Modulation of conduction slowing in ischemic rabbit myocardium by calcium-channel activation and blockade

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ABSTRACT Ventricular action potentials and longitudinal conduction times over short distances of epicardium were recorded in isolated rabbit hearts. Global ischemia produced a progressive decrease of resting membrane potential, depression of action potentials, and conduction slowing to approximately 50% of control values over 8 to 10 min. Verapamil (2 x 10\(^{-6}\)M) markedly attenuated ischemia-induced conduction slowing in association with less depression of maximum upstroke velocity (V\(_{\text{max}}\)) and slightly less change in resting membrane potential. In contrast, Bay K 8644 (10\(^{-7}\)M), a calcium-channel agonist, exacerbated ischemia-induced conduction slowing and depression of V\(_{\text{max}}\) but did not significantly affect resting membrane potential. Regression analysis of V\(_{\text{max}}\) vs resting membrane potential and the square of conduction velocity vs V\(_{\text{max}}\) demonstrated that verapamil and Bay K 8644 shifted these relationships in opposite directions. The results indicate that the calcium-channel activation state can modulate slowing of conduction during early ischemia. This is most likely due to alterations in calcium influx before and/or during ischemia. There appear to be three possible components to this effect: (1) a small alteration in the magnitude of ischemia-induced depolarization, (2) alterations of membrane responsiveness at depolarized values of resting membrane potential, and (3) alterations in “nonactive” components of conduction during ischemia, such as changes in excitability or cell-to-cell coupling.


THE PROTECTIVE EFFECT of calcium channel-blocking drugs against early ischemic ventricular arrhythmias has been demonstrated in several experimental studies.1–5 These agents may act, in part, by affecting hemodynamics and regional myocardial blood flow to improve the metabolic supply-demand imbalance.6,7 However, a major factor appears to be a direct effect, possibly related to decreased calcium entry into cells, which alters the electrophysiologic response to ischemia.5,8

Cellular electrophysiologic changes occurring in the first few minutes of myocardial ischemia have been well described,9 but the relationship of these changes to the genesis of ventricular tachyarrhythmias remains incompletely understood. Proposed mechanisms include various forms of reentry10–14 and enhancement of automaticity or triggered activity by depolarizing injury currents.15,16 Considerable evidence has correlated conduction slowing and delayed activation in the ischemic zone with the appearance of arrhythmias,17,18 and the antiarrhythmic effect of calcium channel-blocking drugs in this setting appears to be associated with preservation of conduction in the ischemic zone.2,8,19–21

The present study was undertaken to more clearly define the role of the calcium channel in modulating ischemia-induced conduction slowing in relation to changes in resting potential and action potential characteristics. A combination of intracellular and extracellular recording techniques was used to measure transmembrane potentials and longitudinal conduction times over short distances of epicardium in globally ischemic, isolated rabbit hearts. The calcium antagonist verapamil markedly attenuated ischemia-induced conduction slowing, whereas Bay K 8644, an agent that activates calcium channels by a noncyclic-AMP mechanism, exacerbated conduction slowing. Analysis of the results suggests three separate components to
the effect of calcium-channel activation state on factors related to impulse propagation during acute ischemia. These include the magnitude of ischemia-induced depolarization of resting membrane potential, alteration in membrane responsiveness, and alteration in the relation of conduction velocity to maximum upstroke velocity ($V_{ma}$).

