Modulation of the Purkinje-Ventricular Muscle Junctional Conduction by Elevated Potassium and Hypoxia

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Action potential transmission in the canine ventricle normally occurs from the Purkinje (P) system into the ventricular muscle (VM) at specific P-VM junction sites. Transitional (T) cells are located between the Purkinje and the ventricular (V) cells at these P-VM junction sites. It has been shown that exposure to elevated \([K^+]_0\) in combination with hypoxia produces an increase in the P-VM conduction time. To examine this increase in P-VM conduction time, simultaneous measurements of the action potential upstrokes of T cells and the activation times of the local P and V cells at P-VM junctional sites were obtained from in vitro canine papillary muscles. The effects of elevated \([K^+]_0\) and hypoxia on conduction from P cells to T cells was then compared with the conduction from T cells to V cells to assess the relative contribution of each to the increase in the P-VM conduction time. We found that this intervention has approximately equal effects on the two sequential steps involved in P-VM conduction. We then analyzed the increased delay from T cells and V cells on the basis of three hypothetical mechanisms: 1) increased coupling resistance, 2) decreased V cell excitability, and 3) decreased cellular responsiveness of the T cells. Our results show that the effects of elevated \([K^+]_0\) and hypoxia on T-VM delay can be accounted for by a decreased responsiveness of the T cells without any significant electrical uncoupling between T and V cells or decrease in VM excitability. (Circulation 1989;79:1100–1105)

The normal activation sequence of the ventricular tissue requires that an action potential propagating through the His-Purkinje system initiate activation of the underlying subendocardial ventricular muscle (VM). Numerous in vitro studies have focused on the process of Purkinje–ventricular muscle (P-VM) transmission, generally in canine preparations.\(^1\)\(^-\)\(^5\) It has been clearly shown that transitional (T) cells are positioned between the Purkinje (P) and ventricular (V) cells at the P-VM junctional sites. However, the specific membrane properties of these T cells and the pattern of electrical coupling between P and T cells and between T and V cells is not known. For normal orthodromic conduction, it appears that the T cells have action potential upstrokes with two phases. The first phase reflects the local activation of the T cells due to a depolarizing current from the P cells. This first phase of the T cell action potential has a quite variable amplitude and maximum \(dV/dt (V_{\text{max}})\) due to a considerable electrical load imposed on the T cells by the underlying V cells. When the V cells become activated, the second phase of the T cell action potential upstroke occurs due to a removal of the electrical load imposed by the V cells and, to some extent, a depolarizing current coming from the V cells back to the T cells. The effect of this electrical loading of the T cells was further demonstrated by Mendez et al.,\(^1\) showing that the action potential upstroke of the T cells became monophasic if the direction of propagation was reversed, thus removing the electrical loading of the T cells by the V cells.

This overall process of conduction through the P-VM junction can then be divided into two sequential processes of P-T conduction followed by T-VM conduction. A number of interventions that either lower tissue excitability or increase coupling resistance have been shown to increase the P-VM conduction delay or, in some cases, even block conduction between the P and V cells.\(^3\)\(^-\)\(^5\) We recently evaluated\(^6\) the effects of hypoxia, elevated \([K^+]_0\), low pH, and rapid pacing on the time delay that occurs at P-VM junctions. One finding of considerable interest is that exposure of the syncytium to a steady depo-
larization (induced by elevating extracellular K\(^+\) from 4 to 8 mM) or a moderate hypoxia had small effects on increasing P-VM delay. However, the combination of elevated [K\(^+\)]\(_0\) and hypoxia, both known to be present during acute myocardial ischemia, had a significantly larger effect on increasing P-VM delay.

