Effects of Cellular Uncoupling on Conduction in Anisotropic Canine Ventricular Myocardium


Experiments were performed on canine superfused ventricular epicardial tissue slices to determine the effects of 1.0–2.0 mM heptanol, an uncoupling agent, on conduction longitudinal and transverse to myocardial fiber orientation. Conduction velocities were measured between proximal and distal pairs of epicardial electrodes oriented transverse and longitudinal to the direction of a conducted wavefront evoked by pacing at a basic cycle length of 2,000 msec from one margin of the tissue before and after the addition of heptanol. In a separate group of tissues, the dual bipolar orthogonal electrode was used to sequentially map epicardial activation at 40 to 45 sites in a 1 cm x 2 cm area before and 30 minutes after the introduction of heptanol. In a third group of tissues, transmembrane potentials were recorded with standard microelectrode techniques to determine the effects of heptanol on action potential characteristics. Heptanol did not significantly effect action potential amplitude or maximum rate of depolarization. After 1.0 mM heptanol, conduction velocity began to decrease in 1–2 minutes and reached a steady state in 15–20 minutes. Conduction velocity in the longitudinal direction decreased from a control value of 0.56 ± 0.13 to 0.46 ± 0.10 M/sec (±SD) at 30 minutes after heptanol (p = 0.005). In the transverse direction, it decreased from 0.24 ± 0.09 to 0.17 ± 0.05 M/sec (p = 0.002). The ratio of longitudinal to transverse conduction velocities increased from 2.54 ± 1.00 to 2.94 ± 0.82 (p = 0.042). Thus, heptanol preferentially slowed conduction in the transverse direction. Because heptanol did not greatly influence active membrane properties, we used cable equations to calculate the time course of the change in effective junctional resistivity, which rose from 133.2 Ω · cm before heptanol to 312.2 Ω · cm 30 minutes after heptanol administration. We conclude that heptanol slows conduction velocity by selectively increasing junctional resistivity. The preferential slowing of conduction in the transverse direction is most likely due to the fact that more junctional resistances are encountered per unit distance in the transverse than in the longitudinal direction. (Circulation Research 1988;63:879–892)

While the anatomic anisotropic nature of canine ventricular myocardium has been well described, the electrophysiological implications of these characteristics have only been recently investigated. Several investigators have demonstrated directional differences in the conduction velocity of impulse propagation relative to fiber orientation and have explained that the slower velocity in the transverse direction is due to a greater effective axial resistance in the transverse direction. In addition, Spach et al have shown that ouabain, which increases junctional resistivity, preferentially depressed impulse conduction transverse to fiber orientation. Using a mathematical model of anisotropic cardiac muscle, they explained the directional differences of ouabain on conduction velocity in terms of an equivalent circuit in which many more junctional resistances per unit distance of impulse conduction are present transverse than longitudinal to fiber orientation. Thus, pharmacological agents that affect junctional resistance can be expected to have a greater effect on the impulse propagation transverse to fiber orientation. Recent studies by Kadish et al have shown that procainamide, which increases the space constant as well as decreases inward sodium current, has differential effects on impulse conduction relative to fiber orientation in anisotropic ventricular muscle. One

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possible mechanism could involve procainamide's effect on junctional resistance. Further support of a preferential effect on transverse conduction was provided by a recent study by Delmar et al., who used the uncoupling effect of heptanol in sheep epicardial muscle to demonstrate that conduction block occurs first transverse to myocardial fiber orientation. However, in their study, the preparations eventually progressed to complete block in both longitudinal and transverse directions. One objective of the present study was to establish a preparation in which conduction is slowed to a steady state by an increase in junctional resistance without progression to complete block. The advantages of such a system are twofold. First, in normal anisotropic cardiac muscle, the degree of the preferential slowing of conduction transverse to fiber orientation should allow indirect calculation of the magnitude of the increase in junctional resistance through the use of standard cable equations applied in two dimensions. Currently, direct measurement of nerval resistance can only be accomplished through the use of isolated cardiac myocyte pairs. Second, if the degree of conduction slowing induced by a given concentration of heptanol can be quantified for normal myocardium, then heptanol can be used as a pharmacological tool to investigate the role of high junctional resistance in various types of slow conduction. In this regard, Joyner and Overhold have recently verified that there is a restricted pathway for intercellular current flow across the Purkinje-muscle junction (a slow conduction region) by showing that octanol preferentially slows propagation from Purkinje to muscle.

In an attempt to test the hypothesis that changes in junctional resistance preferentially influence propagation transverse to fiber orientation in cardiac muscle and to establish a tissue model for studying cell coupling, we evaluated the effects of heptanol, an aliphatic alcohol, on conduction in anisotropic canine ventricular muscle. Since heptanol has been shown to decrease cellular coupling and is reported to have minor effects on action potential depolarization in mammalian heart, observed alterations in conduction velocity may be attributed primarily to changes in junctional resistance. The pharmacological modification of junctional resistance could provide a tool to evaluate the role of cell coupling in conduction, which may be crucial in elucidating the mechanisms of some arrhythmias and antiarrhythmic interventions.

