Influence of Rate-Dependent Cellular Uncoupling on Conduction Change During Simulated Ischemia in Guinea Pig Papillary Muscles: Effect of Verapamil


This study was performed to determine if the changes in cellular coupling induced by simulated ischemia were rate-dependent and if they contributed to the rate-dependent conduction slowing that occurs in this setting. We also sought to determine if the known ability of verapamil to prevent ischemia-induced conduction changes might be related to the preservation of cellular coupling. We studied the effects of increasing stimulation frequency from 0.5 to 2.0 Hz on the simultaneous changes in the maximum rate of rise ($V_{\text{max}}$) of the action potential upstroke, conduction velocity, and internal longitudinal resistance ($r_l$) determined by the voltage ratio method in superfused guinea pig papillary muscles under conditions of simulated ischemia (SI). When stimulation frequency was 0.5 Hz, 30 minutes of SI caused a 16.5% decrease in $V_{\text{max}}$, a 16% increase in $r_l$, and a 12.9% decrease in conduction velocity. When stimulation frequency was increased to 2.0 Hz, 30 minutes of SI caused a 30% decrease in $V_{\text{max}}$, a 72.9% increase in $r_l$, and a 21.4% decrease in conduction velocity. Thus, the changes were rate-dependent. Verapamil ($1 \times 10^{-4}$ M) did not influence the changes in these parameters during SI at 0.5 Hz nor the decrease in $V_{\text{max}}$ during SI at 2.0 Hz, but it did prevent the rate-dependent increase in $r_l$. Verapamil also prevented the rate-dependent decrease in conduction velocity induced by SI. Our results suggest that during simulated ischemia the rate-dependent component of the increase in $r_l$ contributes to the rate-dependence of the conduction slowing. Our results also suggest that the effects of verapamil on ischemia-induced conduction changes are mediated, at least in part, by the prevention of rate-dependent cellular uncoupling. (Circulation Research 1989;65:95-102)

Acute myocardial ischemia results in a slowing of conduction$^1$-$^3$ that becomes more pronounced when the stimulation frequency is increased (i.e., the slowing is rate-dependent)$^4$.$^5$ These changes in conduction contribute to the genesis of lethal ventricular arrhythmias. It is known that the maximum rate of rise of the action potential upstroke is decreased in acute ischemia$^6$ due primarily to a decrease in resting membrane potential that occurs as myocardial extracellular potassium rises.$^7$.$^8$ Acute ischemia also causes an increase in internal longitudinal resistance ($r_l$), indicating cellular uncoupling.$^9$.$^{10}$ These changes underlie the changes in conduction induced by acute ischemia, but it is not known if these changes are rate-dependent.

The calcium channel-blocking agent verapamil slows the rate and magnitude of the changes in extracellular myocardial potassium and pH associated with acute ischemia,$^1^1$ diminishes the conduction slowing induced by acute ischemia,$^1^1$,$^1^2$ and prevents ischemia-induced ventricular fibrillation.$^1^3$ However, the effects of verapamil on ischemia-induced changes in cellular coupling have not been defined.

The purposes of our study were to test the hypotheses that the rate dependence of the ischemia-induced conduction slowing was due, at least in part, to rate-dependent cellular uncoupling and that the rate-dependent effect was prevented by verapamil. We used a superfused papillary muscle...
preparation that permitted us to correlate changes in the maximum rate of rise (V_max) of the action potential upstroke, r_t, and conduction velocity under conditions designed to simulate ischemia. We were also able to determine the effects of changing stimulation frequency on these parameters before and after the addition of verapamil.

