Characteristics of Junctional Regions Between Purkinje and Ventricular Muscle Cells of Canine Ventricular Subendocardium

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The normal cardiac activation sequence requires propagation of the action potential from the subendocardial Purkinje network into the underlying ventricular muscle cells. This process occurs at specific junctional sites distributed over the endocardial surface of both ventricles. At these junctional sites, action potentials can be recorded from cells that appear to be interposed between the Purkinje cells and the ventricular muscle cells. The action potential upstrokes recorded from these "transition" cells have characteristic double phases produced by electrotonic interactions with the Purkinje cells and the ventricular muscle cells. We have shown that these junctional regions in the canine subendocardium appear to be fixed anatomic sites with locations independent of the activation sequence of the Purkinje network. In addition, the activation delay between the Purkinje cells and the ventricular muscle cells at a junctional site and the patterns of the action potential upstrokes of transitional cells at a junctional site are independent of the activation sequence of the Purkinje network. We have also demonstrated that at some locations there are multiple Purkinje activation signals recorded with a surface electrode and that these multiple activation signals represent discrete groups of Purkinje cells, some of which contribute to the junctional process while others appear to be substantially uncoupled from neighboring Purkinje cell groups and the underlying transitional cells.

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The normal cardiac excitation sequence requires the propagation of an action potential from the subendocardial Purkinje (P) network into the underlying ventricular muscle (VM) cells. The general pattern of this propagation process consists of three stages of excitation: 1) Propagation within the Purkinje layer progresses rapidly from the site of stimulation as a wavefront which activates the entire Purkinje layer. 2) At specific sites for each preparation, termed Purkinje–ventricular junctions (PVJ), the electrical coupling between the P cells and the underlying VM cells is such that, with a delay of a few milliseconds, localized VM cell activation occurs. 3) Propagation within the VM layer progresses from these PVJ sites to activate the rest of the VM layer of the preparation. Myerburg et al.1,2 showed that there was a spatial distribution of PVJ sites in papillary muscles, with PVJs located preferentially toward the base of the muscle. These studies were confirmed and expanded by two-dimensional surface mapping of papillary muscles and other endocardial regions.3,4

The process of action potential propagation through PVJ sites has been studied by numerous investigators with both intracellular microelectrode recordings and extracellular surface mapping.3,4 This process seems to involve a group of cells that appear to be "specialized" with respect to the time course of their action potential rising phase. These cells, which appear to be interposed between the P cells and the VM cells at the PVJ sites, were termed "transition" (T) cells by Alanis and Benitez.5 From their electrophysiologic studies, Alanis and Benitez6,7 and Matsuda et al.8 identified two depolarization components in the T cells' action potential during P to VM action potential propagation. They proposed that the first component was due to active depolarization of the T cell, while the second, slower rising component was due to electrotonic depolarization of the T cell by the adjacent, actively depolarizing VM cells. Subsequently, Mendez et al.9 used these findings to justify measuring PVJ delay by recording from a single T cell (called a "terminal Purkinje cell") and using the time delay between the depolarization components as the PVJ delay time.

Several important electrophysiologic features of T cell action potentials were illustrated by microelectrode recordings at junctional sites during premature P layer stimulation or with altered Tyrode's solutions.9 When junctional delay was increased by raising extracellular calcium ion concentration, the increased junctional delay was associated with an increase in the time delay between the two components of the T cell depolarization phase. Premature excitation of the Purkinje layer produced a diminished early component of depolarization in the T cell action potential, even when the rising phase of the Purkinje cell was the same as for
the regularly occurring excitation, suggesting a prolonged refractoriness of the T cell. Current injection into T cells at a junctional site was shown to decrease or increase junctional delay with depolarizing and hyperpolarizing current injections, respectively.

One possibility not excluded by previous work is that the cells recorded as T cells are not actually specialized in terms of their intrinsic membrane properties but that the appearance of their rising phase is simply due to electrotonic interactions that modulate the cell into a T cell action potential configuration. If this hypothesis is true, the actual location of PVJ sites and the action potential upstroke of cells recorded as T cells could vary with changes in the activation sequence of the P layer. The alternative hypothesis is that PVJ sites are fixed anatomic entities with a specialized structure of T cells such that when local P activation occurs, the T cells at the PVJ site follow a standard activation sequence, thereby producing a PVJ delay and a set of T cell action potential configurations that is independent of the activation sequence within the P layer. The results presented in this paper support the latter hypothesis.