**Methods**

**Experimental preparation.** White New Zealand rabbits (6 to 8 kg) were premedicated with ketamine (25 mg/kg). The animals were killed by cervical dislocation and the hearts were rapidly removed, rinsed with iced Tyrode’s solution, and perfused via an aortic cannula at constant flow (3 to 5 ml/min/g) in a modified Langendorf preparation (Figure 1). The Tyrode solution had the following composition: NaCl 137 mM, KCl 5.5 mM, MgCl$_2$ 1.5 mM, CaCl$_2$ 2.0 mM, NaHCO$_3$ 11.9 mM, Na$_2$HPO$_4$ 0.42 mM, glucose 11 mM, pyruvate 2.0 mM, EDTA 0.57 mM, and ascorbic acid 1.42 μM. The solution was gassed with 95% O$_2$, 5% CO$_2$ (95% N$_2$, 5% CO$_2$ during the ischemic period); pH was 7.40. The mitral valve and pulmonary artery were cut and the sinoatrial node and a portion of the right atrium were removed. The heart was submerged in a jacketed 4 × 6 × 4 cm tissue bath and positioned with the anterior left ventricle up. Temperature was maintained at 35°C by a thermostatically controlled pump.

With a micromanipulator, pacing and recording electrodes were positioned in a linear array on the anterior surface of the left ventricle parallel to fiber orientation as determined visually through a dissecting microscope. The pacing electrode was constructed from two Teflon-coated stainless steel wires (0.003 inch diameter) with an intraelectrode distance of 0.2 mm. Stimuli were delivered by a Grass S-88 stimulator and SIUSA isolation unit (4 msec duration, two times diastolic threshold). Activation times were measured with unipolar electrograms recorded by Teflon-coated tungsten wire (0.002 inch diameter) electrodes and amplified and displayed with use of Tektronix 5A26 A-C coupled differential amplifier and a 5113 dual-beam storage oscilloscope. Injury potentials, which were frequently noted initially, subsided after the first few minutes of equilibration. Electrode positions were verified and interelectrode distances were measured by ocular micrometer before and after each episode of ischemia. Interelectrode distance ranged from 1.5 to 2.1 mm between pacing and proximal recording electrodes and 1.5 to 4.0 mm between proximal and distal recording electrodes. Apparent conduction velocity ($\theta$) was calculated by the equation: $\theta = d/d\tau$, where $\Delta t$ is the difference in activation time and $d$ is distance between proximal and distal electrodes.

Transmembrane potentials were recorded with flexibly mounted microelectrodes. Tips from glass micropipettes filled with 3M KCl were suspended from fine tungsten wire (0.001 in), with Ag-AgCl wires serving as reversible half cells. Electrode resistance ranged from 20 to 40 MΩ. Signals were fed to a high-impedance WPI750 preamplifier and $dV/dt$ was obtained with an electronic differentiator. Calibration was achieved with a ramp generator and response was linear from 5 to 200 V/sec.

Both transmembrane potential and its first derivative were amplified with Tektronix 5A26 preamplifiers and displayed on a Tektronix 5113 dual-beam storage oscilloscope simultaneously with extracellular electrograms. Records were obtained on Polaroid film and with a Gould 2200S strip-chart recorder.

In vigorously beating perfused hearts, stable recordings of transmembrane potentials were easily obtainable, but generally

![Figure 1](http://circ.ahajournals.org/)

**FIGURE 1.** Experimental preparation. The isolated rabbit heart is positioned horizontally with the anterior left ventricle up and perfused via an aortic cannula. Extracellular stimulating and recording electrodes are positioned in a linear array parallel to fiber orientation. A flexibly mounted microelectrode was used to make multiple impalements between proximal and distal recording sites (see text for details).
only for brief intervals ranging from several seconds to a few minutes. However, after the first few minutes of ischemia it was often possible to hold impalements for several minutes. In a few instances, a single stable recording was maintained during perfusion and throughout a 10 min period of ischemia. For the purposes of this study, multiple sites between the extracellular recording electrodes were sampled. A minimum of three impalements were made during normal perfusion and seven to 20 were performed at random times during each 10 min period of global ischemia. Corresponding activation times for proximal and distal extracellular electrograms were simultaneously recorded for each stable impalement. Recordings were generally stable for 15 sec or longer and in most instances were intentionally terminated to obtain data from multiple impalements throughout the 10 min period of ischemia.