Materials and Methods

In all experiments, right ventricular papillary muscles from 300–350-g guinea pigs anesthetized with methohexitol were used. Our preparation was identical to that which we have previously described. We required that the papillary muscles have a length of at least 5.5 mm, a mean diameter of less than 1.3 mm, and no branches. The preparations were covered by a thin layer of nylon mesh and superfused with Tyrode’s solution at a constant rate of 2.6–3.2 ml/min. Suction applied at the tendonous end of the preparation was adjusted to provide a thin layer of fluid on the surface of the fiber. The preparation was stimulated at the cut end of the fiber by bipolar, tungsten electrodes placed 0.5 mm apart using constant current pulses of 1-msec duration and two times diastolic threshold strength. Our modified method for determining r_t from the voltage ratio method originally reported by Weidmann was also identical to that which we have recently described. In all experiments, 3 M KCl-filled glass microelectrodes with a resistance of 15–25 MΩ were used. They were placed no closer than 1 mm to each end. The local transmembrane potential (V_m) was recorded between an intracellular and extracellular electrode placed closely together near one end of the preparation. The intracellular potential (V_i) was recorded between the intracellular electrode and a second extracellular electrode placed near the opposite end of the preparation. The extracellular potential (V_o) was determined from the first derivative of the V_o signal, and conduction velocity was calculated by dividing this time into the interelectrode distance.

Intracellular resistivity (R_i) during the control superfusate was calculated from the equation

\[ R_i = r_i \cdot 0.7A/l \]

where A is cross-sectional area determined as previously described and I is interelectrode distance.

The millimolar composition of the normal Tyrode’s solution was as follows: NaCl 125, KCl 5.4, CaCl_2 1.8, MgCl_2 1.05, NaHCO_3 24, NaH_2PO_4 0.42, and glucose 5. The solution was gassed with 95% O_2-5% CO_2. In the superfusion chamber, pH was 7.35–7.40 and pO_2 was greater than 350 mm Hg. The temperature of the preparation was maintained at 36°–37°C.

After placement of the electrodes and the recording of baseline values, the fiber was superfused with 9 mM KCl solution in which sodium chloride was reduced to 121.4 mM to maintain constant total ionic strength but which was otherwise similar to the 5.4 mM KCl/Tyrode’s solution. We chose the 9 mM KCl/Tyrode’s solution as the “control” superfusate for the simulated ischemia experiments to eliminate the supernormal conduction that occurs in guinea pig papillary muscles when superfusion with solution having a potassium concentration of 5.4 mM is changed to solution having a potassium concentration of 9.0 mM (Table 1). The millimolar composition of the solution used to simulate ischemia (SI) was KCl 9.0, NaCl 142.4, CaCl_2 1.8, MgCl_2 1.05, NaHCO_3 3.0, NaH_2PO_4 0.42, and no glucose. This solution was gassed with 95% N_2-5% CO_2. Nitrogen was also blown across the top of the preparation and filled the Perspex-lined Faraday cage under slight positive pressure. During superfusion with the SI solution, the pO_2 in the chamber was 20–30 mm Hg and the pH was 6.5. In the verapamil experiments, baseline values were first obtained in the absence of verapamil. Verapamil (1 × 10^-6 M) was then added to the control superfusate. Twenty minutes later, the SI superfusate, to which 1 × 10^-6 M verapamil had been added, was instituted.

Unless otherwise indicated, the duration of the SI was 30 minutes. The preparation was superfused with the 9.0 mM K+ control solution for 30–60 minutes after the first exposure to the SI for values to return to pre-SI levels. Those experiments in which the values failed to return to within 5% of the control levels were not accepted for comparative analysis of the data at 0.5 and 2.0 Hz. We also required that the intracellular electrode used to determine V_m, V_i, and V_max remain in the same cell throughout the total duration of the experiment. The values shown are expressed as the mean ±1 SD. Statistical significance was determined by t tests applied to either within-preparation changes in logarithms (which are equivalent to percent changes, i.e., 10 minutes of 2.0 Hz SI compared with 10 minutes of 0.5 Hz SI; Figures 1, 2, and 3; Tables 1, 2, and 3) or within-preparation differences for changes in logarithms (which are equivalent to ratios of percent changes, i.e., 30 minutes of 2.0 Hz SI versus 30
TABLE 1. Effect of Increasing K⁺ From 5.4 to 9.0 mM in Control Solution at Stimulation Frequency of 0.5 Hz

