Conduction between isolated rabbit Purkinje and ventricular myocytes coupled by a variable resistance

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2Department of Medicine, Cardiac Rhythm Management Lab, and 3Department of Biomedical Engineering, University of Alabama-Birmingham, Birmingham, Alabama 35294; 4Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah School of Medicine, Salt Lake City, Utah 84112; and 5Department of Physiology and Biophysics, University of Calgary School of Medicine, Calgary, Alberta T2N 4N1, Canada

Huelsing, Delilah J., Kenneth W. Spitzer, Jonathon M. Cordeiro, and Andrew E. Pollard. Conduction between isolated rabbit Purkinje and ventricular myocytes coupled by a variable resistance. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1163–H1173, 1998.—Conduction at the Purkinje-ventricular junction (PVJ) demonstrates unidirectional block under both physiological and pathophysiological conditions. Although this block is typically attributed to multidimensional electrotonic interactions, we examined possible membrane-level contributions using single, isolated rabbit Purkinje (P) and ventricular (V) myocytes coupled by an electronic circuit. When we varied the junctional resistance (Rj) between paired V myocytes, conduction block occurred at lower Rj values during conduction from the smaller to larger myocyte (115 ± 59 MΩ) than from the larger to smaller myocyte (201 ± 51 MΩ). In Purkinje-ventricular myocyte pairs, however, block occurred at lower Rj values during P-to-V conduction (85 ± 39 MΩ) than during V-to-P conduction (912 ± 175 MΩ), although there was little difference in the mean cell size. Companion computer simulations, performed to examine how the early plateau currents affected conduction, showed that P-to-V block occurred at lower Rj values when the transient outward current was increased or the calcium current was decreased in the model P cell. These results suggest that intrinsic differences in phase 1 repolarization can contribute to unidirectional block at the PVJ.

Purkinje (P)-to-ventricular (V) conduction is an essential step in the cardiac excitation sequence. The P network is a thin layer of tissue that is coupled to the much thicker ventricular myocardium at numerous, discrete sites in the subendocardium that are termed Purkinje-ventricular junctions (PVJ s) (28, 30, 39). Electrotonic interactions within this multidimensional synctium contribute to asymmetric (19) and discontinuous (45) conduction at these PVJ s. Asymmetric conduction refers to the directional difference in safety factor for propagation at the PVJ; this is manifested in unidirectional (P to V) block under both pathophysiological (12, 45) and physiological (26, 28) conditions. Unidirectional block is thought to occur because the large, less excitable V mass imposes an electrical load on the smaller, more excitable P layer (30). Purkinje-ventricular (PV) conduction is described as discontinuous because the conduction delay between the two regions is quite large (3–6 ms) (39, 41, 45), considering the relatively short distance (0.1–1.0 mm) traveled by the impulse (39). Sparse intercellular connections between the P and V regions form a high-resistance barrier (29, 39) that contributes to the discontinuity in PV conduction (19). Although this barrier may benefit P-to-V conduction by shielding the P layer from the electrical load imposed by the V mass (17, 19, 30), ischemic conditions increase the resistance of the barrier, thereby uncoupling the P and V regions (41). This may produce triggered arrhythmias secondary to prolonged P action potentials (22) or reentry due to the combined presence of unidirectional block and slow conduction (13, 32).

Although unidirectional block at the PVJ has traditionally been attributed to these electrotonic interactions, intrinsic differences between P and V myocytes may also underlie directional differences in conduction at the PVJ. For example, P myocytes are typically larger than V myocytes (8). Joyner et al. (18) showed that conduction from a large V cell to a small V cell succeeded at higher values of junctional resistance (Rj) than conduction from a small V cell to a large V cell. Early partial repolarization of the stimulated, or source, cell limited the Rj that could be imposed before conduction failed, i.e., the critical Rj. This repolarization, termed source loading, was due to the electrical load imposed by the nonstimulated, or sink, cell. Ionic mechanisms that may induce directional differences in conduction include P and V differences in the inward rectifier current (IK1), Na+ channel density, the L-type calcium current (ICaL), and the transient outward current (Ito). IK1 is smaller in P myocytes than in V myocytes, resulting in a larger diastolic membrane resistance (Rm) in P myocytes (7). This larger Rm should yield a smaller current threshold for P myocytes. Additionally, P myocytes have a greater density of Na+ channels (10) and, therefore, a faster maximum upstroke velocity (Vmax) than V myocytes (8, 44). In computer simulations of conduction between regions of differing excitability separated by a resistive barrier, unidirectional block occurred in the direction of high to low excitability (19). Intrinsic P and V differences during early repolarization may also influence the success of PV conduction. Blocking ICaL decreased the critical Rj for conduction between electrically coupled V myocytes by reducing the source available for conduction (37). Additionally, in canine papillary muscle prepa-
rations that included PVJs, blocking I_{Ca} increased P-to-V conduction delay, whereas enhancing I_{Ca} decreased P-to-V delay (45). I_{L} is responsible for the early, rapid repolarization during phase 1 of the action potential. Because cellular differences in I_{L} cause greater phase 1 repolarization in P than in V myocytes (1, 6, 8, 9), P-to-V and V-to-P conduction may be differentially modulated by I_{L}.

Because electrotonic interactions within the multidimensional syncytium make it difficult to assess how these intrinsic cellular properties influence PV conduction in intact preparations, we used a two-myocyte experimental system (38). Because this system does not require functional gap junctions, it was straightforward to systematically vary R_{j} between the cells and measure conduction delay and early partial repolarization during P-to-V and V-to-P conduction. Companion numerical simulations provided insight into how differences in intrinsic phase 1 repolarization influenced P-to-V conduction. Our results demonstrate that, even in a two-myocyte system, V-to-P conduction is highly favored over P-to-V conduction. The directional difference in critical R_{j} likely resulted from differences in diastolic R_{m} and intrinsic phase 1 repolarization.