Materials and Methods

The recording and stimulating methods used have been previously described in detail. A left ventricular (LV) papillary muscle or a portion of the LV septum or free wall was obtained from the heart of a mongrel dog after the heart had been removed under sodium pentobarbital anesthesia. The preparation was allowed to recover for 1 hour in the experimental chamber before data collection began. The chamber was perfused with Tyrode’s solution of the following composition (in mM): NaCl 125, NaH₂PO₄ 0.4, NaHCO₃ 24, KCl 4, MgCl₂ 1, CaCl₂ 2.7, and dextrose 5.5, pH 7.35. The solution was continuously gassed with a 95% O₂-5% CO₂ mixture, and chamber temperature was maintained at 36–37° C. All preparations were stimulated at a frequency of 1 Hz using square wave pulses 0.5–1 msec duration with amplitudes 1.5–2 times diastolic threshold. In preparations on which two pacing sites were used, the second stimulating electrode was placed so that at the PVJ site, the direction of propagation of the P layer action potential wavefront stimulated by the second electrode was at approximately right angles to that stimulated by the first electrode.

Extracellular recordings were made on the endocardial surface of the preparation, using a monopolar electrode with a reference electrode located approximately 1 mm above the preparation surface. Intracellular recordings were obtained with conventional glass microelectrode techniques. Data from both types of recordings were amplified and digitally sampled by an analog to digital converter system (12-bit resolution, sampling rate 5,000–10,000 samples/channel/sec) interfaced to a VAX 11/780 computer system. Data analysis was done by interactive cursor controlled measurements of activation times using a Tektronix 4014 graphics terminal.

Results

In 11 experiments (5 papillary muscles and 6 endocardial preparations), we examined whether PVJ sites were functionally determined or represented anatomically fixed sites. For each experiment, after identifying a PVJ region, 2 stimulating electrodes were placed so that the directions of propagation of the P activation wavefront into the PVJ region produced by the 2 stimulating electrodes were approximately orthogonal. P and VM activation maps with 0.5- or 1.0-mm spacings between the surface recording points were then constructed using each stimulating electrode. Figure 1 shows the results from detailed (0.5-mm spacing) mapping of 1 preparation. For stimulation at location X, Figure 1A shows the P activation time map, B shows the VM activation time map, and C shows the P-V difference map. D, E, and F show comparable maps for stimulation at location Y. Note that despite the differences in P layer activation direction, 4 P-V difference minima (identified by the 5-msec isochromes in C and F) are present in the same locations with X or Y stimulation and that the same overall delay is present at each minima.

Table 1 gives the results for all 11 experiments. For each experiment, whether 0.5-mm or 1-mm map spacing was used, minima in P-V delay were determined for each stimulation direction from the P-V difference maps. P-V difference minima were then defined as representing separate PVJ regions if the individual minima centers were separated by at least 4 mm. Finally, the actual P-V difference times for each recording point within the PVJ region were used to calculate the average PVJ delay for each PVJ region. In every experiment, the location of the PVJ region(s) did not change when the P layer was stimulated at the two selected sites. In addition, as shown in Table 1, the PVJ delays at these regions were virtually the same regardless of the direction of P layer action potential propagation into the PVJ regions.

In 8 of these experiments, microelectrode recordings were made from individual cells to see what happened to the cells’ action potentials and activation time with altered PVJ activation direction. Some of the cells fit the description of the T cells of Alanis and Benitez and Matsuda et al. Figure 2 shows the initial portions of action potentials of P, T, and VM cells sequentially penetrated at a PVJ site (as determined from extracellular mapping). For these experiments, an action potential was defined as a T cell action potential if it had the following characteristics: 1) Its phase 0 depolarization was not smooth but had variable depolarization rates (i.e., phase 0 had bumps or notches), and/or there was a “hump” on the early portion of the plateau. 2) The time of occurrence of its [dV/dt]ₘₐₓ fell between the times of P layer and VM layer activation at the PVJ (as determined from an extracellular recording made at the PVJ). 3) Its overall appearance and duration were similar to those of a VM cell action potential. As in Figure 1, the X and Y stimulation sites were located so that the P layer action wavefronts induced by these two bipoles entered the PVJ in directions approximate-
**Figure 1.** Isochronal activation maps for a canine left septal endocardial preparation. Surface recordings were sequentially made at 0.5-mm spacings (as indicated by dots) during repetitive 1 Hz pacing of Purkinje system from 2 stimulus sites (X and Y). As each surface point was recorded, data were taken with stimulus alternatively applied to location X and location Y. Parts A, B, and C: P activation times, VM activation times, and P-V difference times, respectively, when stimulus was applied to location X. Parts D, E, and F: P activation times, VM activation times, and P-V differences for same surface locations but recorded during stimulation at location Y. See text for discussion.

