Effects of Hypoxia, Hyperkalemia, and Metabolic Acidosis on Canine Subendocardial Action Potential Conduction

R.D. Veenstra, R.W. Joyner, R.T. Wiedmann, Ming-Lon Young, and Rose C. Tan

We have studied the individual and combined effects of elevated external potassium concentration (8 mM [K+]o), metabolic acidosis (pH = 6.8), and hypoxia at different stimulation rates (BCL = 1,000 or 400 milliseconds) on Purkinje (P) and ventricular (V) conduction velocities and on Purkinje-ventricular junctional conduction delay (PVJ delay) in in vitro preparations from canine ventricles. Elevated [K+]o had opposite effects on P and V velocities, increasing V velocity by 8% while reducing P velocity by 7%. Acidosis reduced P velocity by 9% while reducing V velocity by only 4%. Hypoxia and rapid stimulation rates had no significant effect on either P or V velocities. All test solutions (except hypoxia alone) significantly increased the PVJ delay. The magnitude of the increase in PVJ delay was much greater than the effects on either P or V velocity. In addition, hypoxia and rapid stimulation augmented the increase in PVJ delay in the presence of elevated [K+]o and/or acidosis. The special features of conduction at the PV junctional sites may produce altered pathways of excitation of the ventricles during myocardial ischemia. (Circulation Research 1987;60:93-101)

Acute myocardial ischemia produces alterations in the ventricular excitation sequence that can lead to arrhythmias. Although other factors may be involved in in vivo myocardial ischemia, it is clear that acute ischemia produces an elevation in extracellular [K+]o, a decrease in extracellular pH, and a relative hypoxia. It is possible to recreate in vitro the changes in [K+]o and pH at values observed in in vivo ischemia. The creation of hypoxia in vitro can easily be done, but is difficult to equate to the ischemic state due to differences between Tyrode's solution and blood in regard to oxygen-carrying capacity. However, it is impossible to measure activation times in vivo sufficiently to quantitatively assess the regional changes in conduction velocity. Since the subendocardial surface of the ventricles survives well in vitro, canine subendocardial preparations from papillary muscles and from the upper right septal surface were used to investigate the effects of hypoxia, elevated [K+]o, lowered pH, and decreases in the basic cycle length for stimulation on the conduction velocity (or conduction delay) on the three components of the subendocardial activation sequence: 1) the layer of Purkinje cells that exist over most of the ventricular endocardial surface; 2) the junctional regions between the superficial Purkinje cells and the underlying muscle cells; and 3) the subendocardial layer of muscle cells.

The Purkinje-ventricular junction (PVJ) is a region of the heart that exhibits a localized delay in conduction from the Purkinje layer into the ventricular layer. This PVJ conduction delay (PVJ delay) has been shown to be sensitive to various factors such as [Ca2+], rapid stimulation rate, and quinidine. Mendez et al. reported that, although increases of [K+]o to 6 mM slightly decrease PVJ delay, increases in [K+]o to 8 mM increase the PVJ delay of canine papillary muscles while further elevations in [K+]o to 10–11 mM can create orthodromic conduction block. Compared to the discrete delay observed at junctional sites, conduction through the Purkinje layer of cells and through the underlying ventricular cells (away from junctional sites) can be characterized as a wavefront of activation that may be circular or elliptical but has a clearly defined conduction velocity along a given line of recording sites progressing outward from a site of stimulation.

Studies of the effects of elevated [K+]o, on conduction velocity in the heart have shown that moderate elevations in [K+]o increase the conduction velocity in cardiac Purkinje fibers, and in guinea pig papillary muscles. Kagiyama et al. showed that further elevations in [K+]o begin to decrease conduction velocity in guinea pig papillary muscles and that extracellular acidosis reduces the conduction velocity at all levels of [K+]o without blocking the increase in conduction velocity seen with slight elevations in [K+]o. From these studies on many different preparations, it appears that moderate increases in [K+]o increase the PVJ delay although the opposite effect occurs in the myocardium as an increase in the conduction velocity. Metabolic acidosis appears to have a suppressant effect on conduction that is independent of the [K+]o. In this paper, the effects of 8 mM [K+]o and metabolic acidosis (pH 6.8) alone, and in combination, on PVJ delay and conduction ve-

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locity in the canine endocardium are compared using the technique described in Veenstra et al.\textsuperscript{10} This extracellular recording technique allows us to definitively locate the PVJ sites and quantify the effects of $[K^+]_o$ and metabolic acidosis on PVJ delay. The additional effects produced by hypoxia and decreases in the basic cycle length (BCL) for stimulation both with normal Tyrode’s solution and in solutions with elevated $[K^+]_o$ and/or lowered pH are also examined.