<table>
<thead>
<tr>
<th>Solution</th>
<th>Vᵢ (mV)</th>
<th>Vₒ (mV)</th>
<th>Vₓ/Vₒ</th>
<th>rₒ (KΩ)</th>
<th>rᵢ (KΩ)</th>
<th>rᵢ (Ω centrifugal)</th>
<th>Θ (cm/sec)</th>
<th>Vₓ/Vₘₐₓ (V/sec)</th>
<th>RMP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.4 mM K⁺</td>
<td>108.6±4.6</td>
<td>13.2±2.0</td>
<td>83±1.2</td>
<td>1.03±0.11</td>
<td>1.15±0.13</td>
<td>10.4±2.3</td>
<td>68.0±4.9</td>
<td>198.8±15.6</td>
<td>-86.7±2.3</td>
</tr>
<tr>
<td>9.0 mM K⁺</td>
<td>96.8±3.0</td>
<td>11.6±1.1</td>
<td>84±0.9</td>
<td>1.00±0.11</td>
<td>1.12±0.12</td>
<td>10.1±2.2</td>
<td>73.1±5.9</td>
<td>168.5±15.6</td>
<td>-74.8±3.5</td>
</tr>
</tbody>
</table>

Vᵢ, intracellular potential; Vₒ, extracellular potential; rₒ, total resistance; rᵢ, extracellular resistance; rᵢ, internal longitudinal resistance; Rᵢ, intracellular resistivity; Θ, conduction velocity; Vₓ/Vₘₐₓ, maximum rate of rise; RMP, resting membrane potential.

n=5.

*p<0.05.

Results

The changes associated with the substitution of 9 mM K⁺/Tyrode’s control solution for 5.4 mM K⁺/Tyrode’s control as determined in five experiments are shown in Table 1. Resting membrane potential and Vₓ/Vₘₐₓ decreased, rₒ and rᵢ were unchanged, and conduction velocity increased. These changes are similar to those which have been previously described. The effects of SI at stimulation frequencies of 0.5 and 2.0 Hz were determined in five experiments. In two additional experiments, the intracellular electrode did not remain in the same cell, and conduction velocity was the only parameter included for analysis. Figure 1 shows the time course of the changes in conduction velocity, Vₓ/Vₘₐₓ, and rᵢ in these experiments. The values of all measured parameters after 30 minutes of exposure to SI are shown in Table 2. Figure 1 illustrates that during SI at 0.5 Hz, the decreases in Vₓ/Vₘₐₓ and conduction velocity began immediately after the onset of SI. rᵢ increased by 16.0% after 30 minutes, but the increase did not begin until 10 minutes after the onset of SI. Stimulation at a rate of 2.0 Hz during superfusion with the 9 mM K⁺ control caused an 11.2% fall in Vₓ/Vₘₐₓ (p=NS) and a 5.2% decrease in conduction velocity (p<0.05) but did not change rᵢ. Thirty minutes of SI at 2.0 Hz caused a 30% decrease in Vₓ/Vₘₐₓ, a 21.4% decrease in conduction velocity, and a 72.9% increase in rᵢ as compared with the 2.0 Hz control. The changes in conduction velocity and rᵢ were significantly greater than those induced by SI at 0.5 Hz. However, the change in Vₓ/Vₘₐₓ did not quite reach statistical significance (p=0.057). The increase in rᵢ, like the fall in Vₓ/Vₘₐₓ and conduction velocity, began immediately after the onset of SI. rᵢ did not change by more than 6% throughout the course of these experiments.

Three experiments were performed in which the effects of SI at 2.0 Hz were studied without a prior exposure at 0.5 Hz. These experiments were performed to determine if intracellular changes persisting after SI at 0.5 Hz could have influenced the results observed during SI at 2.0 Hz. The results of these experiments are shown in Figure 2 and illustrate that the changes in rᵢ, conduction velocity, and Vₓ/Vₘₐₓ were not influenced by the prior exposure to SI.