Procedure. After a 30-min period of equilibration, perfusion was stopped and the chamber was superflushed with Tyrode's solution gassed with 95% N2 and 5% CO2. To ensure constant temperature during ischemia, each heart was totally submerged and recording sites were just beneath the surface of the Tyrode's solution. An initial trial period of ischemia lasted 5 to 10 min and allowed for minor adjustments of electrode position and verification of stable extracellular recordings. Data were taken only from subsequent ischemic runs, each of 10 min duration, with a 30 to 60 min recovery period between runs. Experiments were excluded from analysis if extracellular electrode position was not maintained throughout the experiment or if block or discontinuous changes in electrogram configuration and Δt occurred before 8 min of ischemia.

Three series of experiments were performed:
(1) Control (5 preparations): Three serial ischemic runs separated by recovery periods were performed in each preparation to verify the reproducibility of electrophysiologic changes.
(2) Verapamil (8 preparations): The first control run was performed in the absence of the drug. Verapamil (2 × 10^-6 M) was then added to the Tyrode's solution and a second ischemic run was performed after an additional 30 min of equilibration.
(3) Bay K 8644 (6 preparations): The first two control runs (see below) were performed in the absence of the drug. Bay K 8644 (10^-7 M) was then added to the Tyrode's solution and a third ischemic run was performed after an additional 30 min of equilibration.

All experiments were performed at a constant pacing rate of 3.3 Hz, with the exception of the third series in which Bay K 8644 was evaluated. In initial experiments at a pacing rate of 3.3 Hz, Bay K 8644 produced profound slowing of conduction and early conduction block during ischemia. Therefore, Bay K 8644 experiments were performed at a constant pacing rate of 2.0 Hz after control runs at both 3.3 and 2.0 Hz.

Data analysis. During each ischemic period a variable number (seven to 20) of intracellular recordings was obtained at random times and for each time point action potential variables and extracellular conduction times were recorded. For the purposes of analysis, data were grouped into 2 min intervals and expressed as mean ± SEM. Values were compared by analysis of variance and differences in means were considered significant at p < .05. For each time interval, there were no significant differences in the mean times of data acquisition during corresponding time intervals of serial ischemic runs.

The relationships of Vmax to resting membrane potential and of conduction velocity squared (θ^2) to Vmax were evaluated by linear regression analysis and slopes were compared by analysis of covariance.23 The θ^2 was plotted because this yielded a more linear relationship as predicted by theoretical considerations and experimental findings in models that more closely approximate unidimensional linear conduction.24, 25

Drugs. Verapamil (Sigma) was weighed fresh daily and dissolved in Tyrode's solution. Bay K 8644 (Miles Laboratories) was diluted from a concentrated stock solution (10^-3 M in DMSO). Care was taken to protect solutions from direct light. Preliminary experiments indicated that the final concentrations of DMSO, 1:10,000 (0.01% by volume), produced no significant electrophysiologic effects.

Results

Electrophysiologic changes during global ischemia. Hearts contracted vigorously during normal perfusion. However, contractions quickly diminished and hearts became mechanically quiescent during the first minute of ischemia. In a few instances, recordings were made from a single impalement maintained under control conditions and throughout a 10 min period of global ischemia. Tracings from one of these continuous recordings are illustrated in figure 2 and are representative of data obtained with multiple serial impalements. There was a progressive loss of resting membrane potential, shortening of action potential duration, and a decrease in action potential amplitude. The rate of rise of the action potential also progressively decreased and the difference in activation times between proximal and distal electrograms was increased as conduction slowed. These changes are illustrated in figure 3.

When the duration of global ischemia was brief (10 min or less), action potential variables and conduction times rapidly returned to control values after reperfusion. This allowed multiple ischemic episodes to be studied in each preparation. As illustrated in table 1, the electrophysiologic changes induced by global ischemia were quite reproducible in three serial periods of global ischemia when a 30 min recovery period of perfusion was allowed between each period of ischemia. At 8 to 10 min of ischemia, apparent conduction velocity decreased by approximately 50% and changes in all variables relative to control were statistically significant (p < .01). Ventricular arrhythmias were never observed either during ischemia or reperfusion.