There is growing evidence that the PVJ is a region of discontinuous propagation, which can be attributed to the existence of spatial asymmetries in electrical load and excitability in addition to an increased coupling resistance between the Purkinje and ventricular cells. These factors produce different results in the actions of low pH, elevated $[K^+]_o$, hypoxia, and lowered BCL on conduction through the PVJ as compared to the conduction through either the Purkinje (P) or the ventricular (V) layer of the canine subendocardium.

Material and Methods

Adult mongrel dogs were anesthetized with sodium pentobarbital (Nembutal, 30 mg/kg, i.v.) and ventilated by a respirator during a left lateral thoracotomy to remove the heart. Either a left ventricular papillary muscle or a portion of the upper right septal ventricular surface were rapidly removed. In each case, the preparation was placed in a plexiglass chamber and continuously superfused with a Tyrode’s solution as described below. A bipolar teflon-coated silver wire was used for stimulation and bipolar teflon-coated silver wires (wire diameter 75 µm, A-M Systems, Everett, Wash.) for surface recordings. As previously described,\textsuperscript{10} the recording electrodes had one wire touching the surface of the preparation and the other wire about 1 millimeter above the preparation, producing essentially a monopolar surface recording with a close reference to eliminate noise. Each surface recording was amplified by an AC-coupled instrumentation operational amplifier (ADS21, Analog Devices, Inc., Norwood, Mass.) with a band pass of 0.1–10,000 Hz before data acquisition by a 12-bit A/D converter system on a VAX 11/780 computer (Digital Equipment Corporation, Maynard, Mass.) at a rate of 5,000–10,000 samples/channel/sec.

Placement of Electrodes

The measurements of Purkinje conduction velocity and PVJ delay were made simultaneously on papillary muscles. A stimulating electrode was placed on a free-running P strand near the apex of the muscle (Figure 1). Surface recordings were made serially over a 10 mm $\times$ 10 mm area at 1 or 2 mm spacing during repetitive 1 Hz pacing. During this period (about 30 minutes), at least 2 surface electrodes remained at fixed locations and showed constant waveforms indicating a stable propagation sequence during the recording period. The activation map produced by the serial recordings served to document the uniformity of propagation in the P layer and the general location of PVJ sites. After the mapping procedure, one surface electrode was placed near the attachment of the P strand and another placed toward the base of the muscle to monitor the P layer conduction velocity. Several other electrodes were located at sites of P to V junctions. These sites were identified as previously described\textsuperscript{10} as sites with a short delay between P and V activation and an all negative signal produced by V activation, indicating local activation as opposed to the bipolar signals produced by V activation at sites where propagation within the V layer was occurring. Identifying several PVJ sites in the control solution was necessary because many sites that were PVJ sites in the control solution became nonjunctonal in a reversible fashion in the test solutions. Since the PV delay at a nonjunctonal site is not a true measure of the local delay, the data included only those sites that remained junctional in all of the test solutions at BCL 1,000 and BCL 400 milliseconds. Therefore, the changes in PVJ delay reported here are an underestimation of the effects of the test solutions on the “average” PVJ site.