**MATERIALS AND METHODS**

Cell isolation. Single P and V myocytes were isolated from rabbit hearts using previously published techniques (7, 33). Adult, male rabbits weighing 2.0–3.0 kg were anesthetized with 1 ml/kg pentobarbital sodium and 0.5 ml heparin to prevent clotting. After rapid isolation of the heart, the aorta was cannulated for Langendorff perfusion. The heart was agitated with a stream of 100% O_{2}. The temperature was maintained at 37°C. Single P myocytes were periodically removed from the bath and stored in 0.1 mM Ca_{2+} solution, and enzyme solution was added to the remaining P fibers in the bath to maintain a 2-ml volume. Cell dissociation required 15–60 min under these conditions. After the P fibers were dissected from both ventricles, the left ventricle was minced and gently agitated for 6–10 min in 0.1 mM Ca_{2+} Tyrode solution containing no enzymes.

Free-running P fibers were dissected from both ventricles, put into a small bath containing enzyme solution, and agitated with a stream of 100% O_{2}. The temperature was maintained at 37°C. Single P myocytes were periodically removed from the bath and stored in 0.1 mM Ca_{2+} solution, and enzyme solution was added to the remaining P fibers in the bath to maintain a 2-ml volume. Cell dissociation required 15–60 min under these conditions. After the P fibers were dissected from both ventricles, the left ventricle was minced and gently agitated for 6–10 min in 0.1 mM Ca_{2+} Tyrode solution containing no enzymes. The isolated cells were stored at room temperature in 0.1 mM Ca_{2+} Tyrode solution until use.

Solutions. Nominally Ca_{2+}-free Tyrode solution (in mM) 126 NaCl, 5.4 KCl, 5.0 MgCl_{2}, 22 glucose, 1.0 NaH_{2}PO_{4}, 20 taurine, 5 creatine, 5 sodium pyruvate, and 24 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), with pH adjusted to 7.4 with NaOH. The enzyme solution had the same composition, except it also contained 1 mg/ml collagenase (type II, Worthington Biochemical, Freehold, NJ), 0.1 mg/ml protease (type XIV, Sigma Chemical, St. Louis, MO), and 0.1 mM CaCl_{2}.

The normal bathing solution during the experiments contained (in mM) 126 NaCl, 5.4 KCl, 1.0 MgCl_{2}, 1.0 CaCl_{2}, 11 glucose, and 24 HEPES, titrated with 13.0 mM NaOH (pH 7.4). Two pipette filling solutions were used: the ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA) filling solution and the normal filling solution. The EGTA filling solution contained (in mM) 0.1 MgCl_{2}, 5.0 HEPES, 10 EGTA, 5.0 K_{2}ATP, and 90 potassium aspartate, with pH adjusted to 7.2 with KOH. The potassium aspartate caused a liquid junction potential (~10 mV) (9), and results obtained using the EGTA filling solution were corrected by this amount. The normal filling solution contained (in mM) 113 KCl, 10 NaCl, 5.5 glucose, 5.0 K_{2}ATP, 0.5 MgCl_{2}, 10 HEPES, and 11 KOH (pH 7.2).

Electrical recordings. P and V myocytes were placed in a glass-bottom, temperature-controlled bath (36°C) and continuously bathed with the normal solution at a rate of 1–2 ml/min. Only quiescent, rod-shaped cells were studied. Transmembrane potentials were recorded with an Axoclamp 2B amplifier system (Axon Instruments, Foster City, CA). Suction pipettes were made from borosilicate glass (no. 7052, OD 1.65 mm, ID 1.20 mm, A-M Systems, Everett, WA), and after they were fire polished and filled, they had resistances of 3–6 MΩ. Pipette series resistance was carefully compensated before cell attachment. Pipette capacitance was minimized by maintaining a low level (1 mm) of solution in the bath. Action potentials were initiated with intracellular current injection (cycle length = 1 or 2 s). The stimulus duration was 3 ms, and the current stimulus magnitude (I_{stim}) was just sufficient to establish 1:1 pacing during the coupling experiments. The P transmembrane voltage (V_{m,p}) and the V transmembrane voltage (V_{m,v}) were filtered at 1 kHz and digitized at 4 kHz with a 12-bit analog-to-digital converter (Digidata 1200A, Axon Instruments) and recorded with a computer using pCLAMP 6 software (Axon Instruments) for the coupling experiments. In additional experiments, current thresholds were measured in single P and V myocytes with 3-ms (I_{th,3}) and 30-ms (I_{th,30}) pulses to compare how P and V current thresholds changed with pulse duration. Current threshold was defined as the smallest current needed to initiate an action potential and, therefore, represented a measure of excitability in the uncoupled cell. These action potentials were filtered at 5 kHz and digitized at 10 kHz to examine the effect of sampling rate on the action potential upstroke.

Electrical coupling of the two myocytes was achieved using the electronic circuit described by Tan and J oxyer (38). Briefly, two amplifiers with variable gain computed the membrane voltage differences, (V_{m,p} − V_{m,v}) and (V_{m,v} − V_{m,p}). The output was sent to voltage-to-current converters with a fixed gain to simultaneously supply a current of (V_{m,p} − V_{m,v})/R_{j} to the V myocyte and (V_{m,v} − V_{m,p})/R_{j} to the P myocyte. We defined this coupling current, I_{j}, as positive when it flowed into the stimulated cell, either via an external stimulus or from the nonstimulated cell, and negative when it flowed from the stimulated cell to the nonstimulated cell. R_{j} was determined by the gains of the converters and amplifiers and could be varied from 0 to 1,000 MΩ in our system.