Effect of Verapamil. The effects of verapamil on ischemia-induced changes in conduction and action potential characteristics are summarized in table 2. During normal perfusion, verapamil (2 × 10^-6 M) significantly decreased action potential duration but did not affect conduction, resting membrane potential, action potential amplitude, or Vmax. However, during ischemia there was considerably less slowing of conduction, slightly less depolarization, and a significant preservation of upstroke velocity. Action potential duration, which was decreased before ischemia, was slightly longer after 8 to 10 min of global ischemia in
the presence of verapamil relative to ischemia in the absence of the drug.

**Bay K 8644.** Bay K 8644 produced several effects that were essentially opposite to those of verapamil. Initial experiments were performed at a pacing rate of 3.3 Hz. Bay K 8644 consistently produced a marked exacerbation of conduction slowing and depression of action potentials with the rapid development of 2:1 block and inexcitability within 2 to 5 min. Experiments were therefore performed at a constant pacing rate of 2.0 Hz.

The effects of cycle length and Bay K 8644 on conduction and action potential characteristics are summarized in table 3. At the slower pacing rate of 2.0 Hz, there was significantly less depression of conduction velocity and \( V_{\text{max}} \) after 8 to 10 min of global ischemia. Action potential duration was longer and there was slightly less depression in action potential amplitude relative to the faster pacing rate of 3.3 Hz. Bay K 8644 (10^{-7}M) markedly exacerbated ischemia-induced conduction slowing and depression of \( V_{\text{max}} \). Action potential amplitude was also somewhat more decreased, as was action potential duration. However, the change in resting potential during ischemia was not significantly different from control.

**Relationship of \( \frac{dV}{dt} \) to \( V_{\text{max}} \) and of \( \theta^2 \) to \( \frac{dV}{dt} \).** To further evaluate the effects of verapamil and Bay K 8644 on the determinants of conduction velocity, the relation of action potential upstroke velocity to resting membrane potential was examined by linear regression analysis (figure 4). Verapamil significantly shifted the slope of the least squares regression line such that at more depolarized resting potentials, there was a relatively greater value of \( \frac{dV}{dt} \). Bay K 8644 produced the opposite effect, shifting this relationship in the opposite direction such that more depolarized resting potentials were associated with lower \( \frac{dV}{dt} \) relative to control.

The relationship of the \( \theta^2 \) to \( V_{\text{max}} \) was also examined. In figure 5, \( \theta^2 \) is plotted against \( V_{\text{max}} \). In the top panel, it can be seen that verapamil also altered this relationship such that lower \( V_{\text{max}} \) was associated with relatively

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**FIGURE 2.** Effects of ischemia on the cardiac action potential. Tracings are samples of a continuous recording from a single subepicardial impalement maintained throughout a 10 min period of global ischemia. A, During perfusion; B to F, at 2, 4, 6, 8, and 10 min of ischemia, respectively. Zero potential is indicated by the horizontal line in each panel. Ischemia was characterized by progressive depolarization of resting membrane potential, loss of action potential amplitude, and shortening of action potential duration (see text for details).

**FIGURE 3.** Effects of ischemia on conduction time and \( dV/dt \) in the isolated rabbit heart. In both panels, 1 is \( -dV/dt \); 2 and 3 are proximal and distal extracellular recordings, respectively. Calibration is shown for \( -dV/dt \). For extracellular electrograms 1 division = 500 \( \mu \)V.
greater $\theta^2$ compared with control. In the bottom panel, Bay K 8644 produced a smaller but significant shift in the opposite direction such that a lower $V_{\text{max}}$ was associated with a smaller $\theta^2$ relative to control.