For the measurement of conduction velocity in the V layer, separate preparations were used, with the same test protocol, isolated from the upper right septal surface. As previously studied by Spach et al,\textsuperscript{12} this region often has no superficial P layer and the activation

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Isochronal activation maps of the P layer (Panel A) and the underlying V layer (Panel B), with isochrones marked in milliseconds following the stimulus applied to the Purkinje strand as diagrammed. Recordings were made serially at 36 locations (dots) during 1-Hz stimulation. Preparation RJ062485.}
\end{figure}
wavefront following surface stimulation is elliptical with the long axis of the ellipse parallel to the general fiber orientation. In these preparations, activation sequence mapping was performed over a 10 mm × 10 mm area as for the papillary muscle. From the isochronal activation map the long axis of the elliptical wavefront was determined and then 3–5 surface electrodes were placed along the axis. Recordings from these sites during the test protocol determined the effects on conduction velocity. The velocity along this axis was determined by a linear least-squares fit to the activation time for each electrode position plotted against the electrode distances. These septal preparations were used instead of papillary muscle preparations to determine V conduction velocity to avoid altered directions of V conduction by shifts in the location of P to V conduction at PVJ sites on the papillary muscle. In all experiments included in the analysis, the waveform of ventricular activation remained symmetrical and biphasic at all recorded sites, indicating that conduction was not decremental.

**Test Protocol**

The control solution had the following composition (in mM): NaCl 125; MgCl₂ 1.0; NaHCO₃ 24; KCl 4; CaCl₂ 2.7; NaH₂PO₄ 0.4; and dextrose 5.5. The solution was aerated with 95% O₂ and 5% CO₂, with a pH of 7.4 and a temperature of 37°C. The modifications of this solution are shown in Table 1. Low pH (6.8) was produced by lowering the HCO₃⁻ concentration to 4 mM while raising the NaCl to 145 mM to keep [Na⁺]ᵣ constant. Elevated [K⁺]ᵣ was produced by increasing KCl to 8 mM. Hypoxia was produced by aerating with 95% N₂ and 5% CO₂. The aeration in all cases was done outside the experimental chamber. Measurement of oxygen levels in fluid withdrawn from the chamber by a syringe during several experiments showed a Po₂ of 350–400 mm Hg under control conditions and 60–100 mm Hg under hypoxic conditions. The protocol used for each test solution was a 15-minute period of pacing at BCL 1,000 milliseconds in the control solution then a 1-minute period of pacing at BCL 400 milliseconds in the control solution, then a 15-minute period of pacing at BCL 1,000 milliseconds in the test solution followed by a 1-minute period of pacing at BCL 400 milliseconds in the test solution. Extracellular recordings were stored at the end of each of these 4 periods, allowing the effects of the test solution to be assessed at steady-state BCL 1,000-millisecond pacing and at the end of a 1-minute period of BCL 400-millisecond pacing.

**Data Analysis**

Recordings stored on the VAX 11/780 computer system were displayed on a Tektronix 4014 graphics terminal, and activation times for the P layer and V layer were determined by picking the time of the peak negative point of the waveform associated with P and V activation. Since the application of each test solution was preceded by a control period, a paired t test was used to assess the statistical significance of the changes in P velocity, V velocity, and PVJ delay for each solution at each cycle length.

**Results**

Figure 1 shows results for a papillary muscle for which serial recordings at BCL 1,000 milliseconds, in the control solution at the sites indicated by dots, produced isochronal activation maps of the P layer (Panel A) and the underlying V layer (Panel B). In response to a stimulus on a free-running P strand, the P layer conducts an action potential in the direction of apex to base (Panel A). Activation of the underlying ventricular layer does not begin at the point where the P strand attaches to the muscle but in a broad region toward the base of the muscle indicated by the isochrone labelled 12 milliseconds. The 3 sites labelled with stars were continuously monitored during the test protocol for this muscle. Figure 2 shows, in the three upper traces, recordings from these sites during the control solution at BCL 1,000 milliseconds. At each site there is a biphasic signal produced by P layer activation, and these are progressively later for sites 1, 2, and 3. The earliest V activation is at site 3 with a short PV delay and an all-negative V signal. V activation at site 2 closely follows activation at site 1 and is also predominantly negative, indicating primarily local P to V activation at site 2 as well as at site 3. At site 1, V activation produces a delayed biphasic signal characteristic of propagation into site 1 through the V layer in a direction of base to apex (Figure 1B).