Our procedure for studying conduction between P and V myocytes was to first establish pipette attachments in both cells. Before coupling, both cells were stimulated, and the intrinsic action potentials were recorded for each cell. The cells were then electrically coupled with an R_{j} of ~50 MΩ. Pacing stimuli were delivered only to the P myocyte to elicit P-to-V conduction. To determine the critical R_{j} for P-to-V conduction, R_{j} was stepwise increased until conduction failed. To elicit V-to-P conduction, pacing stimuli were delivered only to the V myocyte. We determined the critical R_{j} for V-to-P conduction by stepwise increasing R_{j} until conduction failed. Note that because there was an upper bound on the R_{j} that could be imposed between the cells, we could not measure critical R_{j} above 1,000 MΩ. Therefore, for conduction that did not fail before the upper bound was reached, we considered the critical R_{j} equal to 1,000 MΩ.
Diastolic membrane resistance ($R_m$, MΩ) and capacitance ($C_m$, pF) were determined using intracellular injection of small hyperpolarizing, constant-current pulses (I = 0.1–0.5 nA, 100-ms duration) to elicit small changes in the transmembrane potential ($\Delta V_m = 3–11$ mV) of uncoupled cells. $R_m$ was calculated as $\Delta V_m/I$, and $C_m$ was calculated as $\gamma R_m$, where $\gamma$ was the membrane time constant determined with an exponential fit. Using Clampfit 6 software (Axon Instruments), we differentiated $V_m$ with respect to time and identified the maximum derivative as the maximum upstroke velocity, $V_{\text{max}}$. We defined the time of activation as the time of $V_{\text{max}}$.

Reported conduction delays reflected the difference in activation times between coupled cells. All tabulated data are presented as means ± SD. Statistical analysis included analysis of variance with repeated measures using the SPSS package (SPSS, Chicago, IL).

Computer simulations. To model the coupled cells, we used the DiFrancesco-Noble (DN) membrane equations (4) to describe the ionic currents for a single P cell and the Luo-Rudy (LRd) membrane equations (24, 46) to describe the ionic currents for a single V cell. Action potentials were calculated by numerically solving the following equations

$$\frac{V_{m,v} - V_{m,p}}{R_j} = k \left( C_m \frac{dV_{m,p}}{dt} + I_{\text{ion,p}} \right)$$

$$\frac{V_{m,p} - V_{m,v}}{R_j} = k \left( C_m \frac{dV_{m,v}}{dt} + I_{\text{ion,v}} \right)$$

where $R_j$ was the junctional resistance (MΩ), $C_m$ was the membrane capacitance ($\mu$F/cm$^2$), $I_{\text{ion,p}}$ and $I_{\text{ion,v}}$ were total ionic current in the P and the V myocyte, respectively ($\mu$A/cm$^2$), and $k$ (1.26 × 10$^{-4}$ cm$^3$) depended on the surface-to-volume ratio and the spatial increment. $I_j$, flowing from the model P cell to the model V cell was simply equal to $(V_{m,v} - V_{m,p})/R_j$ from Eq. 1. For V-to-P current flow, $I_j$ was equal to $(V_{m,p} - V_{m,v})/R_j$ from Eq. 2. All simulations were performed on a Sun Microsystems SPARC4 workstation. Solution times were typically 2 min.

Parameter scaling for $V_{\text{max}}$. Because the current that a source cell may provide to a sink cell is related to $V_{\text{max}}$, we wanted to ensure that the ratio of $V_{\text{max}}$ measured in the P myocyte to $V_{\text{max}}$ measured in the V myocyte ($V_{\text{max,p}}/V_{\text{max,v}}$) was maintained in the simulations. Therefore, we used the well-known relationship between the maximum sodium conductance ($g_{\text{Na}}$) and $V_{\text{max}}$ and we increased $g_{\text{Na}}$ in the DN model from 11.2 to 12.7 mS/µF to obtain a concomitant increase in $V_{\text{max}}$ from 177 to 200 V/s. In the LRd model, we decreased $g_{\text{Na}}$ from 16.0 to 6.35 mS/µF, which reduced $V_{\text{max}}$ from 365 to 135 V/s. We chose these values to reflect average $V_{\text{max}}$ values measured at the 4-kHz sampling rate in the uncoupled P and V myocyte pairs.

Parameter scaling for $I_{\text{to}}$ and $I_{\text{Ca}}$. Both the DN and LRd models include formulations of $I_{\text{to}}$, but only the DN model has a formulation of $I_{\text{Ca}}$. To achieve different plateau potentials for the model P cell, we varied either $I_{\text{to}}$ or $I_{\text{Ca}}$ of the model P cell. This allowed us to determine relative effects on critical parameters, such as early partial repolarization, and conduction delay during P-to-V conduction.

RESULTS

Cell size and excitability. Conduction was studied in a total of eight cell pairs, four Purkinje-ventricular (PV) myocyte pairs and, for comparison, four ventricular-ventricular (VV) myocyte pairs. Ten additional uncoupled P and V myocytes were used to compare the effect of short and long duration pulses on current threshold and to examine the effect of sampling rate on $V_{\text{max}}$. Table 1 summarises diastolic membrane resistance, membrane capacitance, and resting potential measured in uncoupled myocytes from the PV and VV pairs. The mean $R_m$ in the P myocyte ($R_m$) was 3.4 times greater than the mean $R_m$ in the V myocyte ($R_m$) (98.0 vs. 28.9 MΩ, respectively). Because this largely reflects the much smaller $I_{\text{K1}}$ in rabbit P myocytes (7), we used $C_m$ instead of $R_m$ as a measure of cell size. In two of the four PV myocyte pairs, the P myocyte was larger than the V myocyte. On average, the P myocytes were 16% larger than the V myocytes (67.1 vs. 57.7 pF, respectively). For each VV myocyte pair, the data presented in Table 1 are ordered by membrane capacitance, such that V denotes the larger myocyte and V' the smaller myocyte. The mean ratio of $C_m$ in the V myocyte to $C_m$ in the V' myocyte ($C_mV/C_mV'$) was 1.6 (58.5 pF/36.2 pF). The mean $V_{\text{rest}}$ values were consistent with reported values for resting potential in the literature (5, 8).