For the present study, we have obtained micro-electrode recordings from T cells at P-VM junctions while simultaneously monitoring the activation times of the P cells and V cells. This was done to assess the relative contributions of the P-T delay and the T-VM delay to the overall enhancement of P-VM delay during conditions of elevated [K\(^+\)]\(_0\) and hypoxia. Our results show that this intervention affects both processes to a nearly equal extent. We then focused specifically on the increase in T-VM delay and proposed three hypothesis that could explain the effects of elevated [K\(^+\)]\(_0\) and hypoxia on the T-VM delay. First, the coupling resistance between T and V cells could be increased, thereby restricting the current flow from T to V cells. Second, the excitability of the V cells at the junctional site could be lowered, thus requiring more current from the T cells to be delivered to the V cells to achieve VM activation. Third, the responsiveness of the T cells may be decreased in that they are less able to provide current to the V cells, thus requiring more current for a critical amount of charge to be passed from the T cells to the V cells. Our experiments have been designed to test which of these three hypotheses can account for the increase in T-VM delay that occurs with exposure to elevated [K\(^+\)]\(_0\) and hypoxia.

**Methods**

Healthy adult mongrel dogs of either sex weighing 20–25 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v. Nembutal) and artificially ventilated via a cuffed endotracheal tube. A left lateral thoracotomy was performed to expose the heart, which was then rapidly removed and placed into a beaker of oxygenated, ice-cold Tyrode’s solution. The left ventricle was opened along the interventricular septum to remove by sharp dissection one or both papillary muscles, which were then placed in a Plexiglas experimental chamber (volume, 20 ml). The preparation was continuously superfused with warmed (36–37°C) Tyrode’s solution of the following composition (mM): NaCl 125, NaH\(_2\)PO\(_4\) 0.4, NaHCO\(_3\) 24, KCl 4, MgCl\(_2\) 1, CaCl\(_2\) 2.7, and dextrose 5.5. The control solution, flowing at a rate of 20 ml/min, was aerated with 95% O\(_2\)-5% CO\(_2\) to maintain pH of 7.35. The test solution (elevated [K\(^+\)]\(_0\) and hypoxia) used for this work consisted of the control Tyrode’s solution with the [K\(^+\)]\(_0\) raised from 4 to 8 mM and aerated with 95% N\(_2\)-5% CO\(_2\). The resulting PO\(_2\) in the experimental chamber was 350–400 mm Hg with control aeration and 60–80 mm Hg with the "hypoxic" aeration. All preparations were stimulated at a frequency of 1 Hz using square-wave pulses of 1 msec duration and 1.5–2.0 times diastolic threshold. Bipolar stimulation was accomplished through a pair of 125-μm diameter Teflon-coated silver wires via a stimulus isolation unit (Grass Medical Instruments). The preparation was repetitively stimulated on a free-running Purkinje strand.

Extracellular recordings were made from the endocardial surface using modified bipolar electrodes made from a twisted pair of Teflon-coated 75-μm diameter silver wires (A-M System, Inc), with one lead touching the surface and the other lead about 1.0 mm above the surface of the preparation. The signal from the modified bipolar electrode was amplified differentially with a gain of 100 and a bandpass of 5 Hz–10 kHz. Intracellular recordings were made with conventional glass microelectrodes filled with 2.5 M KCl with a tip resistance of 15–25 MΩ. The micro-electrode was connected to the probe input of a DAGON preamplifier with careful adjustment of capacitance neutralization after each cell penetration to ensure a rise time of the measuring electrode that was less than 50 μsec. The signals from the surface electrodes and from the microelectrodes were sampled by an RC Electronics A/D converter system on an IBM PC AT computer, with a minimal sampling frequency of 20,000 samples/channel/sec.

**Results**

For each preparation, a P-VM junctional site was located by activation mapping of the superficial P and the underlying VM layer. Our use of surface recordings allowed us not only to find a P-VM junction site under control conditions but also to verify that this particular site remained a P-VM junction site during exposure to our test solution of elevated [K\(^+\)]\(_0\) and hypoxia. Only those sites that maintained an all-negative VM activation signal were used to analyze the effects of the intervention because we have previously shown that some sites were reversibly blocked with elevated [K\(^+\)]\(_0\).