The lower 3 traces of Figure 2 are recordings from the same 3 sites but after 15 minutes of pacing at BCL 1,000 milliseconds in the low pH, 8K hypoxia solution. The P activation signals are reduced in size and the P layer conduction velocity is decreased (the difference in activation times for sites 1 and 3 is greater). Site 3 still has the earliest V activation and the V signal at site 3 is still all negative but reduced in amplitude. The V activation at site 2 is considerably delayed after activation at site 3, and the V waveform at site 2 is clearly biphasic, indicating that activity is now propagating into site 2 through the V layer instead of by local P to V activation at site 2. Site 1 shows a large delay in V activation and a reduced amplitude of the V activation signal. All of the changes at the 3 sites were completely reversed after 15 minutes in the control solution. For this preparation, site 3 was used for the

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**Table 1. Modifications of Control Solution for Study**

<table>
<thead>
<tr>
<th>Solution</th>
<th>[K⁺]ᵣ₀</th>
<th>[HCO₃⁻]ᵣ₀</th>
<th>Gas mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>24</td>
<td>95% O₂, 5% CO₂</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>4</td>
<td>24</td>
<td>95% N₂, 5% CO₂</td>
</tr>
<tr>
<td>Low pH, 8K hypoxia</td>
<td>8</td>
<td>4</td>
<td>95% N₂, 5% CO₂</td>
</tr>
<tr>
<td>Low pH, 8K</td>
<td>8</td>
<td>4</td>
<td>95% O₂, 5% CO₂</td>
</tr>
<tr>
<td>8K Hypoxia</td>
<td>8</td>
<td>24</td>
<td>95% N₂, 5% CO₂</td>
</tr>
<tr>
<td>8K</td>
<td>8</td>
<td>24</td>
<td>95% O₂, 5% CO₂</td>
</tr>
<tr>
<td>Low pH hypoxia</td>
<td>4</td>
<td>4</td>
<td>95% N₂, 5% CO₂</td>
</tr>
<tr>
<td>Low pH</td>
<td>4</td>
<td>4</td>
<td>95% O₂, 5% CO₂</td>
</tr>
</tbody>
</table>
PVJ delay analysis during the test protocol. Figure 3A shows sequential recordings from site 3 of Figure 1 during each of the test solutions. Two traces are superimposed as "controls." These are the control records taken before the first test solution ("hypoxia") and after the last test solution ("low pH, 4K"), separated by nearly 4 hours. In Panel B of Figure 3, the same data as in Panel A is displayed at a faster sweep speed and with the individual traces shifted in time, to align the P activation signals for each of the traces to emphasize the changes in PVJ delay. The largest effect is produced by the low pH, 8K hypoxia solution. The test solutions hypoxia or low pH, 4K produce almost no effect.

Table 2 presents a summary of the data from papillary muscle experiments. The first 4 columns of the table list the measurements or values of the test solutions used; [K⁺]₀ was 4 or 8 mM, pH was 7.4 or 6.8, oxygen level was normal (N) or hypoxic (H), and the cycle length was 1,000 milliseconds applied for 15 minutes or 400 milliseconds applied for 1 minute. The first two rows of the table compare the results for PVJ delay and P velocity in the control solution at BCL 1,000 vs. 400 milliseconds. No significant change in
PVJ delay or P velocity was produced by the decreased cycle length in the control solution. In the following rows of the table, the values (mean ± SD) for each test solution are compared to the values for the immediately preceding control period. For instance, the PVJ delay after 15 minutes of low pH, 8K hypoxia at BCL 1,000 milliseconds was 8.85 ± 2.4 milliseconds while during the preceding control period at the end of 15 minutes pacing at BCL 1,000 milliseconds the PVJ delay was 4.29 ± 1.27 milliseconds. This represents a 106% increase in PVJ delay and was statistically significant at p < 0.005. For the same test solution, the PVJ delay increased to 17.75 ± 9.2 milliseconds during the 1 minute of pacing at BCL 400 milliseconds while in the preceding control period the application of pacing at BCL 400 milliseconds for 1 minute raised the PVJ delay to only 4.45 ± 1.36 milliseconds. Comparing the PVJ delay values at BCL 400 milliseconds, the low pH, 8K hypoxia solution produced a 199% increase in PVJ delay.