Tables 2 and 3 present values of the stimulus current used during conduction in PV and VV myocyte pairs, current thresholds assessed with 3-ms and 30-ms pulses in uncoupled P and V myocytes, and maximum upstroke velocity measured in uncoupled myocytes at 4- and 10-kHz sampling rates. On average, $I_{\text{stim}}$ in the V myocyte to $V_{\text{max}}$ measured in the V myocyte ($V_{\text{max,p}}/V_{\text{max,v}}$) was maintained in the simulations. Therefore, we used the well-known relationship between the maximum sodium conductance ($g_{\text{Na}}$) and $V_{\text{max}}$ and we increased $g_{\text{Na}}$ in the DN model from 11.2 to 12.7 mS/µF to obtain a concomitant increase in $V_{\text{max}}$ from 177 to 200 V/s. In the LRd model, we decreased $g_{\text{Na}}$ from 16.0 to 6.35 mS/µF, which reduced $V_{\text{max}}$ from 365 to 135 V/s. We chose these values to reflect average $V_{\text{max}}$ values measured at the 4-kHz sampling rate in the uncoupled P and V myocyte pairs.

**Table 1. Membrane resistance, capacitance, and resting potential measured in uncoupled myocytes**

<table>
<thead>
<tr>
<th>PV Myocyte Pair</th>
<th>$R_m,p$ MΩ</th>
<th>$R_m,v$ MΩ</th>
<th>$C_m,p$ pF</th>
<th>$C_m,v$ pF</th>
<th>$V_{\text{rest,p}}$ mV</th>
<th>$V_{\text{rest,v}}$ mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1-V1*</td>
<td>120.0</td>
<td>11.0</td>
<td>52.7</td>
<td>101.7</td>
<td>81.2</td>
<td>78.4</td>
</tr>
<tr>
<td>P2-V2*</td>
<td>79.0</td>
<td>25.0</td>
<td>110.3</td>
<td>57.1</td>
<td>79.4</td>
<td>67.8</td>
</tr>
<tr>
<td>P3-V3*</td>
<td>107.0</td>
<td>41.0</td>
<td>76.7</td>
<td>38.9</td>
<td>-92.4</td>
<td>-83.9</td>
</tr>
<tr>
<td>P4-V4*</td>
<td>85.0</td>
<td>28.9</td>
<td>28.9</td>
<td>33.1</td>
<td>-70.9</td>
<td>-74.7</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>98.0 ± 19.1</td>
<td>28.9 ± 13.8</td>
<td>67.1 ± 34.8</td>
<td>57.7 ± 31.1</td>
<td>-78.4 ± 5.2</td>
<td>-79.4 ± 4.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VV Myocyte Pair</th>
<th>$R_m,p$ MΩ</th>
<th>$R_m,v$ MΩ</th>
<th>$C_m,p$ pF</th>
<th>$C_m,v$ pF</th>
<th>$V_{\text{rest,p}}$ mV</th>
<th>$V_{\text{rest,v}}$ mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>V5-V6*</td>
<td>23.0</td>
<td>60.0</td>
<td>52.7</td>
<td>28.1</td>
<td>-82.6</td>
<td>-80.5</td>
</tr>
<tr>
<td>V7-V8t</td>
<td>37.5</td>
<td>39.6</td>
<td>25.4</td>
<td>61.5</td>
<td>-77.3</td>
<td>-76.7</td>
</tr>
<tr>
<td>V9-V10t</td>
<td>41.0</td>
<td>44.1</td>
<td>29.9</td>
<td>61.5</td>
<td>-83.1</td>
<td>-76.2</td>
</tr>
<tr>
<td>V11-V12t</td>
<td>28.5</td>
<td>97.8</td>
<td>61.5</td>
<td>66.1</td>
<td>-77.3</td>
<td>-72.4</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>32.5 ± 8.2</td>
<td>47.0 ± 14.5</td>
<td>58.5 ± 26.7</td>
<td>36.2 ± 17.0</td>
<td>-77.3 ± 7.9</td>
<td>-76.4 ± 3.3</td>
</tr>
</tbody>
</table>

*EGTA filling solution; †normal filling solution. $R_m,p$, $R_m,v$, and $R_m,v$, membrane resistance of Purkinje (P) and ventricular (V and V') myocytes, respectively; $C_m,p$, $C_m,v$, and $C_m,v$, membrane capacitance of P myocyte, V myocyte, and V' myocyte, respectively; $V_{\text{rest,p}}$, $V_{\text{rest,v}}$, and $V_{\text{rest,v}}$, resting potential of P myocyte, V myocyte and V' myocyte, respectively.
Table 2. Current stimulus and maximum upstroke velocity values in myocyte pairs

<table>
<thead>
<tr>
<th>Myocytes</th>
<th>( I_{\text{stim},p} )</th>
<th>( I_{\text{stim},v} )</th>
<th>( V_{\text{max},p} )</th>
<th>( V_{\text{max},v} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV pairs</td>
<td>1.0 ± 0.1</td>
<td>1.6 ± 0.3</td>
<td>200.2 ± 73.6*</td>
<td>135.9 ± 74.9*</td>
</tr>
<tr>
<td>VV’ pairs</td>
<td>1.4 ± 0.4</td>
<td>1.3 ± 0.2</td>
<td>122.2 ± 26.0*</td>
<td>110.8 ± 38.0*</td>
</tr>
</tbody>
</table>

Current stimulus \((I_{\text{stim}})\) and maximum upstroke velocity \((V_{\text{max}})\) values are mean ± SD. In PV myocyte pairs, \( I_{\text{stim},p} \) and \( I_{\text{stim},v} \) are stimulus currents used during P-to-V and V-to-P conduction, respectively. In VV’ myocyte pairs, \( I_{\text{stim},p} \) and \( I_{\text{stim},v} \) are stimulus currents used during V-to-V’ and V-to-V’ conduction, respectively. \( V_{\text{max},p} \), \( V_{\text{max},v} \), and \( V_{\text{max},v’} \) are maximum upstroke velocities of P, V, and V’ myocytes, respectively. *Digitized at 4 kHz.