An example of recordings from a P-VM junctional site that met our acceptance criteria is shown in Figure 1. For these experiments, three successive exposures of the preparation to the test solution were made. Each exposure lasted 15 minutes with a control period of 15 minutes between each successive application of the test solution. The superimposed traces at the bottom of Panel A of Figure 1 are the surface potential recordings at the P-VM junction site during the control periods (with a P-VM delay of 3.5 msec) and during the three interventions (with P-VM delays of 5–5.5 msec). Because the test solution had a small effect on P velocity, we have aligned all of the traces in time so that the P activation signals all occur at the 2-msec mark on the time scale. It is important to note that the surface potential recordings show that the test solution effects are consistently reproducible and reversible in terms of the overall process of P-VM conduction. The three upper parts of Panel A illustrate action potentials recorded from a P cell, a T cell, and a V cell at this P-VM junction site. During the first exposure of the P-VM junction to the test
EFFECTS OF $8K^+$ AND HYPOXIA

**A**

- **P CELL**
  - Membrane potential (mV)
  - Time (ms)
- **T CELL**
  - Membrane potential (mV)
  - Time (ms)
- **V CELL**
  - Membrane potential (mV)
  - Time (ms)

**B**

- **CONTROL RECORDINGS**
  - Membrane potential (mV)
  - Time (ms)
- Panel A: Action potentials recorded with a microelectrode from a P cell (top panel), a T cell (second from top panel), and a V cell (third from top panel) along with the simultaneously recorded surface potential recordings from a P-VM junctional site. The trace labeled with an asterisk was obtained during the test solution, whereas the other two traces in each panel are records obtained in control solution and after the recovery period, with all traces aligned such that the P activation signal occurred at the 2-msec time point on the time axis. Panels B and C: Overlays of the separately recorded P, T, and V cell action potentials under control conditions (B) and in the test solution (C), with the $V_{max}$ values (V/sec) indicated on each trace.

**C**

- **TEST RECORDINGS**
  - Membrane potential (mV)
  - Time (ms)

solution, we recorded with a microelectrode from a P cell, with results shown in the upper part of Panel A. The trace labeled with an asterisk is the test recording, while the other two traces of each panel are the control recording and the subsequent recording during the recovery period from this P cell using a sustained microelectrode penetration.

During the second exposure to the test solution, we had the microelectrode in a T cell at this P-VM junction site. As for the upper part of Panel A, the control and recovery traces shown in the middle part of Panel A show complete reversibility of the effects of our test solution on the T cell action potential waveform. For the third exposure to our test solution, we had the microelectrode in a V cell at this same P-VM junction site. The effects of our test solution on the V cell action potential waveform are also completely reversible with washout.

Because the surface potential recordings show that the effects of our intervention were reproduc-
TABLE 1. Effects of Elevated [K+]o and Hypoxia at Canine Purkinje–Ventricular Muscle Junction Sites