Similarly, the P velocity decreased, for the same preparations, in response to the low pH, 8K hypoxia solution, decreasing by 26% at BCL 1,000 milliseconds and also by 26% at BCL 400 milliseconds, both statistically significant at p < 0.05. The hypoxia solution produced no statistically significant effect on PVJ delay or P velocity at BCL 1,000 or BCL 400 milliseconds. The low pH solution had small (< 10%) effects on both PVJ delay and P velocity.

The percent increase in PVJ delay with each test solution is shown in Figure 4. In this bar graph, the 4 horizontal groups correspond to the combinations of pH and [K+]o: NL pH, 4K (pH 7.4, [K+]o 4 mM); low pH, 4K (pH 6.8, [K+]o 4 mM); NL pH, 8K (pH 7.4, [K+]o 8 mM); and low pH, 8K (pH 6.8, [K+]o 8 mM). For each group, the 4 shaded bars (from left to right) indicate BCL 1,000 milliseconds, normal O2; BCL 400 milliseconds, normal O2; BCL 1,000 milliseconds, hypoxia; and BCL 400 milliseconds, hypoxia. Several important features are apparent. First, with normal pH and [K+]o, neither decreasing the BCL nor the oxygen has much effect on PVJ delay. Second, when [K+]o is elevated to 8 mM (the two groups on the right) there is a significant effect on PVJ delay produced by a decrease in BCL or the oxygen level, and these effects are clearly additive. The percent changes in P velocity for the same preparations are shown in Figure 5. Note the difference in the vertical scale between Figures 4 and 5, since P velocity in all cases was less affected than PVJ delay. A decrease in P velocity is produced by lowering the pH or by increasing [K+]o with neither decreasing the BCL nor the oxygen level had no effect on P velocity under any conditions of pH and [K+]o, while these effects were very significant on PVJ delay.

Table 3 summarizes the results of the velocity measurements for the V layer of the right septal preparations in the same format as Table 2. As was seen for the P layer of the papillary muscle, a change in cycle length from 1,000 to 400 milliseconds had no significant effect on V conduction velocity. Hypoxia produced a statistically significant 8% increase in V velocity. The combination of low pH, 8K hypoxia produced a statistically significant decrease in V velocity of 9% (BCL 1,000) and 16% (BCL 400). Elevated [K+]o, with normal pH and oxygen level produced statistically significant increases in V velocity of 8% (BCL 1,000) and 6% (BCL 400). These results are shown graphically in Figure 6 in the same format as Figures 4 and 5. Several points are apparent: First, all of the changes in V velocity are much smaller than in PVJ delay with the same test solutions. Second, elevated [K+]o produces an increase in V velocity in the septal preparations while producing a decrease in P velocity and
an increase in PVJ delay in the papillary muscle preparations.

Discussion

Rationale for Experimental Protocols

There are several reasons why this particular protocol was used for the studies. First, all test solutions were required to produce completely reversible effects on all measured parameters. Four independent variables (pH, $[K^+]$, oxygen level, and pacing cycle length) were used. In preliminary trials, the use of the shorter BCL (400 milliseconds) for long periods of time, particularly under hypoxic conditions, produced severe alterations in PVJ delay that were only partially reversible. Therefore, only 1 minute of BCL 400 pacing was used for all solutions and all preparations. Measurements at 30 seconds and 1 minute of BCL 400 pacing produced nearly identical results so that, while the 1 minute of rapid pacing cannot be considered a "steady state," it did produce a reversible and reproducible effect. Second, the level of hypoxia obtained is not as low as that obtained by other investigators (cf. Gil- mour et al.,' Wotjczak). There is no way to compare quantitatively the hypoxia produced in a superfusion chamber with that produced in ischemia. In the superfusion chamber, the tissue at increasing depths from the surface becomes progressively hypoxic, regardless of the $P_{O_2}$ of the superfusing solution. The objective was to assess the relative effects of the hypoxia, alone and in combination with acidosis and elevated $[K^+]$ on the Purkinje velocity, the ventricular velocity, and the PVJ delay. The degree of hypoxia obtained was sufficient to produce large, easily measured effects on PVJ delay in combination with low pH and/or elevated $[K^+]$ and was therefore sufficient to assess the relative effects of hypoxia on the three subendocardial regions. However, it cannot be extrapolated from these data on the effects of mild hypoxia that the V layer and P layer are not susceptible to more severe hypoxic interventions. Third, only one level of elevated $[K^+]$ and low pH, respectively, were tested. Since 15 minutes was needed to evaluate each test solution, the standard protocol (with a control period between each test period) required about 4 hours to complete. The low pH and elevated $[K^+]$ selected were comparable to those measured by Hill and Gettes during myocardial ischemia in the pig heart.