The effects of verapamil, 1x10⁻⁶ M, on all measured parameters were determined in five experiments. In two additional experiments, conduction velocity was the only parameter measured. This concentration of verapamil was chosen because it did not influence Vₓ/Vₘₐₓ during the control or the change in Vₓ/Vₘₐₓ induced by SI at 0.5 Hz. Figure 3 and Table 3 show the results of these experiments, while Table 4 compares the changes induced by SI at the two frequencies before and after verapamil.

FIGURE 1. Plot of time course of changes in conduction velocity (Θ, upper solid circles), maximum rate of rise of the action potential upstroke (Vₓ/Vₘₐₓ, upper open circles), and internal longitudinal resistance (rᵢ, lower closed circles) during simulated ischemia (SI, shaded areas) at 0.5 and 2.0 Hz. *p<0.05 compared with control at same stimulation frequency. †p<0.05 compared with 0.5-Hz SI. ‡p<0.05 compared with 0.5-Hz control.
FIGURE 2. Plot of time course of changes in conduction velocity (\(\Theta\)), maximum rate of rise of the action potential upstroke (\(V_{\text{max}}\)), and internal longitudinal resistance (\(r_i\)) during simulated ischemia (SI, shaded area) at 2.0 Hz. \(n=5\) for \(V_{\text{max}}\) and \(r_i\), \(n=7\) for \(\Theta\). *\(p<0.05\) compared with control at 2.0 Hz.

Table 4 also shows the rate-dependent changes obtained by subtracting the changes induced by SI at 0.5 Hz from those induced by SI at 2.0 Hz. Verapamil did not significantly influence any of the measured 0.5-Hz control values. Nor did it significantly alter the changes in \(V_{\text{max}}\) (–20.1%), conduction velocity (–12.5%), and \(r_i\) (+22.1%) induced by SI at 0.5 Hz. The decrease in \(V_{\text{max}}\) during SI at 2.0 Hz after the addition of verapamil (–29.3%) was similar to that occurring in its absence. However, after verapamil the changes in \(r_i\) (+26.2%) and conduction velocity (–14.2%) during SI at 2.0 Hz were significantly less than those occurring before verapamil and not significantly greater than those that occurred during SI at 0.5 Hz. In addition, the onset of the rise in \(r_i\) was delayed in the presence of verapamil.

Our final three experiments were performed to assure that the apparent effects of verapamil on the changes in \(r_i\) and conduction velocity during SI at 2.0 Hz were not due to unanticipated differences in the preparation. In these experiments, we compared the effects of SI at 2.0 Hz before and after the addition of verapamil. Figure 4 shows the results of one of these experiments and confirms the protective effect of verapamil on the increase in \(r_i\) and the decrease in conduction velocity. Similar results were observed in the other two experiments.

The rate-dependent effects of SI before and after verapamil, determined as shown in Table 4 subtracting the percent changes in these parameters at 0.5 Hz from those occurring at 2.0 Hz, are shown in Figure 5. The decrease in the rate-dependent effects of SI on \(r_i\) and conduction velocity following verapamil were statistically significant.

Discussion

Our experiments were designed to gain insight into the causes of the rate-dependent changes in conduction that have been observed in intact hearts made either regionally or globally ischemic\(^3\)–\(^5\) and into the ability of verapamil to prevent ischemia-induced conduction changes\(^11\)–\(^12\) and ventricular fibrillation.\(^13\) We used the superfused papillary muscle preparation in which ischemia was simulated by altering the composition of the superfusate and our modification\(^14\) of the voltage ratio method described originally by Weidmann.\(^15\) This preparation and methodology permits the simultaneous determination of longitudinal conduction velocity, \(V_{\text{max}}\), of the action potential upstroke, \(r_o\), and \(r_i\). Our preparation differs from the blood-perfused rabbit papillary muscle preparation recently described by Kléber and associates.\(^10\)–\(^18\) In our superfused preparation, the extracellular space is expanded by the layer of superfusate on the surface of the fiber, and \(r_o\) is significantly lower than that observed in the perfused preparation. However, by maintaining the ionic strength of the superfusate and the size of the