Materials and Methods

Tissue Preparation

Fourteen adult mongrel dogs weighing between 10 and 20 kg were anesthetized with intravenous sodium pentobarbital (30 mg/kg). The hearts were excised through a left lateral thoracotomy, and 19 rectangular tissue samples approximately 1.5 x 2 cm were shaved from the epicardial surface of either the right or left ventricle. To minimize the effect of the change in fiber orientation with increased depth from the epicardial surface, all tissue samples were thinly sliced to a depth of only 2–3 mm from the epicardial surface. Slices were removed so that the fiber orientation was parallel to the long axis of the cut tissue. Tissue samples were harvested from areas without epicardial fat, large epicardial blood vessels, or irregularities in fiber orientation such as those seen at the apex. The ability to obtain parallel fiber orientation over the length of these small tissues has been confirmed previously.

Cut slices were placed in the tissue bath with the epicardial surface facing upward. The bath was superfused with Tyrode's solution containing 1.6 mM calcium and equilibrated with 95% O2-5% CO2 at 37 °C. Tissues were stimulated with a Teflon-coated bipolar silver electrode with constant current rectangular pulses 2 msec in duration, twice diastolic threshold at a cycle length of 2,000 msec.

Extracellular Potential Recording

Extracellular potentials were recorded in two ways. A custom-made dual bipolar recording electrode probe containing four 0.4-mm diameter electrodes arranged in two orthogonal pairs with 0.6-mm interelectrode spacing was used to record two simultaneous bipolar electrograms. The axis of the two orthogonal bipolar pairs of the probe was oriented so that one (designated X) was parallel to the long axis of the tissue, while the other (designated Y) was perpendicular. In addition to monitoring the individual bipolar electrograms, the X and Y pairs were placed into the X and Y axes of an oscilloscope and summed so that a loop could be generated. This loop has been shown previously to indicate the direction of impulse propagation at the recording site. The interpretation was verified in vivo by the comparison of vector loops with wavefront directions generated from maps of epicardial activation sequences. The inherent assumptions are as follows: The conducted wavefront approximates a uniform moving dipole, and each pair of electrodes are sufficiently close, so that the unipolar signals are morphologically identical, though shifted in time. The magnitude and polarity at each instant in time of the resultant difference signals of the X and Y bipoles is determined by the interelectrode distance, the conduction velocity, and the angle of the boundary of the dipole wavefront relative to the axes of the X and Y electrode pairs. Since, in the small area of the vector probe, the magnitude of the conduction velocity is assumed to be constant and both X and Y bipoles have the same interelectrode distance, the overall local direction of the wavefront can be derived from the maximum of the vector sum of the two orthogonal difference signals. This also assumes that conduction has no component that is not normal to the surface, which was assured with thin epicardial strips. Vector loop directions were measured from their origin to the
maximum X-Y amplitude and were arbitrarily defined in terms of their angle relative to the long axis of the tissue and therefore myocardial fiber orientation. Angles changed from 0° to 180° as the loop changed in the positive (upward) direction from leftward to rightward directed along the long axis and from 0° to −180° in the negative (downward) direction. As measured, the vector angle indicates the wavefront direction in the “field of view” of the orthogonal electrodes. We have previously estimated the field of view for bipolar electrodes 0.6 mm apart to be approximately 1–2 mm² in vitro.17

Standard close bipolar electrograms filtered between 1 and 1,000 Hz were also obtained using 28-gauge Teflon-coated silver wires exposed only at the tip in contact with the tissue.

Intracellular Potential Recording

Through the use of standard 3 M potassium-filled glass microelectrodes, transmembrane action potentials were recorded from cells in the superficial epicardial surface. The recorded action potentials and electronically differentiated rate of depolarization were displayed on an oscilloscope and photographed on 35-mm film. Action potential parameters were measured to the nearest 0.1 mV and 0.1 msec with a digitizing tablet (GTCO, Rockville, Maryland) interfaced with a computer (Hewlett-Packard, Sunnyvale, California). These measurements included the resting membrane potential, action potential amplitude, and the maximum rate of depolarization.

Experimental Protocol

After the tissues were placed in the bath, conduction was monitored continuously to verify stability. When conduction velocity remained stable for 30 minutes, experiments were performed in one of the following three ways.

Protocol A. This protocol, as schematized in Figure 1, was performed in five tissues. One corner of the tissue (ST) greater than 2 mm from either boundary edge was paced at a basic cycle length of 2,000 msec. The vector probes (V) and bipolar electrodes (BP) were positioned along each axis so that conduction times could be determined longitudinal (BP$_L$–VL) and transverse (BP$_T$–VT) to impulse propagation from the stimulation electrode. Conduction velocities longitudinal and transverse to fiber orientation were calculated from these conduction times and the measured distance between the recording sites. In addition, the measured distance between the recording sites. In addition, the longitudinal direction of impulse propagation was monitored through the generation of a vector loop representation of the direction of wavefront activation obtained through the vector sum of the two component bipolar electrodes contained in the vector probe (VL). This protocol enabled the continuous beat-to-beat recording of conduction velocities while vector loops were continually monitored to ensure the stability of the direction of impulse propagation.

