic stimulation (0.5 Hz) at the center of the preparation, and another for premature stimulation. After the eighth basic stimulus (S1), single (S2) or double (S2 + S3) premature stimuli were applied to the test site with various coupling intervals. Pulses for stimulation were 2 msec in duration. The intensity was 1.2 times and 3 times the diastolic threshold for S1 and for S2 and S3, respectively. Activation sequences were recorded with the use of the multiple electrode grid for extracellular potential mapping (figure 2). Therefore, the test site was limited to an area of 5 mm in diameter around the center of the preparation. Distant bipolar electrograms were recorded through two pairs of Ag-AgCl wire electrodes (1.0 mm in diameter) placed on each margin of the preparation in both the horizontal and vertical directions to monitor the overall excitation.

**Morphology and statistical analysis.** At the end of each experiment the preparation was fixed in 10% formalin and sectioned parallel to the epicardium at 100 μm intervals to verify the fiber orientation histology (figure 1). The tissue was stained with Mallory-azan.

Values were expressed or plotted as the mean ± SE unless otherwise stated. Statistical analysis of measured variables was performed with Student’s t test for paired data. Analysis of variance was used when multiple unpaired data were compared. p values of less than .05 were considered to indicate a significant difference.

More details on each procedure are given in Results.

**Results**

**Effects of activation sequences on action potential configuration.** Effects of five patterns of activation sequence on the action potentials recorded from the center of the preparation were examined at either 30° or 35° C. A single impalement of microelectrode was maintained throughout each experiment.

Figure 3 illustrates representative results at 30° C. When the stimuli were applied to the center of the tissue, just adjacent to the recording microelectrode, the activation front proceeded nearly centrifugally, with a higher speed in the longitudinal direction (along the long axis of the muscle fibers) than in the transverse direction (perpendicular to the long axis). Stimulation through the electrodes of ST-L1 caused longitudinal propagation with parallel isochrones at a conduction velocity of 0.34 m/sec. Stimulation through the electrodes of ST-T1 caused transverse propagation with more crowded parallel isochrones at a conduction velocity of 0.15 m/sec. Collision of longitudinal or transverse wavefronts (longitudinal or transverse collision) at the action potential recording site was produced by the application of stimuli to both ST-L1 and ST-L2 or to both ST-T1 and ST-T2. The time delay of electrical stimuli between ST-L1 and ST-L2 (longitudinal collision) or between ST-T1 and ST-T2 (transverse collision) was adjusted in 1 msec steps until the highest values for Vmax and amplitude of action potential upstroke for the stimulation mode were obtained. These various activation sequences caused a marked difference in the upstroke phase of action potential despite the same resting membrane potential (-82 mV). Thus, Vmax and amplitude at phase 0 (APA) were highest during transverse collision (113 V/sec, 112 mV). The values then decreased in the following order: longitudinal collision (106 V/sec, 101 mV), transverse propagation (103 V/sec, 101 mV), and longitudinal propagation (75 V/sec, 97 mV). During centrifugal propagation, APA showed the lowest
value (92 mV), while a distinct Vmax value was not obtained because of the superimposed stimulus artifact. The repolarization phase of the action potentials was also affected by the changes of activation sequence. The APD at -60 mV (APD -60mV), which corresponds approximately to the effective refractory period, was longest during centrifugal propagation (386 msec). APD -60mV then decreased in the following order: longitudinal propagation (371 msec), transverse propagation (361 msec), and transverse collision (359 msec).

Table 1 summarizes the results obtained from six preparations at 30° C. Average values of Vmax and APA were highest during transverse or longitudinal collision. The values during transverse propagation were significantly higher than those during longitudinal propagation. The average value of APD -60mV was longest during centrifugal propagation, and shortest during longitudinal or transverse collision. The value during longitudinal propagation was significantly longer than during transverse propagation.

The results obtained at 35° C were qualitatively similar to those at 30° C (table 2). Thus, Vmax and APA were largest during transverse or longitudinal collision, and then decreased in the following order: transverse propagation, longitudinal propagation, and centrifugal propagation. On the other hand, APD -60mV was longest during centrifugal propagation, and then decreased in the order longitudinal propagation, transverse propagation, and longitudinal or transverse collision. Conduction velocity during longitudinal or transverse propagation at 35° C was 26% to 34% higher than at 30° C, whereas the extent of the change in APD at 35° C was appreciably less than at 30° C.