FIGURE 3. Time course of conduction velocity (\(\Theta\)), maximum rate of rise of the action potential upstroke (\(V_{\text{max}}\)), and internal longitudinal resistance (\(r_i\)) during simulated ischemia (SI, shaded areas) at 0.5 and 2.0 Hz after addition of \(1\times10^{-6}\) M verapamil. Symbols as in Figure 1. \(n=5\) for \(V_{\text{max}}\) and \(r_i\), \(n=7\) for \(\Theta\).
fluid layer on the surface of the preparation, we are able to maintain a constant \( r_\text{c} \). Therefore, any changes in \( r_\text{e} \) or \( V_\text{r}/V_\text{c} \) presumably reflect a change in \( r_\text{e} \) due primarily to the resistance at the gap junction.\(^\text{19}\) However, we cannot eliminate the possibility that changes in cell volume, perhaps related to inhibition of the Na-K* pump, contributed to the observed changes in \( r_\text{e} \).

\( V_\text{max} \) of the action potential upstroke can be measured in the superfused preparation but has not been measured in the perfused preparation. This permits a direct correlation of the changes in conduction velocity to the simultaneous changes in \( V_\text{max} \) and other passive membrane properties. The superfused preparation also permits modifications of the superfusate that can be held constant throughout the protocol. Our modification of the superfusate permitted us to use as control, solutions with 9 mM KCl to avoid the supernormal conduction that results in the guinea pig papillary muscle on increasing extracellular potassium from 5.4 to 9.0 mM.\(^\text{17}\)

In spite of the differences in preparation, the values of \( R \), that we obtained during the control superfusions are similar to those in the perfused preparation,\(^\text{10,18}\) as is the value for conduction velocity during the 5.4 mM K* control. Furthermore, the delay in the rise of \( r \) after the onset of simulated ischemia at 0.5 Hz is similar to that reported in the perfused preparation when arterial flow is interrupted, although the magnitude of the rise in \( r \) is less. This lesser rise in \( r \) can be attributed to the composition of the solution used to simulate ischemia. We intentionally limited the pH decrease to 6.5 and the potassium rise to 9.0 mM to avoid the rapid development of irreversible changes that occur in the setting of no-flow ischemia\(^\text{10}\) when potassium is higher and pH is lower.\(^\text{11}\) For the same reason, the decrease in conduction velocity that we observed is less than that reported during no-flow ischemia in perfused preparations.\(^\text{5,10}\)

An increase in stimulation frequency by itself resulted in a decrease in \( V_\text{max} \) with no significant change in resting potential, a decrease in conduction velocity, and no change in \( r \). These changes are similar to those we have reported previously.\(^\text{20}\) The decrease in \( V_\text{max} \) can be attributed to an increase in

### Table 2. Effect of 30 Minutes Simulated Ischemia at 0.5 and 2.0 Hz

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0.5-Hz Control</th>
<th>0.5-Hz SI</th>
<th>2.0-Hz Control</th>
<th>2.0-Hz SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_\text{i} ) (mv)</td>
<td>98.0±2.7</td>
<td>94.1±2.3*</td>
<td>93.3±2.7</td>
<td>86.9±4.6*</td>
</tr>
<tr>
<td>( V_\text{o} ) (mv)</td>
<td>9.3±1.8</td>
<td>7.3±1.4*</td>
<td>8.8±2.3</td>
<td>4.8±2.7*</td>
</tr>
<tr>
<td>( V_\text{r}/V_\text{c} )</td>
<td>10.9±2.4</td>
<td>13.3±2.8*</td>
<td>11.2±2.5</td>
<td>18.4±3.0*</td>
</tr>
<tr>
<td>( r_\text{e} ) (Kf)</td>
<td>0.85±0.34</td>
<td>0.83±0.32</td>
<td>0.83±0.37</td>
<td>0.87±0.37</td>
</tr>
<tr>
<td>( r_\text{c} ) (Kf)</td>
<td>0.94±0.37</td>
<td>0.89±0.35*</td>
<td>0.91±0.41</td>
<td>0.92±0.39</td>
</tr>
<tr>
<td>( \Theta ) (cm/sec)</td>
<td>70.8±8.5</td>
<td>61.4±5.1*</td>
<td>67.2±8.6</td>
<td>52.6±5.8*</td>
</tr>
<tr>
<td>( V_\text{max} ) (V/sec)</td>
<td>164.6±33.2</td>
<td>136.4±23.3*</td>
<td>145.4±29.0</td>
<td>102.6±25.8*</td>
</tr>
<tr>
<td>RMP (mv)</td>
<td>-76.6±3.5</td>
<td>-74.2±4.2*</td>
<td>-80.0±7.6</td>
<td>-75.0±11.2</td>
</tr>
<tr>
<td>APA (mv)</td>
<td>109.1±2.7</td>
<td>102.0±3.2*</td>
<td>104.9±4.1</td>
<td>93.7±6.1*</td>
</tr>
</tbody>
</table>