Ensure that the direction of impulse propagation had not changed during the experiment.

After control measurements were obtained, heptanol was added to the tissue bath to achieve a concentration of 1.0 mM. Electrograms and vector loops were recorded for 30 minutes. An additional dose of heptanol was then added to bring the concentration to 2.0 mM, and data were recorded for 30 minutes. Heptanol was then washed out of the tissue bath, and data were recorded for 30 minutes during washout.

Protocol B. In five tissues, pacing at a basic cycle length of 2,000 msec was initiated midway along the short edge, greater than 2 mm from the cut edge (Figure 2). A roving vector probe was used to record X and Y bipolar electrograms and vector loops at 40–45 points with a clear soft plastic template to guide the probe. The template was 1 x 2 cm and was suspended over, but not touching, the tissue surface. The 45 equally spaced sites were separated by 0.25 cm and arranged in a 9 x 5 array. Each map required approximately 10 minutes to complete. From these points, isochronal activation
maps were constructed with an interpolation algorithm. With a linear regression analysis, conduction velocities were calculated from the slope of a plot of activation times and distances at multiple points taken along a line constructed in the direction of wavefront propagation in both the longitudinal and transverse directions. Examples of such lines are shown in Figure 2A.

After control activation maps were obtained, 1.0 mM heptanol was added to the tissue bath, and after 30 minutes, activation maps were repeated. After a 30-minute washout period, maps were again obtained. The advantage of this protocol was that conduction was determined from multiple sites across the tissue surface to verify uniform anisotropic conduction and to confirm the absence of gross irregularities in impulse propagation after the addition of heptanol. The disadvantage was that the time course of the effect of heptanol could not be determined.

Protocol C. To verify the relatively negligible effects of heptanol on active membrane properties transmembrane potentials were recorded from eight epicardial tissues. Five to eight sequential recordings were made in each tissue in a 2–4-mm line parallel to fiber orientation and longitudinal to the direction of the conducted wavefront during constant pacing at a basic cycle length of 2,000 msec. Thirty minutes after the addition of heptanol at a concentration of 1.5 mM, action potentials were recorded in the same area.

Data Analysis

We were able to select recording sites with appropriate orientation relative to pacing site and fiber orientation to provide areas that had preferential longitudinal or transverse conduction. In Protocol A, the vector loops were monitored in a manner that allowed the pairs of electrodes to be placed so...
that their axis was in the direction of longitudinal or
transverse conduction (Figure 1). In Protocol B, we
displayed conduction as isochronal activation maps
and selected data corresponding to respective lon-
gitudinal and transverse conduction directions. Tab-
ular data were derived from measurements in these
directions. Tabular data are expressed as mean ± SD.
In Table 1, experiments 1–5 were derived from
Protocol B and experiments 6–10 were derived from
Protocol A. The data of Table 2 were derived from
Protocol C, and means and standard deviations are
presented for each tissue. Conduction velocities
were compared before and after heptanol with a
paired t test. Mean values for action potential
parameters derived from each tissue before and
after heptanol were compared by a nonpaired t test
since these were not from the same impalements.
Values of p < 0.05 indicated significance.

### Results

#### Effects of Heptanol on Longitudinal and Transverse Conduction

During control measurements, the ventricular
epicardial strips exhibited marked anisotropy of the
spread of excitation. Figure 2 shows isochronal
activation maps from Protocol B for a typical
epicardial strip. The regularly spaced isochrones
demonstrate uniform anisotropic conduction
throughout the preparation. Conduction velocities
for each tissue are shown in Table 1. Mean con-
duction velocities longitudinal to fiber orientation
were two to three times faster than conduction
velocities transverse to fiber orientation, with a
mean ratio of ΩL/T = 2.54 ± 1.00. This is similar to
what others have reported in mammalian myo-
cardium.1–3,6

### Table 1. Effects of Heptanol on Conduction Longitudinal and Transverse to Fiber Orientation

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Control (m/sec)</th>
<th>Heptanol (mM)</th>
<th>Washout (m/sec)</th>
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<tr>
<td></td>
<td>ΩL</td>
<td>ΩT</td>
<td>ΩL/T</td>
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<tr>
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<td>0.17</td>
<td>2.76</td>
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<td>2</td>
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<td>0.19</td>
<td>2.00</td>
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<td>0.47</td>
<td>0.33</td>
<td>1.42</td>
</tr>
<tr>
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<td>0.74</td>
<td>0.22</td>
<td>3.36</td>
</tr>
<tr>
<td>5</td>
<td>0.64</td>
<td>0.23</td>
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<td>1.61</td>
</tr>
<tr>
<td>8</td>
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</tr>
<tr>
<td>10</td>
<td>0.33</td>
<td>0.17</td>
<td>1.96</td>
</tr>
<tr>
<td>*Mean ± SD</td>
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<td>0.24</td>
<td>2.54</td>
</tr>
<tr>
<td></td>
<td>±0.13</td>
<td>±0.09</td>
<td>±1.00</td>
</tr>
<tr>
<td>p</td>
<td>0.005</td>
<td>0.002</td>
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</tr>
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</table>

θL, conduction velocity longitudinal to fiber orientation; ΩT, conduction velocity transverse to fiber orientation; and ΩL/T, the ratio of longitudinal to transverse conduction velocity.