<table>
<thead>
<tr>
<th>Experiment</th>
<th>P-VM delay (msec)</th>
<th>T cell V_max (V/sec)</th>
<th>T cell APA (mV)</th>
<th>T-VM time (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Test Ratio</td>
<td>Control Test Ratio</td>
<td>Control Test Ratio</td>
<td>Control Test Ratio</td>
</tr>
<tr>
<td>1</td>
<td>5.2 7.5 1.44</td>
<td>163 53 3.07</td>
<td>85 56 1.52</td>
<td>2.2 3.2 1.45</td>
</tr>
<tr>
<td>2</td>
<td>3.9 6.3 1.62</td>
<td>113 73 1.55</td>
<td>82 58 1.41</td>
<td>1.9 2.7 1.42</td>
</tr>
<tr>
<td>3</td>
<td>4.6 6.7 1.46</td>
<td>183 71 2.58</td>
<td>101 66 1.53</td>
<td>1.9 2.9 1.53</td>
</tr>
<tr>
<td>4</td>
<td>3.5 5.1 1.46</td>
<td>121 59 2.05</td>
<td>101 69 1.46</td>
<td>1.8 2.7 1.50</td>
</tr>
<tr>
<td>5</td>
<td>4.2 5.9 1.40</td>
<td>135 96 1.41</td>
<td>94 79 1.19</td>
<td>1.9 2.1 1.11</td>
</tr>
<tr>
<td>6</td>
<td>3.4 4.9 1.44</td>
<td>136 66 2.06</td>
<td>86 58 1.48</td>
<td>2.3 3.0 1.30</td>
</tr>
<tr>
<td>7</td>
<td>3.4 4.9 1.44</td>
<td>136 66 2.06</td>
<td>96 58 1.66</td>
<td>2.3 3.8 1.65</td>
</tr>
<tr>
<td>Mean</td>
<td>4.0 5.9 1.47</td>
<td>150 71 2.11</td>
<td>96 64 1.46</td>
<td>2.0 2.9 1.42</td>
</tr>
<tr>
<td>SEM</td>
<td>0.3 0.4 0.03</td>
<td>12 5 0.23</td>
<td>4 3 0.06</td>
<td>0.1 0.2 0.07</td>
</tr>
</tbody>
</table>

P, Purkinje; VM, ventricular muscle; T, transitional; APA, action potential amplitude.
*p < 0.05 for comparison of test with control.

Table 1 shows the effects of Elevated [K+]o and Hypoxia at Canine Purkinje–Ventricular Muscle Junction Sites. The table presents data on the P-VM delay, T cell V_max, T cell APA, and T-VM time for different experimental conditions. The table includes control and test values, along with the ratio of test to control. The data indicate that Elevated [K+]o and Hypoxia have a significant effect on the parameters measured, with changes ranging from 14% to 35%. The data are presented in a tabular format, making it easy to compare the different conditions and their effects on the parameters of interest.

**Figure 2.** Bar graphs of elevated [K+]o and hypoxia causes changes in the Purkinje–ventricular muscle (P-VM) delay, the V_max of the transitional (T) cells, the action potential amplitude (APA) of the T cells, and the T-VM time delay. Solid bars, average percent changes in these parameters (n = 7; see Table 1); shaded bars, values during the recovery period.
on P-VM delay is less than a 10% increase in P-VM delay, while the combination of elevated $[K^+]_o$ and hypoxia produces a 40–50% increase in P-VM delay. The action of the elevated $[K^+]_o$ produces a depolarization that decreases the magnitude of the sodium inward current by some degree of inactivation. The action of hypoxia is unclear as to what extent it produces a further decrease in excitability or some degree of electrical uncoupling of the cells.\textsuperscript{7} During the time between the initial activation of the T cell and the activation of the V cell, the driving potential for current flow from T cells to V cells is approximately equal to the T cell APA (see Figure 1). The resistance that limits this current flow is the gap junctions between T cells and V cells. If the coupling resistance is increased or if the VM excitability is decreased during the change from control solution to our test solution, then the increase in T-VM time would be greater than could be accounted for by a decrease in the T cell APA. If, however, the coupling resistance and the VM excitability stays constant, then the increase in T-VM time should be inversely proportional to the decrease in the T cell APA. Another way of expressing these hypothetical relationships is illustrated in Figure 3. For each of the seven preparations, we computed (for the abscissa) the ratio of T-VM time under the test solution to the T-VM time under the control solution. For the ordinate, we computed the ratio of T cell APA under control conditions to the value under the test solution. The hypothesis that the increase in T-VM time with our test solution can be explained by a decreased responsiveness of the T cells without an increase in coupling resistance or a decrease in V cell excitability is illustrated by the dashed line of unity slope. The data from these seven preparations fall very nicely along this line. Either of the first two hypotheses, an increase in coupling resistance, or a decrease in VM excitability occurring during exposure to elevated $[K^+]_o$ and hypoxia, would predict that the points would fall below the dashed line such that increases in T-VM time would not be accompanied by a corresponding decrease in T cell APA.