**Discussion**

**Electrophysiologic effects of ischemia.** The effects of acute ischemia on resting membrane potential and action potentials recorded from the subepicardium of isolated rabbit hearts in the present study are qualitatively similar to findings of other investigators in similar experimental preparations. A progressive loss of resting potential was observed in association with decreased action potential amplitude and upstroke velocity as well as a marked shortening of action potential duration.

Experimental studies in vivo have demonstrated an association between early ventricular arrhythmias and ischemia-induced conduction slowing and regional activation delay. However, in the present investigation no arrhythmias were observed either during ischemia or after reperfusion. This is possibly due to the fact that the duration of each ischemic period was brief (10 min or less) and ischemia was global rather than regional. One could speculate that heterogeneity of conduction, refractoriness, and/or resting potential was not sufficient to produce arrhythmias by reentry or other mechanisms. Calcium overload during ischemia or reperfusion, particularly in the presence of Bay K 8644, might favor the production of arrhythmias due to early afterdepolarizations and triggered activity. However, there was no evidence for this under the experimental conditions of this study.

Apparent conduction velocity, which was calculated by dividing the distance between two closely positioned unipolar recording sites by the difference in conduction time, slowed to approximately 50% of control values over 8 to 10 min of global ischemia. This method assumes a constant linear pathway between recording electrodes. However, the actual pathway of propagation is unknown. Although detailed mapping of activation was not performed in this study, the method used probably yields a good estimate of the changes in mean conduction velocity for the following

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tr>
<td>Effects of verapamil (2 x 10^-6M) on conduction and action potential characteristics in ischemia</td>
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</table>

<table>
<thead>
<tr>
<th>Run 1</th>
<th>Control</th>
<th>0.55 ± 0.03</th>
<th>81 ± 1</th>
<th>143 ± 9</th>
<th>101 ± 5</th>
<th>149 ± 8</th>
<th>171 ± 9</th>
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<tr>
<td>8-10 min</td>
<td>0.24 ± 0.03</td>
<td>61 ± 2</td>
<td>52 ± 7</td>
<td>69 ± 4</td>
<td>52 ± 7</td>
<td>66 ± 6</td>
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<tr>
<td>Run 2</td>
<td>Control</td>
<td>0.55 ± 0.03</td>
<td>80 ± 2</td>
<td>148 ± 9</td>
<td>99 ± 6</td>
<td>146 ± 9</td>
<td>163 ± 10</td>
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<td>Verapamil</td>
<td>0.50 ± 0.03</td>
<td>80 ± 2</td>
<td>146 ± 7</td>
<td>94 ± 5</td>
<td>118 ± 8 #</td>
<td>139 ± 8 #</td>
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<tr>
<td>8-10 min</td>
<td>0.45 ± 0.03 #</td>
<td>65 ± 2 #</td>
<td>107 ± 8 #</td>
<td>83 ± 4 #</td>
<td>80 ± 6 #</td>
<td>96 ± 6 #</td>
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</table>

Values are mean ± SEM; n = 8; abbreviations as in table 1.

\#p < .05 vs run 1; \#p < .05 vs control.
TABLE 3
Effects of Bay K 8644 (10^{-7}M) on conduction and action potential characteristics in ischemia