*Mean and SD for experiments 3 through 10.
†By paired t test versus control for experiments 3 through 10.
Initially, we searched for a concentration of heptanol high enough to produce a consistent depression in conduction velocity without changes in conduction direction. Conduction velocities both longitudinal and transverse to fiber orientation were inconsistently affected by 0.2 and 0.5 mM heptanol. As shown in Figure 2 for 0.2 mM heptanol, the isochronal maps demonstrate little effect on conduction but emphasize the stability of epicardial activation patterns over the 2-hour time course of this experiment. Based on these initial experiments, doses of 1 to 2 mM were chosen for the subsequent studies.

With addition of 1.0 mM heptanol, conduction velocity was generally delayed both transverse and longitudinal to fiber orientation. Figure 3 shows isochronal maps and superimposed vector angles at each site during control (A), 30–40 minutes after heptanol (B), and 30–40 minutes after washout (C) for one experiment. Because of heptanol's greater reduction in transverse conduction velocity, the shape of the isochrones is elongated. On the macroscopic scale of our measurements, the regularly spaced isochrones suggest uniform anisotropy both before and after the addition of heptanol. The regions of the isochrones that represent predominately longitudinal and transverse conduction do not change, suggesting that slowing was not associated with overall changes in wavefront direction or areas of block. However,
Effects of Uncoupling on Conduction

Figure 4. An example of analog records from experimental Protocol A. In each panel the upper two electrograms represent conduction longitudinal to fiber orientation and the lower two electrograms represent conduction transverse to fiber orientation. Electrogram $L_p$ was obtained from the proximal bipolar electrode ($BP_L$, Figure 1). Electrogram $L_d$ is the X bipolar electrogram obtained from the vector probe ($VL$, Figure 1). Electrograms $T_p$ and $T_d$ were obtained from corresponding bipolar electrodes and the Y axis electrode of the vector probe oriented in the transverse direction ($BP_T$ and $VT$, Figure 1). The distance between the longitudinal pair of electrodes was 7.5 mm, and the distance between the transverse pair was 3.4 mm. At the right of each panel, the vector loop obtained from the longitudinal vector probe ($VL$, Figure 1). Open arrows indicate the activation times for each electrogram and the numbers represent the time in milliseconds between the proximal and the distal electrograms in each direction. The electrogram and time course calibrations are to the right of panel one and are the same for each panel. During control, it required 12.7 msec for the impulse to conduct between proximal and distal recording sites in a longitudinal direction and 9.3 msec in a transverse direction. The right to left orientation of the vector loop verifies the direction of longitudinal wavefront at the distal site.

B: Thirty minutes after the addition of 1.0 mM heptanol.

C: Thirty minutes following heptanol at a level of 2.0 mM.

Dose Dependence and Time Course of Heptanol's Effect

Figure 4 presents analog records derived from a study using Protocol A, schematically represented in Figure 1. In each panel of Figure 4, the upper two electrograms were obtained from areas of longitudinal conduction in the tissue and the lower two from areas of transverse conduction. Electrogram $L_p$ was obtained from the proximal bipolar electrode ($PB_L$, Figure 1), electrogram $L_d$ is the X bipolar electrogram obtained from the vector probe ($VL$, Figure 1). Electrograms $T_p$ and $T_d$ were obtained from corresponding bipolar electrodes and the Y axis electrodes of the vector probe oriented in localized areas not directly on the axis of longitudinal conduction or not directly along the transverse axis, the local wavefront direction must change slightly as the isochrones elongate due to the change in the ratio of longitudinal to transverse conduction velocity. These local changes in vector directions can be seen by comparing the directions of the arrows in the maps in Figures 3A and 3B. While conduction velocity was slowed in both longitudinal and transverse directions for all tissues at a heptanol dosage of 1 mM, there was a relatively greater decrease in conduction velocity of the wavefronts moving transverse to fiber orientation, resulting in a significant increase of the ratio of $\Theta L/T$ from a mean of 2.54 to 2.94 (Table 1).
the transverse direction (BP and Vp of Figure 1). At the right in each panel, the vector loop obtained from the longitudinal vector probe (Vl of Figure 1) is displayed. During control, it required 12.7 msec for the impulse to conduct between proximal and distal recording sites in the longitudinal direction and 9.3 msec in the transverse direction. The right-to-left orientation of the vector loop shows the direction of the longitudinal wavefront at the distal site. The transverse vector loop (not shown) was oriented downward. Thirty minutes after the addition of 1 mM heptanol to the superfusate (Figure 3B), longitudinal conduction time had increased by 24% to 15.8 msec, while transverse conduction time increased 40% to 13 msec. Both longitudinal and transverse (not shown) vector loops indicated unchanged wavefront directions. In Figure 3C, 30 minutes after 2.0 mM heptanol, both longitudinal and transverse conduction times had increased further. However, as indicated by the vector loop, the direction of the wavefront had changed by approximately 40° at the distal longitudinal site, indicating a change in the direction of the wavefront at this level of heptanol. In addition to increasing conduction time, Figure 4 indicates that heptanol caused a decrease in electrogram amplitude. This is most apparent for the transverse electrograms (Tp and Td in Figures 4B and 4C). In all five tissues, the mean decrease in amplitude 30 minutes after 1.0 mM heptanol was 9.6% for the electrograms recorded in the longitudinal axis and 21.5% for those in the transverse axis (p = 0.220 and p = 0.056, respectively, by paired t test). This decrease failed to reach significance because four of 10 longitudinal axis electrograms and three of 10 transverse axis electrograms showed increases in amplitude. A consistent finding was a change in morphology of the electrograms recorded in the transverse axis. With increasing doses of heptanol (from 1.0 to 2.0 mM), these tended to exhibit increasing superimposed small deflections or notches on the waveforms. This was not seen in the electrograms recorded in the longitudinal axis (compare Lp and Ld with Tp and Td in Figures 4A, 4B, and 4C).