Discussion

We have now resolved the large increase in P-VM delay produced by a combination of elevated $[K^+]_o$ and hypoxia into the relative contributions of P-T delay and the subsequent T-VM delay. An important caveat that must be expressed is that the overall process of P-VM conduction must eventually be expressed as a three dimensional process involving many different cells. The division of these cells into three groups (P, T, and V cells) with sequential activation of the three groups is a necessary oversimplification of the overall process. We have recently\textsuperscript{8} evaluated the extent to which the P-VM junction process can be adequately expressed as a serial activation of cells in a localized region. We identified P-VM junction sites in papillary muscles, the interventricular septum, or the free wall of the ventricle. We activated the superficial P layer using alternate stimulations at two sites arranged such that the two directions of propagation through the P layer, in response to the two stimuli, were orthogonal. Using both surface potential recordings and microelectrode recordings from individual cells, we showed that the overall process of P-VM transmission was independent of the direction of propagation within the P layer. Once local activation of the P layer had occurred, the temporal sequence of activation of individual T cells and the V cells was invariant. One complication that was found for P-VM junction sites on the septum and free wall (but not on papillary muscles) was that at some sites there were multiple P activation signals, with only one P activation signal locked in time with the subsequent T and V cell activation, suggesting some degree of longitudinal dissociation within the P layer.

Another important caveat with regard to our analysis of T cell activation time is that at each P-VM junction site there are multiple T cells and the activation times of these T cells are not simultaneous.\textsuperscript{3,8} In our experiments, we recorded from only one T cell at each P-VM junctional site as a comparison of test versus control conditions. The consistency of our results for seven experiments suggests that our findings represent an appropriate average response for T cells at P-VM sites.

To the extent that the P-VM conduction process can be divided into two sequential processes of P-T...
conduction and T-VM conduction, we can develop a model system for each of the two sequential delays. For two groups of cells, A and B, which are coupled to each other through a resistance R, the current flow from group A to group B will be \((V_A - V_B)/R\), where \(V_A\) and \(V_B\) represent the membrane potential (relative to the resting potential) of cells in groups A and B, respectively. If group B requires a charge transfer \(Q_B\) to reach threshold, then we can estimate the time delay between activation of group A and activation of group B as: \(T_D = \frac{(Q_B R)}{(V_A - V_B)}\), where during this time period, \(V_A\) represents the APA of group A and \(V_B\) is very small, representing the rise of potential in group B to the threshold potential. With regard to the P-VM conduction, the T and V cells can be considered as groups A and B, respectively, during P-T conduction. During T-VM conduction, the T and V cells can be considered as groups A and B, respectively. We can directly measure \(T_D\), \(V_A\), and \(V_B\). If both \(Q_B\) and R remain constant, \(T_D\) will vary inversely with \(V_A - V_B\), which is approximately equal during this period of time to the T cell APA. If either \(Q_B\) or R increases due to the intervention, then \(T_D\) will increase more than the amount predicted by a simple inverse relation between \(T_D\) and \(V_A\). These relations formed the basis of our test of the three hypotheses for the increased T-VM delay, as presented in Figure 3.

For the in situ P-VM junction, we cannot directly measure the resistive coupling between T and V cells or the excitability of the individual T or V cells. We recently evaluated the effects of elevating \([K^+]_o\) from 4 to 8 mM on the cellular excitability of isolated rabbit V cells, finding that the cellular excitability actually increased by about 5% with this intervention, even though the \(V_{max}\) of the cells decreased by about 20%. If these relations also hold for canine T and V cells, these data are consistent with our indirect argument that the responsiveness (ability to generate inward current) of the T cells is the critical parameter that determines the increase in the T-VM delay during our interventions.

References


KEY WORDS • action potential propagation • electrical coupling
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