Table 3. Current threshold and maximum upstroke velocity values in single P and V myocytes

<table>
<thead>
<tr>
<th>Myocytes</th>
<th>( I_{\text{th},3} )</th>
<th>( I_{\text{th},30} )</th>
<th>( V_{\text{max},v} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>P myocytes</td>
<td>0.31 ± 0.11</td>
<td>0.04 ± 0.02</td>
<td>517.6 ± 35.8*</td>
</tr>
<tr>
<td>V myocytes</td>
<td>0.84 ± 0.22</td>
<td>0.35 ± 0.09</td>
<td>391.8 ± 24.9*</td>
</tr>
</tbody>
</table>

Values are means ± SD. \( I_{\text{th},3} \) and \( I_{\text{th},30} \) threshold currents for single P and V myocytes during 3-ms and 30-ms pulses, respectively. *Digitized at 10 kHz.

myocyte \((I_{\text{stim}})\) required during V-to-P conduction was 60% higher than \( I_{\text{stim}} \) in the P myocyte \((I_{\text{stim},p})\) during P-to-V conduction (1.6 vs. 1.0 nA, respectively). This was consistent with higher current thresholds in uncoupled V myocytes than in P myocytes. With 3-ms duration pulses, V myocytes required 2.7 times (0.84 vs. 0.31 nA) more current than P myocytes to initiate an action potential. Long-duration pulses, representative of long conduction delays, revealed an even larger excitability difference, as \( I_{\text{th},30} \) was 8.75 times (0.35 vs. 0.04 nA, respectively) higher in the V myocytes. \( V_{\text{max},p} \) averaged 1.47 times higher than \( V_{\text{max},v} \) \((200.2 \text{ vs. 135.9 V/s, respectively})\) in the PV myocyte pairs when sampled at 4 kHz. Action potentials digitized at 10 kHz had faster upstrokes, but neither the timing of \( V_{\text{max}} \) nor the ratio \( V_{\text{max},p}/V_{\text{max},v} \) were significantly affected by the sampling rate. In the VV’ myocyte pairs, the mean values of \( I_{\text{stim},v} \) and \( I_{\text{stim}} \) in the V’ myocyte \((I_{\text{stim},v})\) were not significantly different. Similarly, there was no significant difference between \( V_{\text{max},p} \) and \( V_{\text{max},v} \).

P-to-V and V-to-P conduction. Figure 1 shows action potentials and coupling currents recorded at \( R = 100 \text{ MΩ} \) from a PV myocyte pair (P3-V3) during P-to-V and V-to-P conduction. In Fig. 1, each panel depicts the complete record (either \( V_{m} \) or \( I_{c} \)) in insets at left and the initial portion of the record (encompassing the upstroke and early repolarization) in an expanded timescale at right. During P-to-V conduction (Fig. 1, top left), the P action potential demonstrated a spike-and-dome configuration (25). The P upstroke was followed by a large, early partial repolarization of 71.4 mV, forming the spike (open arrow). On activation of the V myocyte, the large, secondary depolarization of the P myocyte formed the dome (closed arrow). P-to-V conduction delay measured 9.3 ms. During V-to-P conduction (Fig. 1, top right), there was no measurable early partial repolarization of the V myocyte because conduction was completed during the upstroke rather than after the action potential peak. V-to-P delay measured only 1.3 ms. The directional difference in conduction delay was due largely to the difference in diastolic \( R_{m} \) between the P and V myocytes. \( R_{m,p} \) measured 107.0 MΩ, whereas \( R_{m,v} \) measured 41.0 MΩ. As a result, the stimulus current required for activation was smaller for the P myocyte (1.0 nA) than for the V myocyte (1.5 nA). Because the P myocyte reached threshold faster than the V myocyte, V-to-P delay was shorter than P-to-V delay.

The coupling current \( I_{c} \) supplied from the pipettes to the myocytes by the coupling circuit was larger during P-to-V (Fig. 1, bottom left) than V-to-P (Fig. 1, bottom right) conduction. The initial positive deflection, marked a at Fig. 1, bottom left, shows the stimulus applied to the P myocyte. As the V myocyte loaded the P myocyte during P-to-V conduction, \( I_{c} \) rapidly decreased to −1.0 nA (b). This outward current contributed to the large, early partial repolarization of the P action potential. \( I_{c} \) became less negative as \( V_{m,v} \), slowly approached threshold. When the V myocyte fired an action potential and \( V_{m,v} \) rose above \( V_{m,p} \) the coupling current became positive as the V myocyte supplied current to the P myocyte (c). This formed the dome of the P action potential. As shown in the inset, \( I_{c} \) reversed direction again (d) as \( V_{m,p} \) fell below \( V_{m,v} \) during late repolarization. In Fig. 1, bottom right, the initial positive deflection, marked a, shows the stimulus applied to the V myocyte to initiate V-to-P conduction. The P myocyte exerted a smaller electrical load upon the V myocyte, as \( I_{c} \) fell to −0.7 nA (b). \( I_{c} \) was positive briefly (c) when the P action caused the notch in the upstroke of the V action potential, then became negative again (d) as \( V_{m,v} \) remained higher than \( V_{m,p} \) during the plateau. As shown in the inset, \( I_{c} \) reversed direction (e) during late repolarization, as \( V_{m,v} \) fell below \( V_{m,p} \).