Effects of Test Solutions on Conduction Velocity

Elevations in $[K^+]$ to 4-5 mM are known to increase conduction velocity in cardiac Purkinje fibers. This effect is attributed to a decrease in the rheobasic current required for activation. The mechanism for the decrease in the rheobasic current appears to be depolarization of the membrane potential toward the voltage threshold for activation without an accompanying positive shift in the voltage threshold. Further membrane depolarization, induced by elevations in $[K^+]$ to $>7$ mM, leads to a significant reduction in the availability of inward sodium current and a subsequent fall
Effect of Hypoxia, Hyperkalemia, and Metabolic Acidosis on Conduction

Table 3. Modulation of Ventricular Conduction Velocity

<table>
<thead>
<tr>
<th>[K⁺]₀</th>
<th>pH</th>
<th>Normal</th>
<th>Hypoxic</th>
<th>BCL</th>
<th>V Velocity (m/sec)</th>
<th>% Increase in V Velocity</th>
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</thead>
<tbody>
<tr>
<td>4</td>
<td>7.4</td>
<td>N</td>
<td>1000</td>
<td>0.55±0.17</td>
<td>-5</td>
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<td>4</td>
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<td>N</td>
<td>400</td>
<td>0.52±0.17</td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td>7.4</td>
<td>H</td>
<td>1000</td>
<td>0.57±0.18/0.53±0.16</td>
<td>8*</td>
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<tr>
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<td>7.4</td>
<td>H</td>
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<td>0.52±0.21/0.50±0.16</td>
<td>4</td>
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</tr>
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<td>8</td>
<td>6.8</td>
<td>H</td>
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<td>-9*</td>
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<td>6.8</td>
<td>H</td>
<td>400</td>
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<td>-16*</td>
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<td>6.8</td>
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<td>8</td>
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</tr>
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<td>N</td>
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<td>N</td>
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<td>6*</td>
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<td>H</td>
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<td>0.47±0.15/0.49±0.17</td>
<td>-4</td>
<td></td>
</tr>
</tbody>
</table>

All of the tests are compared to controls at the same BCL as the test. n ≥ 6 for all tests.

*Indicates p < 0.05, †indicates p < 0.01.

in conduction velocity. Our observation of a slight (< 10%) rate-independent decrease in P velocity in the presence of 8 mM [K⁺] is consistent with these observations.

Contrary to the findings in the subendocardial P layer, 8 mM [K⁺] produces a slight increase (8%) in V velocity. Decreasing the BCL from 1,000 to 400 milliseconds had no additional effect on V velocity. Supranormal conduction is known to occur in ventricular tissue with moderate elevations in [K⁺], and V velocity is observed to decline to below control values only with elevations of [K⁺] to > 10 mM. Another mechanism of action involves the positive displacement of the voltage threshold by proton interaction with the negative surface charges surrounding the cardiac sodium channel. Metabolic acidosis could also mediate its effect on conduction velocity through alterations in the cable properties of cardiac strands. According to the cable equation, conduction velocity is inversely related to the square root of internal longitudinal resistivity under conditions of continuous propagation in a spatially

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FIGURE 5. Alteration of Purkinje conduction velocity by combinations of low pH, elevated K⁺, hypoxia, and a change in cycle length from 1,000 to 400 milliseconds. The data are plotted as percent change for each test solution compared to the preceding control period. The four horizontal groups are for the four combinations of low or normal pH and 4 or 8 mM K⁺. The cross-hatch pattern divides each group into the four combinations of BCL (1,000 or 400 milliseconds) and oxygen level (normal or hypoxic).