\*p<0.05 compared with control at same stimulation frequency.

### Table 3. Effect of 30 Minutes of Simulated Ischemia at 0.5 and 2.0 Hz After Addition of 1x10^-4 M Verapamil

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0.5-Hz Control +verapamil</th>
<th>0.5-Hz SI +verapamil</th>
<th>2.0-Hz Control +verapamil</th>
<th>2.0-Hz SI +verapamil</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_\text{i} ) (mv)</td>
<td>95.4±4.6</td>
<td>90.4±4.7*</td>
<td>90.7±4.2</td>
<td>83.3±5.5*</td>
</tr>
<tr>
<td>( V_\text{o} ) (mv)</td>
<td>9.9±3.0</td>
<td>7.6±2.5*</td>
<td>9.7±2.6</td>
<td>7.0±2.4*</td>
</tr>
<tr>
<td>( V_\text{r}/V_\text{c} )</td>
<td>10.2±4.4</td>
<td>13.0±4.9*</td>
<td>10.1±4.1</td>
<td>13.2±5.3*</td>
</tr>
<tr>
<td>( r_\text{e} ) (Kf)</td>
<td>0.87±0.19</td>
<td>0.83±0.18</td>
<td>0.84±0.17</td>
<td>0.83±0.18</td>
</tr>
<tr>
<td>( r_\text{c} ) (Kf)</td>
<td>0.98±0.21</td>
<td>0.92±0.18</td>
<td>0.95±0.18</td>
<td>0.92±0.19</td>
</tr>
<tr>
<td>( \Theta ) (cm/sec)</td>
<td>72.2±4.1</td>
<td>63.3±4.6*</td>
<td>70.1±5.9</td>
<td>60.1±4.7*</td>
</tr>
<tr>
<td>( V_\text{max} ) (V/sec)</td>
<td>163.6±15.6</td>
<td>130.6±24.5*</td>
<td>142±10.1</td>
<td>100.6±9.4*</td>
</tr>
<tr>
<td>RMP (mv)</td>
<td>-75.6±3.0</td>
<td>-68.6±5.0*</td>
<td>-78.4±4.0</td>
<td>-72.4±3.6*</td>
</tr>
<tr>
<td>APA (mv)</td>
<td>108.2±1.3</td>
<td>100.9±5.3*</td>
<td>104.1±2.9</td>
<td>92.4±3.5*</td>
</tr>
</tbody>
</table>

Control, 9.0 mM K*/Tyrode's solution; SI, simulated ischemia; \( V_\text{i} \), intracellular potential; \( V_\text{o} \), extracellular potential; \( r_\text{e} \), total resistance; \( r_\text{c} \), extracellular resistance; \( r_\text{e} \), internal longitudinal resistance; \( \Theta \), conduction velocity; \( V_\text{max} \), maximum rate of rise; RMP, resting membrane potential; APA, action potential amplitude. n=7 for \( \Theta \) and 5 for all other parameters.

\*p<0.05 compared with control at same stimulation frequency.

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The increase in stimulation frequency to 2.0 Hz during the SI superfusion caused a rise in \( r_i \) that was more than four times that occurring during SI at 0.5 Hz. In contrast, the decreases in \( V_{\text{max}} \) and conduction velocity during SI at 2.0 Hz were less than two times that observed during SI at 0.5 Hz.