Results from computer simulations (not shown) were consistent with the experimental results, with directional differences in the amount of early partial repolarization, the conduction delay, and the magnitude of the coupling current. During P-to-V conduction, there was 40.6 mV of early partial repolarization of the model P cell. By comparison, there was no measurable early partial repolarization of the model V cell during V-to-P conduction. P-to-V conduction delay was 6.9 ms, and V-to-P conduction delay was 3.8 ms. Peak \( I_{c} \) was −1.8 nA during P-to-V conduction and −1.3 nA during V-to-P conduction.

Conduction at the critical \( R_{j} \). To determine whether conduction in PV myocyte pairs demonstrated unidirectional block, we systematically varied the imposed junctional resistance to identify the critical \( R_{j} \) values for P-to-V and V-to-P conduction. Figure 2 shows action potentials recorded at the critical \( R_{j} \) from myocyte pair P3-V3 and calculated at the critical \( R_{j} \) during simulations. During P-to-V conduction at \( R_{j} = 110 \text{ MΩ} \) in the myocyte pair (Fig. 2, top left), the P action potential again had a spike-and-dome configuration. However, the amount of early partial repolarization increased to 81.9 mV, and P-to-V conduction delay increased to 15.6
ms, with only a 10-MΩ increase in Rj, V-to-P conduction at Rj = 1,000 MΩ (Fig. 2, top right) was slower than at Rj = 100 MΩ. Thus, the P myocyte imposed a load on the V myocyte after the upstroke, and early partial repolarization of the V action potential measured 8.4 mV. Activation of the P myocyte formed a notch in the V action potential as a slight secondary depolarization interrupted early repolarization. V-to-P conduction delay measured 17.6 ms. Though the P myocyte was approximately twice as large as the V myocyte (76.7 vs. 38.9 pF), the directional differences in early partial repolarization and critical Rj indicated that conduction was favored in the V-to-P direction.

Consistent with the experimental results, computer simulations showed large directional differences in the critical Rj and the amount of early partial repolarization. The critical Rj for P-to-V conduction (Fig. 2, bottom left) was 142 MΩ, and the critical Rj for V-to-P conduc-

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**Fig. 2.** P and V action potentials recorded from myocyte pair P3-V3 at critical Rj (top) and calculated at critical Rj in simulations for P-to-V and V-to-P conduction (bottom). Top left: P-to-V conduction in myocytes. Cells were coupled at Rj = 110 MΩ. Top right: V-to-P conduction. Cells were coupled at Rj = 1,000 MΩ. Bottom left: P-to-V conduction in simulations. Model cells were coupled at Rj = 142 MΩ. Bottom right: V-to-P conduction in simulations. Model cells were coupled at Rj = 3,590 MΩ.
Conduction in VV′ myocyte pairs. Compared with conduction in PV myocyte pairs, conduction in pairs of ventricular myocytes demonstrated less directional difference in critical $R_j$, early partial repolarization, and conduction delay. Figure 3 shows action potentials recorded from a VV′ myocyte pair in which the V myocyte was ~1.6 times larger than the V′ myocyte (97.8 vs. 61.5 pF). Figure 3, top left, shows V-to-V′ conduction at the critical $R_j$ (250 MΩ). V-to-V′ conduction delay measured 18.9 ms, and early partial repolarization of the V myocyte measured 30.0 mV. Activation of the V′ myocyte caused a small notch in the V action potential during early repolarization. By comparison, the critical $R_j$ was smaller (110 MΩ) for V′-to-V conduction (Fig. 3, top right). The V′ upstroke was followed by 42.1 mV of early partial repolarization, and on activation of the V′ myocyte, there was a large secondary depolarization of the V′ myocyte. V′-to-V conduction delay measured 12.9 ms. When the two myocytes were coupled at a common $R_j$ of 100 MΩ for both V-to-V′ and V′-to-V conduction, the directional differences in early partial repolarization and conduction delay were more apparent than when measured at the different critical resistances. V-to-V′ conduction (Fig. 3, bottom left) proceeded with a delay of 4.0 ms and 11.1 mV of early partial repolarization, whereas V′-to-V conduction (Fig. 3, bottom right) proceeded with a delay of 9.3 ms and 35.0 mV of early partial repolarization. Thus conduction was favored in the direction of the larger cell to the smaller cell.

The directional differences in conduction delay and early partial repolarization, as measured at $R_j = 100$ MΩ, were smaller in the VV′ myocyte pair than in the PV myocyte pair. For example, V-to-V′ delay/V′-to-V delay measured 9.3 ms/4.0 ms = 2.3, whereas P-to-V delay/V′-to-P delay measured 9.3 ms/1.3 ms = 7.2.

Summary of experimental data. Table 4 summarizes critical $R_j$, conduction delay at the critical $R_j$, and early partial repolarization at the critical $R_j$ from all eight myocyte pairs. The mean critical $R_j$ for P-to-V conduction was 85 MΩ, whereas the mean critical $R_j$ for V-to-P conduction was 912 MΩ. The early partial repolarization averaged 57.3 mV in the P myocyte during P-to-V conduction and 19.0 mV in the V myocyte during V-to-P conduction. Directional differences in conduction delay were similar for PV and VV′ myocyte pairs: V-to-V′ delay/V′-to-V delay averaged 26.6 ms/17.8 ms = 1.5, and V-to-P delay/P-to-V delay averaged 20.4 ms/13.5 ms = 1.5. However, whereas the mean early partial repolarization ratio was just 34.3 mV/32.0 mV = 1.1 in VV′ myocyte pairs, there was a threefold directional difference in early partial repolarization for PV myocyte pairs. The largest difference between PV and VV′ conduction was in the critical $R_j$; the mean critical $R_j$...
myocyte pairs, the critical differences in myocyte size. In three of the four VV
myocyte pairs, the P myocyte was larger than the V myocyte, but the critical Rj was higher for V-to-P conduction in all four PV myocyte pairs.