The data presented here reveal that not only the upstroke phase but also the repolarization phase of

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**TABLE 1**

Effects of activation sequences on action potential configuration at 30° C

<table>
<thead>
<tr>
<th>Conduction velocity (m/sec)</th>
<th>RMP (mV)</th>
<th>APA (mV)</th>
<th>V max (V/sec)</th>
<th>APD -60mV (msec)</th>
<th>ΔAPD (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>82.3 ± 0.5</td>
<td>89.2 ± 1.0</td>
<td>NS</td>
<td>378.8 ± 8.8</td>
<td>25.7 ± 1.5</td>
</tr>
<tr>
<td>L</td>
<td>82.3 ± 0.5</td>
<td>95.3 ± 1.1</td>
<td>A</td>
<td>353.8 ± 8.8</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>T</td>
<td>82.3 ± 0.5</td>
<td>104.3 ± 1.6</td>
<td>A</td>
<td>354.8 ± 8.7</td>
<td>0</td>
</tr>
<tr>
<td>Lc</td>
<td>82.3 ± 0.5</td>
<td>104.5 ± 1.8</td>
<td>A</td>
<td>353.0 ± 8.1</td>
<td>0</td>
</tr>
<tr>
<td>Tc</td>
<td>82.3 ± 0.5</td>
<td>104.5 ± 1.8</td>
<td>A</td>
<td>353.0 ± 8.1</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are mean ± SE from six preparations.

RMP = resting membrane potential; APA = action potential amplitude; ΔAPD = difference in APD -60mV from the one during transverse collision; C = centrifugal propagation; L = longitudinal propagation; T = transverse propagation; Lc = longitudinal collision; Tc = transverse collision.

*Significantly different at p < .05.
TABLE 2
Effects of activation sequences on action potential configuration at 35° C

<table>
<thead>
<tr>
<th>Conduction velocity (m/sec)</th>
<th>RMP (mV)</th>
<th>APA (mV)</th>
<th>Vmax (V/sec)</th>
<th>APD-60mV (msec)</th>
<th>ΔAPD (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>84.3 ± 0.7</td>
<td>85.3 ± 1.4</td>
<td>NS</td>
<td>242.1 ± 7.3</td>
<td>14.1 ± 1.1B</td>
</tr>
<tr>
<td>L</td>
<td>0.47 ± 0.02</td>
<td>84.3 ± 0.7</td>
<td>87.4 ± 1.2</td>
<td>106 ± 3.6</td>
<td>235.8 ± 7.2B</td>
</tr>
<tr>
<td>T</td>
<td>0.19 ± 0.01</td>
<td>84.3 ± 0.7</td>
<td>94.8 ± 1.3</td>
<td>133 ± 2.4</td>
<td>229.9 ± 7.5B</td>
</tr>
<tr>
<td>Lc</td>
<td>84.3 ± 0.7</td>
<td>101.6 ± 1.5</td>
<td>NS</td>
<td>159.0 ± 4.8</td>
<td>228.5 ± 7.4B</td>
</tr>
<tr>
<td>Tc</td>
<td>84.3 ± 0.7</td>
<td>101.6 ± 1.7</td>
<td>NS</td>
<td>162.3 ± 4.1</td>
<td>227.9 ± 7.5B</td>
</tr>
</tbody>
</table>

Values are mean ± SE from eight preparations. Abbreviations are as in Table 1.

*Significantly different at p < .05.
#Significantly different from the value at 30° C at p < .05.

action potentials are significantly affected by the activation sequence. The following experiments were designed to obtain further insight into the mechanism of this repolarization change and its possible roles in the genesis of reentrant arrhythmias. They were carried out at 30° C only because the change in APD is much greater at this lower temperature than at 35° C.

**Spatial distribution of APD and repolarization sequence.**

Figure 4 shows an APD map obtained during centrifugal propagation at 30° C. In this case, 34 action potentials were recorded sequentially from a central area of about 100 mm² surrounding the stimulating electrodes. APD-60mV was longest at the center of the tissue, which was closest to the stimulating electrodes, and shortened gradually as the recording site was moved further from the electrodes. The spatial gradient of change in APD was steeper in the transverse than in the longitudinal direction.