**Table 1. PVJ Delay vs. Direction of Activation**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Preparation</th>
<th>X stimulation (msec)</th>
<th>Y stimulation (msec)</th>
<th>Ratio</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Papillary muscle</td>
<td>3.0</td>
<td>2.9</td>
<td>1.03</td>
</tr>
<tr>
<td>2</td>
<td>Papillary muscle</td>
<td>4.2</td>
<td>4.1</td>
<td>1.02</td>
</tr>
<tr>
<td>3</td>
<td>Papillary muscle</td>
<td>3.6</td>
<td>3.6</td>
<td>1.00</td>
</tr>
<tr>
<td>4</td>
<td>Papillary muscle</td>
<td>4.0</td>
<td>4.4</td>
<td>0.91</td>
</tr>
<tr>
<td>5</td>
<td>Papillary muscle</td>
<td>4.7</td>
<td>4.7</td>
<td>1.00</td>
</tr>
<tr>
<td>6</td>
<td>Septum</td>
<td>2.3</td>
<td>2.2</td>
<td>1.05</td>
</tr>
<tr>
<td>7</td>
<td>Septum</td>
<td>3.8</td>
<td>4.2</td>
<td>0.90</td>
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<tr>
<td>8</td>
<td>Septum</td>
<td>7.5</td>
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<tr>
<td>9</td>
<td>Septum</td>
<td>4.3</td>
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</tr>
<tr>
<td>10</td>
<td>Septum</td>
<td>3.8</td>
<td>3.6</td>
<td>1.06</td>
</tr>
<tr>
<td>11</td>
<td>Septum</td>
<td>4.1</td>
<td>4.1</td>
<td>1.00</td>
</tr>
<tr>
<td>Mean SD</td>
<td></td>
<td>5.0</td>
<td>5.4</td>
<td>0.93</td>
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<td></td>
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<td>5.2</td>
<td>5.7</td>
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<td></td>
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<td>4.5</td>
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</table>

Linear regression: slope, 0.96; intercept, 0.27; r = 0.97; n = 17.

Several important findings regarding T cells are illustrated by Figure 2. First, T cells do not appear to occur as single, isolated cells or a single, electrically well-coupled group of cells linking the P and VM cells at the PVJ. Second, Figure 2 demonstrates that T cells appear to be activated in a linear sequence since the order of action potential recordings shown is the same as the sequence in which the cells were recorded from as the recording microelectrode was advanced at the PVJ site. This same spatial/temporal relation was found in a total of 17 sets of recordings in which two or more T cells were sequentially penetrated; in all cases each successively deeper T cell action potential always fired after its more superficial predecessor.

A third finding illustrated by Figure 2 is that the T cells remained T cells even though the direction of P layer activation into the PVJ changed. This consistency of T cells as T cells was observed in recordings from 54 T cells. Fourth, comparison of the shapes of the T cells' phase 0 with the two activation directions shows them to be very similar, suggesting that the electrical inputs to and loading of the T cells were the same for
both activation directions. Similar results were seen in a total of 54 T cells.

Finally, Figure 2 shows that the relative times of T cell activation with the two stimuli do not change. These findings suggest that the sequence of T cell activation is anatomically defined and thus does not vary with the direction of action potential propagation into the PVJ. Further support for this hypothesis is illustrated in Figure 3. The relative times (with respect to the PVJ delay) of T cell activation for two directions of PVJ activation for 25 T cells from 4 different experiments are plotted in this figure. The graph shows clearly that the relative time of T cell activation remained almost the same, with respect to the temporal sequence of PVJ activation, regardless of the direction of PVJ activation.