I_{Ca}, I_{source}, and source loading. We next considered the contribution of intrinsic phase 1 repolarization to the total early partial repolarization during conduction at the critical Rj. Figure 4 shows action potentials recorded from myocyte pair P3-V3 before and after coupling at the critical Rj. Recall that early partial repolarization was 81.9 mV in the coupled P myocyte during P-to-V conduction (Fig. 2). During this same period, V_{m,p} fell by 72.0 mV in the uncoupled P myocyte (Fig. 4, left), demonstrating that intrinsic phase 1 repolarization accounted for much of the partial repolarization observed during P-to-V conduction at the critical Rj. By comparison, the uncoupled V action potential (Fig. 4, right) demonstrated much less intrinsic phase 1 repolarization. During the time of conduction, V_{m,v} fell by only 4.7 mV in the uncoupled myocyte.

Because intrinsic phase 1 repolarization accounted for a much larger percentage of the total partial repolarization during P-to-V conduction than V-to-P conduction, we determined how alteration of the amount of intrinsic phase 1 repolarization in P myocytes would affect conduction delay, early partial repolarization, and the critical Rj during P-to-V conduction. We used computer simulations to vary I_{to} and I_{Ca} in the model P cell. Increasing I_{to} increased the rate of phase 1 repolarization and lowered the plateau of the uncoupled P cell, whereas decreasing I_{Ca} resulted in a larger degree of phase 1 repolarization and, thus, a lower plateau. These effects caused a progressive increase in both the P-to-V conduction delay and early partial repolarization of the P cell, on coupling to the model V cell. We also examined the relative influences of I_{to} and I_{Ca} on the critical Rj by varying the magnitude of I_{to} and I_{Ca} from zero (representing complete channel block) to five times the nominal model value. Figure 5 shows how the critical Rj for P-to-V conduction varied with the magnitude of I_{to} and I_{Ca} in the model P cell. The critical Rj was highest (169 MΩ) when I_{to} was completely blocked, and it decreased monotonically to 114 MΩ at I_{to} × 5. By comparison, the critical Rj increased monotonically with I_{Ca} from 136 MΩ at zero I_{Ca} to 165 MΩ at I_{Ca} × 5.

### Table 4. Conduction delay and early repolarization at critical Rj for PV and VV myocyte pairs

<table>
<thead>
<tr>
<th>Myocyte Pair</th>
<th>Critical Rj, MΩ</th>
<th>Conduction Delay, ms</th>
<th>Early Repolarization, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P to V</td>
<td>V to P</td>
<td>P to V</td>
</tr>
<tr>
<td>P1-V1</td>
<td>30</td>
<td>1,000</td>
<td>6.8</td>
</tr>
<tr>
<td>P2-V2</td>
<td>85</td>
<td>1,000</td>
<td>15.1</td>
</tr>
<tr>
<td>P3-V3</td>
<td>110</td>
<td>1,000</td>
<td>15.6</td>
</tr>
<tr>
<td>P4-V4</td>
<td>115</td>
<td>650</td>
<td>16.4</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>85 ± 39</td>
<td>912 ± 175*</td>
<td>13.5 ± 4.5</td>
</tr>
</tbody>
</table>

*Early repolarization of stimulated P, V, or V' myocyte is peak potential minus potential at time of conduction. *P = 0.004 when directional differences in critical Rj are compared.*
P-to-V and V-to-P conduction were significantly different, and we examined the P network to the myocardium (P to V), we examined the current generated during the early plateau (18). Because these cells and their ionic currents differ fundamentally, the unidirectional (P to V) block that occurred over a wide range of Rj values could not be ascribed simply to differences in cell size. In fact, mean Cm was not significantly different from mean Cm, although the mean values of critical Rj for P-to-V and V-to-P conduction were significantly different. Additionally, we found that directional differences in early partial repolarization and the critical Rj for conduction between a P myocyte and a V myocyte were greater than directional differences between two V myocytes. Our results suggest that this difference is related to cellular differences in Ito, Ica, and diastolic Rm.

In general, the success of conduction depends on the excitability of the sink, which is largely determined by diastolic input resistance (Rm). During P-to-V conduction in intact tissue preparations, the input resistance of the sink depends primarily on the size, intercellular coupling, and membrane excitability of the ventricular mass. In isolated P and V myocytes, however, the largest fraction of Rm is provided by the diastolic Rm, which is determined mainly by the conductance of I1 (11, 27). I1 helps maintain the resting potential, but because of its marked rectification at depolarized potentials, it is small during the action potential. We have recently found that rabbit I1 current density is two to three times smaller in P than V myocytes (7). This accounts for the higher Rm in P myocytes (98.0 MΩ) than in V myocytes (28.9 MΩ) and contributes to the lower current threshold for P myocytes (Ith,3 = 0.31 nA) than V myocytes (Ith,3 = 0.74 nA). Furthermore, we assessed the excitability of these myocytes with respect to discontinuous conduction by measuring the current threshold with long-duration pulses. Ith,3 was 1.75 times higher in the V myocytes; this difference was statistically significant (P = 0.002). Thus a V myocyte acted as a larger sink during P-to-V conduction than a P myocyte, acting during V-to-P conduction, largely contributing to the nearly 11-fold difference between critical Rj for P-to-V and V-to-P conduction.

By using the coupling-clamp circuit of Tan and Joyner (38), we were able not only to evaluate the source in terms of intrinsic phase 1 repolarization and the sink in terms of excitability, but we were also able to vary the resistive pathway (Rj) between the source and the sink. Low gap junction density at PVJs is consistent with the well-known electrical isolation of P fibers from the underlying V muscle. Thus, we deliberately imposed high values of Rj between the myocytes to yield large conduction delays, thereby modeling discontinuous conduction at the PVJ (25, 26).