In Figure 5, pooled data from five experiments are displayed showing the time course of the effects of heptanol on the conduction longitudinal and transverse to fiber orientation (A) and the ratio of longitudinal to transverse conduction velocity (B). The dark data points are mean values plotted as change from control at every 18-second interval. The light data points indicate plus and minus 1 standard error. In Figure 5A, it can be seen that longitudinal and transverse conduction velocities began to decrease within 1 to 2 minutes after the addition of 1.0 mM heptanol and stabilized after 15 to 20 minutes. Longitudinal conduction velocity was decreased approximately 20% while transverse conduction velocity was decreased 30%. In Figure 5B, this resulted in an increase in the ratio of longitudinal to transverse conduction. At a heptanol concentration of 2.0 mM, there was a further decrease in longitudinal and transverse conduction velocities (A) resulting in a 70% increase in the ratio of longitudinal to transverse conduction velocities. This effect peaked within 15 minutes of the second dose but did not reach a steady state. During washout, conduction velocities returned toward but did not reach control values at 30 minutes.

Figure 6 presents the angle of the longitudinal vector loop plotted in the same manner as the data of Figure 5. Figure 6 shows that with the addition of 1 mM heptanol the conduction wavefront direction remained unchanged in the longitudinal direction. The mean change was less than 10° from control values. However, at 2.0 mM heptanol, vector angles changed by a mean of greater than 30°, indicating a change in the direction of the conducted wavefront. This effect at the higher dose of heptanol is probably due to its greater degree of uncoupling, which unmasks subtle inhomogeneities in the tissues. If conduction in some regions of the tissues slowed inhomogeneously, then circuitous conduction paths between recording sites could result, even in the absence of local conduction block. As mentioned previously, the vector electrodes in Protocol A were arranged so that they were directly on the longitudinal and transverse axes of the conducted wavefront. As such, a pure change in the ratio of longitudinal to transverse conduction velocity due to heptanol would not change the wavefront directions at these recording sites. Thus, conduction velocities measured at 2.0 mM heptanol (Figure 5) were confounded by changes in the overall direction of the wavefronts relative to the electrode orientation. For this reason, data summarized in Table 1 for these experiments (experiment 6 through 10 of Table 1) show only those conduction velocities determined at 1.0 mM heptanol. The present studies demonstrate that following 1.0 mM heptanol, conduction velocity slowed in both the longitudinal and transverse directions and stabilized within 15 to 20 minutes. Conduction velocity was decreased to a greater degree in the transverse direction, resulting in a significant increase in the ratio of longitudinal to transverse conduction velocities. These effects were not accompanied by changes in the overall direction of propagation of the wavefronts.

**Effects of Heptanol on Action Potential Characteristics**

Table 2 presents the means and standard deviations for the action potential parameters recorded during control and after equilibration with 1.5 mM heptanol in a separate group of eight tissues. The maximum rate of depolarization reflecting active membrane properties was variably affected by heptanol in the different tissues; two of the seven showed a nonsignificant increase. In two tissues (numbers 4 and 7), the mean maximum rate of
Depolarization was decreased significantly by 19% and 23%, respectively. However, the pooled data for all impalements showed an overall nonsignificant decrease in mean maximum rate of depolarization of only 7%. Thus, the effects of heptanol on active membrane properties were minimal and cannot account for the decrease in conduction velocity observed in the present study. In a similar study, Delmar et al.\textsuperscript{10} reported no relation between changes in conduction velocity induced by heptanol and the maximum rate of action potential depolarization.