<table>
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<tr>
<th></th>
<th>$\theta$ (m/sec)</th>
<th>$-RP$ (mV)</th>
<th>$V_{\text{max}}$ (V/sec)</th>
<th>AMP (mV)</th>
<th>APD 50 (msec)</th>
<th>APD 90 (msec)</th>
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<tr>
<td>Run 1  (3.3 Hz)</td>
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<tr>
<td>Control</td>
<td>0.54 ± 0.03</td>
<td>82 ± 1</td>
<td>148 ± 8</td>
<td>98 ± 6</td>
<td>163 ± 9</td>
<td>184 ± 11</td>
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<tr>
<td>8–10 min</td>
<td>0.26 ± 0.02</td>
<td>61 ± 2</td>
<td>41 ± 7</td>
<td>70 ± 5</td>
<td>73 ± 10</td>
<td>90 ± 10</td>
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<tr>
<td>Run 2  (2.0 Hz)</td>
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<td></td>
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<tr>
<td>Control</td>
<td>0.55 ± 0.03</td>
<td>81 ± 1</td>
<td>148 ± 9</td>
<td>102 ± 5</td>
<td>214 ±11\textsuperscript{a}</td>
<td>238 ± 13\textsuperscript{a}</td>
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<tr>
<td>8–10 min</td>
<td>0.35 ± 0.03\textsuperscript{a}</td>
<td>63 ± 2</td>
<td>70 ± 9\textsuperscript{a}</td>
<td>77 ± 4</td>
<td>108 ±11\textsuperscript{a}</td>
<td>129 ± 12\textsuperscript{a}</td>
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<tr>
<td>Run 3  (2.0 Hz)</td>
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<td></td>
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<tr>
<td>Control</td>
<td>0.55 ± 0.03</td>
<td>81 ± 1</td>
<td>146 ± 8</td>
<td>103 ± 5</td>
<td>202 ± 14</td>
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<td>BAY K 8644</td>
<td>0.53 ± 0.04</td>
<td>81 ± 2</td>
<td>154 ± 9</td>
<td>105 ± 6</td>
<td>226 ± 11</td>
<td>251 ± 12</td>
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<tr>
<td>8–10 min</td>
<td>0.16 ± 0.02\textsuperscript{b}</td>
<td>60 ± 2</td>
<td>32 ± 6\textsuperscript{b}</td>
<td>65 ± 5\textsuperscript{b}</td>
<td>87 ± 10\textsuperscript{b}</td>
<td>112 ± 11</td>
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Values are mean ± SEM; n = 6; abbreviations as in table 1.
\textsuperscript{a}p < .05 vs run 1; \textsuperscript{b}p < .05 vs control.

![FIGURE 4](http://circ.ahajournals.org/)

**FIGURE 4.** Effect of verapamil and Bay K 8644 on the relationship of $V_{\text{max}}$ to resting membrane potential. Membrane potential (in mV) is indicated on the abscissa and $V_{\text{max}}$ (in V/sec) is indicated on the ordinate. Values for individual impalements and least squares regression lines are plotted. Top, For control values (○) $r = .91$, slope = 4.29; for verapamil (●) $r = .74$, slope = 2.39 (p < .01). Bottom, For control values (△) $r = .84$, slope = 3.45; for Bay K 8644 (▲) $r = .89$, slope = 4.99 (p < .01).

![FIGURE 5](http://circ.ahajournals.org/)

**FIGURE 5.** Effect of verapamil and Bay K 8644 on the relationship of $\theta^2$ to $V_{\text{max}}$. $V_{\text{max}}$ (in V/sec) is indicated on the abscissa and $\theta^2$ (in (m/sec)^2) is indicated on the ordinate. Values for individual impalements and least squares regression lines are plotted. Top, For control values (○) $r = .88$, slope = 2.02 $\times$ 10^{-3}; for verapamil (●) $r = .51$, slope = 7.30 $\times$ 10^{-4} (p < .01). Bottom, For control values (△) $r = .85$, slope = 1.60 $\times$ 10^{-3}; for Bay K 8644 (▲) $r = .91$, slope = 2.10 $\times$ 10^{-3} (p < .01).
reasons: (1) Conduction times were measured over short distances (1.5 to 4.0 mm). (2) During ischemia, conduction times progressively prolonged in a continuous fashion, without abrupt changes in electrogram configuration. In those few instances when these findings were observed before 8 min of ischemia, the data were excluded from analysis because this was believed to be consistent with local conduction block and a change in the pathway of propagation. (3) Mapping studies by others have demonstrated homogeneous epicardial activation with elliptical isochrones and during the first few minutes of ischemia conduction slows in a continuous, uniform fashion before the appearance of local areas of block. Values of apparent conduction velocity obtained in the present study were similar to those reported by others using either multiple epicardial electrodes or high-density mapping techniques.