Verapamil, administered in a concentration (1×10^{-6} M) that did not influence \( V_{\text{max}} \) or conduction during the control superfusion or the changes in these parameters during SI at 0.5 Hz, did prevent the more rapid and greater magnitude of rise in \( r_i \) and the greater decrease in conduction velocity during SI at 2.0 Hz without significantly influencing the change in \( V_{\text{max}} \).

W. Cascio and A.G. Klöber (personal communication) have also observed that the rise in \( r_i \) and decrease in conduction velocity are more pronounced at more rapid rates in the blood-perfused rabbit papillary muscle preparation and that 0.5×10^{-6} M verapamil prevents these rate-dependent effects.

Increases in intracellular sodium, hydrogen, and calcium are known to occur in the setting of acute ischemia,\textsuperscript{24-27} and each is capable of causing cellular uncoupling.\textsuperscript{28-30} Our results do not help to separate these various factors as causes of the rate-dependent increase in \( r_i \) during SI. Verapamil might have prevented both a rate-dependent increase in intracellular acidosi and a rate-dependent increase in intracellular calcium during SI at 2.0 Hz. By decreasing the hydrolysis of ATP, a known effect of verapamil in the ischemic heart,\textsuperscript{31} the drug may have lessened both the rate of development and magnitude of intracellular acidosi. By inducing use-dependent calcium channel blockade,\textsuperscript{22-24} verapamil may have lessened the calcium-induced calcium release from the sarcoplasmic reticulum\textsuperscript{35,36} and, in this way, lessened the rise of intracellular calcium associated with ischemia.\textsuperscript{27} Further studies designed to determine intracellular sodium, hydrogen, and calcium under the conditions of our study are required to clarify the mechanisms underlying our observations. Nonetheless, our study suggests that the ability of verapamil to retard the conduction slowing and the development of ventricular fibrillation\textsuperscript{13} in the setting of acute myocardia\textsuperscript{11,12} ischemia in intact hearts may be related to a decrease in the rate and magnitude of cellular uncoupling.

One of the advantages of our preparation is that it allows analysis of the contribution of the changes in \( V_{\text{max}}, r_i \), and action potential amplitude to the associated simultaneously occurring changes in conduction velocity. Continuous cable theory states that the conduction velocity should be directly proportional to the square root of the changes in \( V_{\text{max}} \), and inversely proportional to the square root of the product of the changes in \( (r_i+r_o) \) and action potential amplitude.\textsuperscript{37} Figure 6 illustrates the comparison between the observed changes in conduction velocity and those predicted by cable theory during SI at

![Figure 6](http://circres.ahajournals.org/)

**Figure 6.** Time course of changes in conduction velocity (\( \Theta \)), maximum rate of rise of the action potential upstroke (\( V_{\text{max}} \)), and internal longitudinal resistance (\( r_i \)) during simulated ischemia (SI, shaded areas) at 2.0 Hz before and after addition of 1×10^{-6} M verapamil.
amplitude. The potential upstroke \( (V_{\text{max}}) \) and conduction velocity \( (\theta) \) to those predicted by cable theory. Comparison of observed changes in conduction velocity \( (\theta) \) to those predicted by cable theory \( (\theta) \) determined from the equation \( \theta = K V_{\text{max}}^{-1}(r_0 + r_1) \) APA, where APA is action potential amplitude.

In summary, our results suggest that simulated ischemia causes rate-dependent uncoupling in guinea pig papillary muscles that contributes to rate-dependent conduction slowing. Our results indicate that the rate-dependent uncoupling and rate-dependent conduction slowing are prevented by the addition of verapamil to the superfused in concentrations that do not affect \( V_{\text{max}} \) of the action potential upstroke. These results may contribute to our understanding of the rate-dependent conduction slowing that occurs in intact ischemic hearts. Moreover, they suggest a possible mechanism, in addition to effects on extracellular potassium accumulation, by which pretreatment with verapamil prevents the conduction slowing and ventricular fibrillation induced by acute myocardial ischemia.

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