**Discussion**

Several investigators have evaluated the implications of anisotropic tissue structure on conduction by developing mathematical models that relate electrical propagation in cardiac muscle to cellular geometry and cellular interconnections.\textsuperscript{5,18} These models propose that impulse propagation in cardiac muscle is influenced by directional differences in the distribution of gap junctions and their associated resistances. The axial resistivity in any direction is composed of the relative contribution of junctional and cytoplasmic resistivity. Because of the elongated shape of cardiac cells, there are more gap junctions per unit distance transverse to the long axis of fiber orientation. Therefore, junctional resistivity has a greater relative contribution to total axial resistivity in the transverse direction. One consequence of this is that conduction transverse to fiber orientation should be affected to a greater degree by conditions that alter junctional resistivity.
Preferential Effect of Heptanol on Transverse Conduction

We have shown that cell uncoupling has a more profound effect on conduction transverse than longitudinal to fiber orientation and that at 1.0 mM heptanol this effect stabilizes after 15 to 20 minutes (Figure 5). As is evident by the actual activation times and vector loop angles (Figures 3 and 6), these effects are clearly not due to alterations in the overall pattern of impulse propagation or to areas of focal block. In experiments with sheep ventricular muscle, Delmar et al.10 reported a greater degree of conduction delay longitudinal to fiber orientation with 1.5 mM heptanol. However, in their experiments, they did not rule out changes in wavefront direction and conduction eventually failed first transverse and then longitudinal to fiber orientation. In our experiments, at 1.0 and 2.0 mM heptanol, we did not observe complete conduction block although at 2.0 mM the direction of the wavefront was altered. Also, in contrast to the findings of Delmar et al.10 who reported the development of “foot potentials,” we did not observe these in our studies at 1.5 mM heptanol. The reason for these differences is not clear. However, in their experiments, Delmar et al.10 used sheep epicardial preparations. Further evidence supporting the hypothesis that an increase in axial resistance is responsible for the greater reduction in conduction velocity transverse to fiber orientation is demonstrated in Figure 4 by the changes in electrogram amplitude and morphology after heptanol exposure. Spach et al.18 have evaluated theoretically and experimentally the factors that affect the amplitude of unipolar extracellular electrograms. These include change in the upstroke velocity of the propagating action potential, change in the direction of impulse propagation relative to fiber orientation, change in cellular geometry, and finally, change in axial resistance. In the present study, only bipolar electrograms were recorded, and therefore, changes in waveform must be interpreted with caution. However, the above considerations may be applied to our findings in a qualitative way. As shown in Table 2, action potential maximum rate of depolarization was relatively unaffected by heptanol. Figure 6 shows that at 1.0 mM heptanol, the overall direction of the wavefront was not changed at a time when the electrograms recorded in the transverse axis exhibited decreases in amplitude (Figure 4B). This decrease in the electrogram amplitudes is consistent with heptanol increasing axial resistance, assuming constant cellular geometry in the setting of unchanged action potential parameters with stability in the direction of wavefront propagation.

It is also obvious from Figure 4 that in addition to amplitude changes heptanol caused a notable change in the shape of the extracellular waveform. With increasing doses of heptanol, both the proximal and distal transverse electrograms developed multiple superimposed deflections or notchings while the shape of both longitudinal electrograms remained relatively smooth and constant compared with the control. Spach and Dolber19 have analyzed such notches in the extracellular waveform during transverse conduction and found that they are caused by microscopic discontinuities in conduction resulting from nonuniform distribution of side-to-side electrical connections between cells. In addition, they suggested that the presence of such notchings can be used as a method for detecting nonuniform anisotropic propa-
gation. As such, the development of notches in the transverse electrograms of Figures 4B and 4C following heptanol indicate a change in the character of transverse conduction from relatively uniform during control to nonuniform propagation at 2.0 mM heptanol. This finding is also corroborative of heptanol’s effect on axial resistance.

Effect of Heptanol on Action Potential Depolarization

There have been few studies dealing with the effect of aliphatic alcohols such as heptanol and octanol on action potential depolarization in mammalian myocardium. Theoretically, the nature of cell coupling in anisotropic myocardium will influence the action potential upstroke of a conducted beat. Spach et al have shown that the maximum rate of depolarization \( V^\text{max} \) of an action potential is greater when the impulse is conducting transverse to fiber orientation. This is a consequence of the nonuniform, side-to-side junctions between myocardial cells producing microscopically discontinuous transverse conduction. During transverse conduction less of the current generated by an active patch of membrane flows to depolarize adjacent inactive membrane than during longitudinal conduction and both depolarization amplitude and \( V^\text{max} \) are consequently increased at the source. The effect would be accentuated by cell uncoupling. Thus, the net effect on \( V^\text{max} \) of an agent that uncouples cells would be the result of the above tendency to increase \( V^\text{max} \) together with any direct effect of the agent on the fast sodium current.