**FIGURE 4.** APD map around the stimulating electrodes during centrifugal propagation. Isochrone interval = 5 msec. Star indicates site of stimulation. Values are APD-60mV at that site (msec). The preparation was constantly driven at 0.5 Hz.
Figure 5 summarizes the results obtained in six preparations. A shortening of APD₆₀mV from the reference level at the center of the tissue was significantly marked along the transverse than the longitudinal direction. For instance, the shortening of APD₆₀mV at 1 mm and 2 mm away from the stimulation site was 12.7 ± 0.7 and 17.3 ± 0.7 msec, respectively, in the transverse direction, compared with 2.0 ± 0.3 and 4.3 ± 0.7 msec in the longitudinal direction.

Figure 6 shows a repolarization sequence. The repolarization time was defined as the interval from the stimulus artifact to the instant of repolarization reaching -60 mV (activation time + APD₆₀mV). Data were obtained from the same preparation as presented in figure 4. If all the recording sites showed uniform APDs, the repolarization sequence would be determined only by the activation time, and a centrifugal pattern analogous to the activation map (figure 3, top left) would be constructed. However, this was not the case because of nonuniform APD distribution. Thus, in the vicinity of the stimulation site (less than about 2 mm away) the repolarization process proceeded more rapidly in the transverse than in the longitudinal direction, resulting in vertically elongated isochrone lines. This is in contrast to the horizontally elongated isochrone lines of the activation pattern. Also, the earliest repolarization site was not at the center of the preparation, but about 1 mm away from the stimulation site in the transverse direction.

Spatial distribution of the RT was examined in four preparations. A representative result is shown in figure 7. The shortest RT was observed at a point about 1 mm away from the center of the tissue in the transverse direction. The isochrone lines of RT were vertically elongated, as shown in the repolarization sequence presented in figure 6.

Reentrant excitation induced by premature stimuli. The RT map presented in figure 7 suggests that if an ectopic premature excitation originates near the center of the tissue, its activation wave will proceed preferentially in the medial and transverse direction (toward an area having a shorter refractory time), whereas activation toward the lateral and longitudinal direction will be inhibited by the longer RT. Such a distortion of activation sequence is considered to lead to one-way block and reentry of excitation. This assumption was tested in six preparations with use of the electrode grid for extracellular potential mapping (figure 2).

A basic stimulus (S₁) applied to the center of the preparation caused a centrifugal propagation with a higher conduction velocity in the longitudinal direction reflected in horizontally elongated isochrones (figure 8). Premature stimuli (S₂, S₃) were applied to a point 1 mm in a right lower direction from the S₁ stimulation site. S₁-S₂ and S₂-S₃ intervals were set at just above the refractory time. Activation caused by S₂ 375 msec after S₁ was characterized by asymmetric isochrones, with an appreciable delay in the right longitudinal direction. When the second premature stimulus (S₃) was applied 355 msec after S₂, it caused a much more distorted activation pattern. Thus, rightward propagation toward "e" electrodes was blocked and a low amplitude potential indicating an electrotonic influence from "a" was recorded in electrogram "e" (figure 8). The S₃ activation front circulated slowly around the blocked area in a clockwise direction, as shown by the order of arrival of local activation at "b," "c," "d," and "e." Within the tissue square covered by the electrode grid, the right upper corner was activated last (226 msec after initiation of S₃). This S₃ activation was followed by a nonstimulated activation (E) initiated from "a," proximal to the area blocked during the S₃ activation. During the E activation the wavefront also circulated in a clockwise direction from "a" to "b," "c," "d," and "e."

Of the 46 trials in six preparations, a single, nonstimulated beat was induced in eight cases in four preparations by two sequential premature stimuli. In six of these eight cases, a local block and a circus
movement of the wavefront (as presented in figure 8) were documented, but in the remaining two cases no such activation sequence indicating reentry of excitation was observed in the area covered by the multiple electrode grid. The sites of premature stimulation in the eight cases showing nonstimulated beats were restricted to four regions, all about 1 mm away from the S1 electrodes in a diagonal direction intermediate between longitudinal and transverse orientations (figure 9). However, no single premature stimulus induced any nonstimulated excitation.