FIGURE 6. Alteration of ventricular conduction velocity by combinations of low pH, elevated K⁺, hypoxia, and a change in cycle length from 1,000 to 400 milliseconds. The data of Table 3 are plotted here as percent change for each test solution compared to the preceding control period. The four horizontal groups are for the four combinations of low or normal pH and 4 or 8 mM K⁺. The cross-hatch pattern divides each group into the four combinations of BCL (1,000 or 400 milliseconds) and oxygen level (normal or hypoxic). These experiments used the same protocol of solution changes as for Table 1 but applied to preparations of the upper right ventricular septum.
Effects of Test Solutions on PVJ Delay

All interventions increased PVJ delay, with the exception of hypoxia alone. Overall, the test solutions had more dramatic effects on PVJ delay than on P or V velocity, with PVJ delay increasing by a maximum of 199% with the combined effects of 8 mM [K⁺], pH 6.8, hypoxia, and BCL 400 milliseconds. Besides the marked increase in PVJ delay, some PVJ sites were reversibly blocked by the test solutions (Figure 2). In addition, hypoxia and BCL 400 milliseconds had pronounced effects on PVJ delay in combination with elevated [K⁺] and/or acidosis. It is proposed that these effects are a consequence of the functional anatomy of the PVJ. PVJ sites are junctions between two well-coupled syncytia with asymmetries in geometry and electrical properties that produce an orthodromic conduction delay and a lowered safety factor across the junctional region. The successful transmission of a propagating action potential across the PVJ in the orthodromic direction will depend on the amplitude and rate of rise of the Purkinje action potential (supplying the current that flows across the PVJ site), the resistive coupling among the cells that make up the junctional region (supplying the resistive pathway for current flow from Purkinje to ventricular cells), and the voltage threshold and input impedance of the subendocardial ventricular cell layer at the junctional site.

The authors previously proposed that the PVJ regions are examples of discontinuous conduction in which a "resistive barrier" exists between the Purkinje and ventricular cells that makes propagation across the junction fundamentally different from propagation with well-coupled syncytia such as the Purkinje and ventricular layers of the subendocardium. The evidence from preparations where two internally coupled syncytia are interconnected through a relatively high resistance and from theoretical studies of such preparations demonstrates that the action potential delay between two such preparations is much more sensitive to the resistive coupling than for preparations in which the resistive coupling between cells is distributed in a linear fashion such as for a cardiac strand. The authors also recently demonstrated this phenomenon by showing that octanol, which partially uncouples gap junctions between cardiac cells, had a much larger effect on PVJ delay than on conduction velocity within the Purkinje or the ventricular layer. A phenomenon much less well understood is that regional changes in the electrical load encountered by the propagating action potential can alter the membrane currents flowing during the depolarization and repolarization phases. Again, the authors demonstrated this phenomenon experimentally and theoretically for the squid axon, showing that for an axon with uniform membrane properties the sodium current flowing into the cell was greatly increased even though the rate of rise of the upstroke of the action potential was decreased in the region just proximal to an increased electrical load. Thus, for a region such as the PVJ it would not be expected that the approximation from uniform cable theory that the conduction velocity would vary as the
square root of the maximum rate of rise (or the square root of the maximum sodium conductance) would apply, but rather that the conduction delay across the junction would be more sensitive to decreases in the intrinsic excitability of the cells on both sides of the PVJ region.28

Other than the demonstration that the PVJ delay is more sensitive to the test solutions used than is the conduction velocity in the P layer or in the V layer, the most important demonstration here is that the effects of hypoxia and decreased cycle length are important additive factors for PVJ delay, while for the P and V layer conduction velocity these interventions produce almost no effect. It is proposed that both interventions produce partial uncoupling of the gap junctions in the preparation, and that the effects are manifested primarily at the PVJ region because the relatively high resistance between the P and V cells, which make up the PV junction, serves as a "resistive barrier" that limits the flow of current to excite the subendocardial ventricular layer. An alternative explanation (or an additive mechanism) is that the presence of an increased electrical load as the action potential propagation progresses across the PVJ may be that the amount of sodium current for each action potential that enters the cells may be substantially increased, and, in the presence of a diminished efficacy of the Na+, K+-ATPase pump or a higher frequency of stimulation there may be a re-

References

Effects of hypoxia, hyperkalemia, and metabolic acidosis on canine subendocardial action potential conduction.

R D Veenstra, R W Joyner, R T Wiedmann, M L Young and R C Tan

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