There are no studies that have directly evaluated the effect of aliphatic alcohols on the fast inward current in mammalian myocardium. However, in voltage-clamped squid giant axon, Oxford and Swenson found that 0.5 mM octanol partially reduces peak sodium conductance by 33%. Hirche, using frog myelinated nerve, found that octanol at a concentration of 0.4 mM reduced peak inward current by 50% and referenced the fact that the modifying action of alcohols on the sodium current in axons appears to be species dependent. In canine ventricular myocardium, Joyner and Overholt report a 23% decrease in \( V^\text{max} \) with 0.2 mM octanol. If we accept \( V^\text{max} \) as an indirect index of peak inward current, the small depression in \( V^\text{max} \) that we observed in canine ventricular myocardium with 1.5 mM heptanol (Table 2) suggests that the modifying action of the alcohols on peak inward current may also be dependent on the alkyl chain length. Heptanol appears to produce less of a depression than octanol. In this regard, Sicouri et al reported in sheep cardiac Purkinje fibers that heptanol (1.2–3.0 mM) reduced \( V^\text{max} \) by 32%, but most of this was attributed to a decrease in maximum diastolic potential and induced foot potentials associated with conduction delay. Delmar et al. using fixed microelectrode impalements before and after heptanol in sheep ventricular myocardium, observed that 1.5 to 3.0 mM heptanol produced variable effects on \( V^\text{max} \). In some cases \( V^\text{max} \) was increased, and in others, it was decreased. They attributed this to the mutually antagonistic effects of changes in cell coupling and direct actions of heptanol on inward current as described above. Our studies with heptanol in canine myocardium (Table 2) are consistent with the above findings. However, in our studies, sequential microelectrode impalements were made only in areas where conduction was proceeding longitudinal to fiber orientation (see “Materials and Methods”); after heptanol, impalements were repeated when conduction was at a steady state and not progressing to block. Thus, the effect of uncoupling on \( V^\text{max} \) was minimized in our experiments. Therefore, the nonsignificant 7% decrease in \( V^\text{max} \) that we observed allows us to conclude that in canine myocardium, heptanol at a concentration of 1.5 mM has minimal direct effects on the fast inward current.

Effects of Heptanol on Effective Junctional Resistivity

Because of the relatively selective effect of heptanol in our preparation we can use the nonnormalized data of Figure 5 to calculate the time course of heptanol’s effect on junctional resistivity using a model of the geometric distribution of intercellular resistances similar to the models of Spach et al and Roberge et al.

If we assume that heptanol acts solely by increasing junctional resistance and that the change in conduction velocity that occurs after the administration of heptanol is due to this change in junctional resistance, we can use cable theory to derive the relative contributions of cytoplasmic and junctional resistivity to total axial resistivity. It should be emphasized that the use of continuous cable theory is a simplification based on assumptions for events at a macroscopic size scale because the development of notches on electrograms during transverse conduction following heptanol indicates that discontinuous conduction occurs on the microscopic scale.

We used an extension of the model assumed by Spach et al in which the current pathway is represented by an alternation of cytoplasmic and junctional resistors. Assuming an idealized geometry consisting of a regular pattern of cells which are longer than they are wide, the relative contribution of axial resistivity to total axial resistivity is greater transverse than longitudinal to cell orientation, since the distance between junctions is smaller in the transverse direction.

Our measurements of longitudinal and transverse conduction velocity in Protocol A (experiments 6–10) were made where the activation wavefront was passing either directly parallel or transverse to fiber orientation. Activation can therefore be considered a plane wave locally and the equations for one-dimensional cable theory then apply. Cable theory yields an inverse squared relation between conduc-
tion velocity and axial resistivity. Thus, for longitudinal and transverse propagation we can write:

\[
\Theta_L = K/R_{el} \\
\Theta_T = K/R_{et}
\]

(1)

(2)

where \(\Theta_L\) and \(\Theta_T\) are conduction velocities in longitudinal and transverse directions and \(R_{el}\) and \(R_{et}\) are the total effective axial resistivity in each direction. \(K\) is a constant which reflects passive membrane properties; from Clerc's data for calf ventricular muscle \(K\) equals 926 \(\Omega \cdot \text{mm}^2/\text{msec}^2\). In each direction, the total effective axial resistivity is the sum of components due to cytoplasmic volume resistivity \(R_c\) and effective junctional resistivity \(R_j\) such that

\[
R_{el} = R_c + R_j
\]

(3)

\[
R_{et} = R_c + AR_j
\]

(4)

We have defined \(R_c\), the effective junctional resistivity, to be the amount of total axial resistivity in the longitudinal direction contributed to by nexal resistance; \(R_j\) is therefore the product of the specific junctional resistivity and the number of nexi per unit length longitudinal to fiber orientation. The anisotropic coupling ratio, \(A\), is the ratio of junctional resistance per unit length transverse to fiber orientation to junctional resistance longitudinal to fiber orientation and thus reflects the elongated cellular structure.