Discussion

The present results reveal that not only the depolarization phase but also the repolarization phase of action potentials in ventricular muscle are substantially affected by the activation sequence. The changes observed in the depolarization phase (APA, Vmax) are consistent with those noted in previous studies by Spach et al.1-5 and Tsuboi et al.10 in atrial or ventricular muscles. These authors proposed an electrotonic interaction between neighboring cardiac cells as a possible mechanism. Thus, during propagation, some of the ionic current that should be used for the construction of phase 0 at the site of excitation is diverted to the local circuit current (axial current) to charge the capacitance of the downstream membrane and to depolarize it to the threshold for excitation. In this way, the downstream cardiac tissue would be a kind of “current sink” slowing down the upstroke phase of action potentials. This concept is supported at least in part by recent simulation studies of propagation of cardiac action potential in which the structural discontinuity of axial resistance (discretization) was incorporated into the model.9,15-17 In two-dimensional cardiac tissue, as used in the present study, the downstream load causing a decrease in Vmax and APA is considered to be largest during centrifugal propagation and smallest during collision of wavefronts. Downstream load during longitudinal propagation may be larger than that during transverse propagation because axial resistivity for local circuit current in the transverse direction is much higher than in the longitudinal direction. Clerc18 demonstrated that the axial resistance of calf ventricular
Refractory Time

FIGURE 7. Top, RT map around the basic stimulation electrodes at the center of preparation. Isochrone interval = 5 msec. Solid star shows the site of basic stimulation. Values indicate RT at that site (msec). RT was measured by the application of a premature test stimulus after the eighth basic stimulus at 0.5 Hz. Excitation of the preparation was monitored via the modified bipolar electrodes at site indicated by open star. Bottom, Actual recordings. In each trace S₁ shows the eighth basic stimulus, and S₂ shows a premature stimulus applied to the S₁ site (C), to a site 1 mm away from the S₁ site in the left longitudinal direction (L₁), or to a site 1 mm away from the S₁ site in the upper transverse direction (T₁). Values indicate S₁-S₂ intervals (msec). RT was defined as the longest S₁-S₂ interval that failed to capture overall excitation of the preparation.

The change in APD in the present study may also be explained by such electrotonic interaction between neighboring cardiac cells.20-22 During centrifugal propagation, repolarization may proceed from the central to the peripheral area of the preparation if the tissue is composed of cells with uniform membrane properties. In this condition, cardiac cells at the center of the tissue would have more negative intracellular potentials during the repolarization phase than cells in the surrounding area, causing a local circuit current from the peripheral to the central area. This depolarizing current seems to prolong the APD (downstream effect). During collision of excitations, the downstream effect is nearly completely eliminated. Instead, a local circuit current in the opposite direction (from the central to the peripheral) would be expected, because repolarization of the central area would be preceded by repolarization in the whole of the remaining tissue. This repolarizing current (upstream effect) may cause shortening of the APD.

With respect to the effect of wavefront collision during the repolarization phase of an action potential, there is some controversy among previous investigators. Spach et al.24 reported that collision did not change the APD in Purkinje strands. Steinhaus et al.,9 however, showed that APD of Purkinje fibers and ventricular muscles was slightly but significantly shorter during wavefront collision than during one-way propagation. Toyoshima and Burgess6 observed that the refractory period of ventricular muscle in intact canine heart was shortened by wavefront collision, and suggested the shortening of APD by an electrotonic interaction. Our data tend to support the latter two groups.

During one-way propagation, the central recording site may simultaneously receive both depolarizing and repolarizing effects from the downstream and the upstream sites, respectively. In the present experiments, however, APD during longitudinal propagation was longer than that during transverse propagation. This suggests that the upstream effect is much weaker than the downstream effect, since electrotonic influence in the longitudinal direction is much greater than in the transverse one.25 In accordance with this assumption, there was no significant difference in the APD during longitudinal collision and during transverse collision despite their different upstream effects. Cranefield and Hoffman26 showed that subthreshold depolarizing current applied during the late phase of repolarization was more effective in prolonging APD than repolarizing current was in shortening APD. The different intensities of the downstream and upstream effects may be related to membrane properties during the late phase of repolarization.