It is important to evaluate our findings with respect to certain limitations of the experimental protocol and the modeling study. We used P myocytes isolated from free-running strands as a model for the subendocardial P myocytes that occur at PVJ s. Locating PVJ s requires extensive mapping of the subendocardium (41), whereas
locating the free-running strands required only visual identification. We felt this approach was justified because a previous comparison of the two cell types in dog hearts revealed close similarity in structure, input resistance, and action potential configuration (2).

Another concern was that the high value of critical $R_j$ for V-to-P conduction reflected pacemaker activity in the P myocyte rather than successful V-to-P conduction. Whereas the pattern of ventricular activation during P-to-V conduction at the critical $R_j$ showed a rapid rise of potential followed by a slow rise to threshold, the P myocyte had a very slow, nearly linear rise in potential during V-to-P conduction at the critical $R_j$ (top panels of Fig. 2). However, single P myocytes isolated from a variety of species including dog, sheep, cow, and rabbit generally do not display phase 4 depolarization and automaticity (5, 8, 31, 33, 34). These cells have stable resting potentials between −80 and −90 mV. For isolated cells with resting potentials above −75 mV, infrequent spontaneous activity consisting of transient depolarization occasionally reached threshold (5, 31, 33). In the present study, cell P4 had a resting potential of −70.9 mV (Table 1). However, we did not observe automaticity in this cell or any of the cells included in this study. Moreover, we would expect that if an underlying pacemaker conductance were to artificially raise the critical $R_j$ for V-to-P conduction, we would see the largest critical $R_j$ in cell pair P4-V4 because the elevated resting potential might predispose the P myocyte to automaticity. In fact, this cell pair demonstrated the lowest critical $R_j$ of the four PV cell pairs. Thus P activation at $R_j = 1,000 \, \text{M} \Omega$ likely represents V-to-P conduction rather than pacemaker activity. A related limitation of the experimental procedure was that critical $R_j$ measurements were bounded at 1,000 MΩ. In three of the four PV myocyte pairs, V-to-P conduction succeeded at $R_j = 1,000 \, \text{M} \Omega$ and may have succeeded at higher $R_j$ values. Thus the directional difference in critical $R_j$ is likely larger than the difference documented here.

The 4-kHz sampling rate used in this study underestimated $V_{\text{max}}$. Under identical conditions except for a 10-kHz sampling rate, mean $V_{\text{max}}$ was 2.6 times higher in single P myocytes and 2.9 times higher in single V myocytes. However, the sampling rate affected only the magnitude rather than the timing of $V_{\text{max}}$, so we were able to use $V_{\text{max}}$ measured with the 4-kHz sampling rate to determine activation times and, thus, the conduction delay between cells.

In the simulations, we assumed that $V_{\text{max,p}}V_{\text{max,v}}$ was equal to that measured in the uncoupled myocytes (1.47) with a 4-kHz sampling rate. Altering $g_{\text{Na}}$ and, therefore $V_{\text{max}}$, in the models to reflect $V_{\text{max,p}},V_{\text{max,v}}$ measured with the 10-kHz sampling rate (1.32) in the myocytes subsequently changed the critical $R_j$ and early partial repolarization values calculated during conduction. However, because the sampling rate affected the magnitudes rather than the ratio of $V_{\text{max}}$, the directional differences in critical $R_j$ and early partial repolarization were not significantly affected in the simulations. For $V_{\text{max,p}}V_{\text{max,v}} = 1.47$, the directional difference in critical $R_j$ was 25.3 (3,590 MΩ/142 MΩ) and in early partial repolarization was 6.1 (77.1 mV/12.7 mV). For $V_{\text{max,p}}V_{\text{max,v}} = 1.32$, the directional difference in critical $R_j$ was also 25.3 (2,820 MΩ/111 MΩ) and in early partial repolarization was 6.0 (71.4 mV/11.8 mV). Another limitation of the modeling studies included the membrane equations chosen to represent P and V action potentials. Neither was developed from isolated rabbit heart cell data, and this may well explain quantitative differences between experimental recordings and the simulated action potentials. However, our primary objective with the simulations was to represent qualitative differences between P and V cells in the upstroke and the early plateau of the action potentials. The most marked difference was that in the magnitude of $I_{\text{to}}$ and the resulting phase 1 repolarization; the DN membrane equations included a formulation of $I_{\text{to}}$, whereas the LRd equations did not. The resulting difference in plateau potentials allowed us to relate intrinsic phase 1 repolarization to the critical $R_j$ for P-to-V conduction.

In normal subendocardial preparations, the safety factor for P-to-V conduction varies among the PVJ’s (26). This may be explained by inherent variation in the structure of each PVJ (26, 39) or in the source current for P-to-V conduction at different PVJ’s. For example, there are large variations (0.1–1.0 mm) in the distance spanned by PVJ’s because the strands connecting the P and V regions vary in length (39). Because $R_j$ depends on the intercellular connections within the strands, this may, in part, explain variations in the P-to-V delay at different PVJ’s in the same preparation. Additionally, Verkerk et al. (43) observed two types of action potentials in single P myocytes, one with large phase 1 repolarization and a relatively negative plateau and another with little phase 1 repolarization and a more positive plateau. These observed differences in action potential configuration were due to differences in $I_{\text{to}}$ and $I_{\text{ca}}$ density. Results from the present study suggest heterogeneity in intrinsic phase 1 repolarization will cause variation in safety factor for P-to-V conduction. Under ischemic conditions, the number of functional gap junctions is reduced (20, 29), and, therefore, $R_j$ is increased. Because the critical $R_j$ will vary from junction to junction, this increase in $R_j$ will likely induce unidirectional block at some junctions, but not others. If conduction over the return pathway through the myocardium is slow enough to allow recovery at the sites of block, that impulse may excite the P network retrogradely, initiating circus movement reentry.
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