We could use Equations 1 and 2 and our measured values of conduction velocity to calculate total effective axial resistivity both before and during application of heptanol to the tissue bath. But at any one point in time we would not be able to separate the contribution of \(R_c\) and \(R_j\) to the total (Equations 3 and 4) without making assumptions about the values of \(R_c\) and \(A\). However, by administering heptanol, which is assumed to alter only junctional resistivity, we can not only derive values for the relative contributions of \(R_c\) and \(R_j\) to the total, but also determine a value for \(A\), the anisotropic coupling ratio, as well. We do this by the following system of simultaneous equations:

\[
\Theta_L^{t=0} = (K/[R_c + R_j^{t=0}])^{1/2}
\]

(5)

\[
\Theta_T^{t=0} = (K/[R_c + AR_j^{t=0}])^{1/2}
\]

(6)

\[
\Theta_L^{t=30} = (K/[R_c + R_j^{t=30}])^{1/2}
\]

(7)

\[
\Theta_T^{t=30} = (K/[R_c + AR_j^{t=30}])^{1/2}
\]

(8)

The superscript \(t\) represents the fact that effective junctional resistivity changes over time after the instillation of heptanol. Taking \(t_0\) to be control and \(r_t\) to be an arbitrary time after heptanol has altered the system by increasing \(R_j\), Equations 5–8 are a system of four equations in four unknowns. Though nonlinear, this system can be solved using an iterative numerical technique.

We used Equations 5–8 with the mean values for \(\Theta_L\) and \(\Theta_T\) from Protocol A (experiments 6–10, Table 1) at \(t_0 = 0\) (control) and \(t_t = 30\) minutes to calculate the results shown in Table 3. Thus, at 30 minutes 1 mM heptanol increased the effective junctional resistivity by 2.3 times from 133 \(\Omega \cdot \text{cm}^2\) to 321 \(\Omega \cdot \text{cm}^2\). The anisotropic coupling ratio is calculated to be 11.4 while the cytoplasmic resistivity equals 205 \(\Omega \cdot \text{cm}^2\).

Our calculated values are similar to those measured by previous investigators. Our value of cytoplasmic resistivity is in accord with that measured by Chapman and Fry23 of 282 \(\Omega \cdot \text{cm}^2\). If we use a value of 70 equivalent junctions per centimeter in the longitudinal direction, the specific junctional resistivity is calculated from the effective junctional resistivity to be equal to 1.9 \(\Omega \cdot \text{cm}^2\) before and 4.5 \(\Omega \cdot \text{cm}^2\) 30 minutes after the introduction of heptanol. These values are similar to measured values reported by Chapman and Fry23 and Woodbury and Crill.2

Since \(R_c\) and \(A\) are assumed to remain constant over time, we can use our calculated values for these parameters and the measured values for \(\Theta_L\) at each 0.3-minute interval (Figure 5) to estimate \(R_j\) at each point in time by applying Equation 5 from \(t = 0 – 30\) minutes. Figure 7 shows a plot of \(R_j\) as a function of time after the instillation of 1 mM heptanol to the bath. We see that effective junctional resistivity rises.
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The relation between measured and predicted \( \Theta_T \) deviates from the line of identity. This likely occurs because, at a concentration of 2.0 mM heptanol, heptanol causes deviations from uniform anisotropy and macroscopic changes in the conduction pathway. There is evidence for this deviation in the vector angle data of Figure 6. Changes in the conduction pathway confound the determination of conduction velocity and violate the assumptions of the model.

**Implications**

We have shown that a uniform alteration in junctional resistivity can result in a directionally dependent alteration in the pattern of impulse propagation as postulated for a model of anisotropic myocardium. For example, in the canine atrium, anisotropy has been shown to provide both slow conduction and unidirectional block required for the development of reentry. In experimentally infarcted canine myocardium, Dillon et al have implicated directional differences in conduction velocity in the establishment of reentrant circuits. Furthermore, disruptions of cellular coupling have been described with many types of cell injury. Most notably, the deleterious effects of myocardial ischemia and infarction on intercellular communication are well documented. Although the precise role of tissue anisotropy and disruptions in cell coupling in clinical arrhythmias remains to be determined, like heptanol, antiarrhythmic drugs may have different effects on conduction relative to fiber orientation, depending on the degree they influence junctional resistivity. In addition, because of heptanol's selective depression of conduction in regions which have higher coupling resistances, it may be a useful tool to evaluate the role of high coupling resistance as a mechanism of abnormal conduction in many pathological states.

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**References**


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**Figure 8.** The relation between the measured transverse conduction velocity (abscissa) and that predicted using the data for longitudinal conduction velocity (derived from Figure 5) and Equation 8. In A, the relation is shown following 1.0 mM heptanol and, in B, at 2.0 mM heptanol. The solid lines are regressions, and the equation and regression coefficients are shown for each. The dashed lines are lines of identity. See text for discussion.

Rapidly during the first several minutes and reaches a plateau after 15 to 20 minutes.

To confirm that longitudinal and transverse conduction velocity are modeled by Equations 5–8, we used our calculated values for \( R_R \) and \( A \) and the calculated \( R_R \) at each point in time for Protocol A with 1 mM heptanol to calculate a predicted \( \Theta_T \) by applying Equation 8 for each 0.3-minute interval. The predicted values for \( \Theta_T \) are compared with the measured values in Figure 8A. Note the excellent relation between the two.

The same procedure was applied to the data for the 2.0 mM heptanol experiment and is plotted in Figure 8B.


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