In 3 preparations (2 endocardial and 1 papillary muscle), analysis was somewhat complicated by the presence of more than one P signal in the extracellular recording. In addition, the temporal relation between these P signals varied with the direction of P layer activation, as illustrated in Figure 4. Figures 4A and C show the VM activation maps produced by X and Y stimulation, respectively; these maps confirm the identical location of the PVJ with the two stimuli. B and D show extracellular recordings from around the actual PVJ site (locations marked a, b, d, e), and from the PVJ site itself (location c, which was determined to be the PVJ site based on our previously published PVJ criteria.) At c, 3 P signals were present, and these signals changed their temporal relation with the two different stimulation sites.

Figure 5 shows the initial portions of 4 action potentials recorded from cells near c. The extracellular recording electrode was moved a short distance (about 200 μm) from c to place it closer to the microelectrode. In addition, the starting time for plotting all recordings with Y site stimulation was shifted a fixed amount to time-align the peak negative deflections of the extracellular VM signals generated by the two stimuli. Note that while the activation time of P cell action potential "P," shifted with the change in PVJ activation direction (expressed with respect to the time of VM activation in the extracellular recording), the activation times of both P cell action potential "P," and T cell action potential "T" remained fixed with respect to each other and to the time of VM activation (and the time of the VM cell activation). These results suggest that at some PVJs, there are portions of the P network that are not directly electrically connected to the T cells of the PVJ at that site but are merely "passers-by."

In the two other preparations (not shown) in which similar recordings were made, it was again found that only one of the P signals shifted in conjunction with the changes in T cell and VM cell activation time, further supporting the notion that specific P "strands" are connected to the VM through the T cells of the PVJ and that the activation sequence of cells through the PVJ are fixed.

Discussion

The results presented here demonstrate that PVJ sites are anatomically fixed and that the sequence of activation times (P cells to T cells to VM cells) is
Location of a PVJ site remains constant even when P layer activation is quite complex, showing multiple P activation times at numerous surface locations. Parts A and C: Diagrams of a septal preparation with isochronal activation maps for VM layer in response to stimulation of P layer at location X (A) or location Y (C). Parts B and D: Surface potentials recorded at the 5 labelled points (A, B, C, D, E) of A and B, respectively. The middle location (c) represents a PVJ site for both directions of P activation as indicated by the all-negative waveform for VM activation and the earlier VM activation of this point than at any of the 4 surrounding points.

independent of the direction of P layer propagation. On the other hand, some endocardial regions were shown to have a superficial Purkinje layer that cannot be represented as an electrically interconnected two-dimensional sheet since at numerous locations there appeared to be multiple P activation times recorded by surface electrodes. Also, the microelectrode recordings showed that these multiple P activation signals corresponded to groups of P cells with no apparent local electrical coupling to each other. This observation raised the interesting hypothesis that at some PVJ sites there could be multiple inputs into the T cells from these discrete groups of P cells. However, in the experiment shown in Figures 4 and 5 (and in 2 additional experiments), there was no evidence to support this hypothesis. In all of these preparations, one group of P cells had an activation time that was fixed in association with the succeeding T cell activation times and the VM cell activation time, while the other group of P cells had an activation time that had a variable relation with the T and VM cell activation times depending on the stimulus location.

However, the demonstration that the complicated upstroke patterns of T cell action potentials did not vary with the direction of action potential propagation in the P layer does not imply that these upstroke patterns are therefore due to the intrinsic properties of the T cell membrane conductances. It has been convincingly shown that these multicomponent upstroke patterns are produced by electrotonic interactions mediated by the anatomic relations between T cells and VM cells. The present study demonstrates that once the process is initiated by the arrival of the action potential in the P cells that couple to the most superficial T cells, the subsequent steps in the junctional process appear to follow a fixed time sequence. This time sequence, however, can be altered at a given PVJ site by altered excitability or by alterations in cell-to-cell electrical coupling.

It is interesting to compare the P to VM junctional sites with the AV node, which can be considered a junctional region between atrial cells and the bundle of His. In the AV node, Janse demonstrated that the sequence of AV nodal activation was altered when the location of atrial stimulation was changed. In fact, not only was the AV nodal conduction time altered, but the
can occur to increase the degree of synchrony of ventricular activation and thus the degree of synchrony of ventricular contraction.

References


KEY WORDS: cardiac activation • Purkinje-ventricular (muscle) junction • action potential propagation
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