An alternative explanation is the different "effec-
tive” distance from the stimulating electrodes to the recording site. Our APD mapping experiments demonstrate that the lengthening of APD, which may be caused by the downstream effect, is more marked in the longitudinal than in the transverse direction, probably due to the different electrical coupling in the two directions. Thus, the effect of a stimulus in prolonging APD reaches more than 5 mm in the longitudinal direction, but is restricted to about 3 mm in the transverse direction (figures 4 and 5). During one-way transverse or longitudinal propagation, the distance from the peripheral stimulating electrodes to the central recording electrodes was 8 mm. Accordingly, there would be sufficient electrotonic influence to prolong APD during longitudinal but not during transverse propagation. A simulation study on APD in anisotropic cardiac tissue by Steinhaus et al. also showed this difference in effective distance. The stimulation sites and cut ends of the tissue will have similar but opposite effects on APD. However, this cut-end effect may be much weaker than the stimulation effect because the distance from the cut ends to the central recording electrodes in our experimental setup was longer than 12 mm.

The activation sequence–dependent change in APD at 35°C was appreciably less than that at 30°C. This may be attributable to increased conduction velocity, which causes a decrease in spatial gradient of intracellular potential between neighboring cells during the repolarization phase. The local circuit current at 35°C would, therefore, be reduced provided that axial resistivity remained unaffected. A simulation study by Steinhaus et al. showed that electrotonic modulation of APD by neighboring excitable membranes is enhanced by lowering the conduction velocity.

The present study also indicated that the repolarization sequence and the regional recovery of excitability
are largely affected by the activation sequence–dependent change in APD. In cases of centrifugal propagation, isochrones of the excitation near the center of the preparation were longitudinally elongated, whereas isochrones of the repolarization sequence and of refractory time were vertically elongated. Moreover, the earliest recovery of excitability was observed not at the center of the preparation, from which excitation was initiated, but approximately 1 mm away from the central stimulation point in the transverse direction. This distortion in the regional recovery of excitability may be a prerequisite for reentry of excitation.

To test this assumption, we applied premature stimuli to various points near the central electrodes used for basic stimulation. No single premature stimulus induced reentry of excitation. However, when two premature stimuli were applied in sequence, nonstimulated excitation could be induced, and a one-way block with accompanying cusp movement of excitation was documented. Kuo et al. investigated the influence of two consecutive premature stimuli (S₂, S₃) on the dispersion of repolarization of monophasic action potentials (MAPs) in canine ventricles. Their results indicate that an S₂ activation front reaching the incompletely repolarized regions of myocardium with longer MAP duration induces slower conduction, resulting in an increase in activation time difference distal to the stimulation site, and that this larger activation time difference facilitates induction of reentrant arrhythmias by the enhancement of the dispersion of repolarization of the S₃ activation. Our results can be explained by similar mechanisms. The basic spatial inhomogeneity of APD during S₁ activation due to the anisotropic cellular geometry would induce regional conduction delay of S₂ activation, resulting in a larger spatial inhomogeneity of repolarization. In this study the sites of premature stimulation inducing nonstimulated beats were restricted to four regions all about 1 mm away from the central electrodes in a diagonal direction intermediate between longitudinal and transverse orientations. Those areas might have the steepest spatial gradient of intracellular potential during the repolarization phase. Further experimental studies including more detailed action potential mapping during S₁ and S₂ activation are required to clarify these points.

Reentry of excitation is a major mechanism for cardiac tachyarrhythmias. It is generally accepted that the requirement for reentry is a spatial variation in propagation based on spatial variations in membrane properties. We have shown above that the activation sequence and anisotropic cellular geometry can distort the repolarization sequence even when the membrane properties are homogenous throughout the tissue. Such distortion, which may be enhanced at lower conduction velocities, would cause a spatial inhomogeneity of refractoriness and set the stage for reentrant arrhythmias. Further clarification of reentry due to spatial inhomogeneity of refractoriness should therefore involve not only spatial variations in membrane properties but also the activation sequence in relation to the structural characteristics of the cardiac tissue.

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