Effects of verapamil and Bay K 8644. Numerous experimental studies have demonstrated that calcium channel–blocking agents can prevent or delay conduction slowing and local conduction block induced by acute ischemia. However, in a recent study by Kimura et al., verapamil was reported to have no effect on conduction slowing in ischemic cat endocardium. In other studies, combined hypoxia, hyperkalemia, and acidosis simulating ischemia have been shown to produce more severe changes in subepicardium than subendocardium and Kimura et al. have previously demonstrated a marked protective effect of verapamil on conduction delay under these conditions in canine subepicardium. In a subsequent study in the isolated cat heart, these same authors confirmed that ischemia produces more severe electrophysiologic changes in subepicardium relative to subendocardium and that verapamil attenuates conduction slowing only in subepicardium. They also noted that although this effect was evident after 10 min of ischemia, after 20 min there was no significant difference from control.

Since the protective effect of calcium channel–blocking agents appears to be largely independent of changes in factors that act to reduce the severity of ischemia such as increases in myocardial blood flow and afterload reduction, it has been suggested that calcium channel–blocking agents are acting directly to modify the electrophysiologic effects of ischemia. The most plausible explanation for this is that these agents act by decreasing the amount of calcium entry into cells before and/or during ischemia.

Although intracellular calcium was not measured in the present study and verapamil is known to have many actions other than its calcium channel–blocking properties, the fact that Bay K 8644, an agent that activates calcium channels, had an effect opposite that of verapamil and exacerbated ischemia-induced conduction slowing is consistent with this hypothesis. Furthermore, a diversity of other calcium channel–blocking agents, including diltiazem, nifedipine, and the inorganic ion Ni\(^{2+}\), have been shown to attenuate subepicardial conduction slowing due to ischemia or simulated ischemic conditions.

Intracellular calcium activity between contractions is normally low and controlled by energy-requiring processes such as sequestration in sarcoplasmic reticulum and mitochondria as well as exchange across the cell membrane. During ischemia, intracellular calcium tends to rise and limited energy supplies are used to maintain low intracellular calcium activity. If total intracellular calcium stores are high at the onset of ischemia or if calcium influx across the cell membrane from outside is relatively high during ischemia, then the metabolic expense of maintaining calcium homeostasis will be high. This will lead to a more rapid depletion of high-energy phosphates and since ATP is produced via glycolysis, intracellular pH will fall faster in settings in which calcium load is high. Agents that modulate calcium-channel activation could be acting before ischemia to affect the total intracellular calcium load at the onset of ischemia or during ischemia to affect additional calcium influx.

Relationship of conduction velocity to resting potential and \(V_{\text{max}}\). The magnitude of the rapid sodium-dependent inward current is a major factor affecting conduction velocity in normal cardiac tissue. \(V_{\text{max}}\) is largely determined by the magnitude of this current as well. However, \(V_{\text{max}}\) is also influenced by other factors such as resistance due to passive membrane and cable properties, and the relationship between sodium current and \(V_{\text{max}}\) is nonlinear. Nevertheless, interventions that change sodium conductance when other factors remain constant change \(V_{\text{max}}\) in the same direction, and in experimental preparations that approximate unidimensional linear conduction, it has been shown that \(\theta^2\) varies proportionally to \(V_{\text{max}}\). Since the magnitude of the rapid sodium-dependent current is voltage dependent, resting membrane potential is a major determinant of both \(V_{\text{max}}\) (as defined by the membrane responsiveness curve) and conduction velocity.

During ischemia, the magnitude of the sodium current presumably decreases due to loss of resting membrane potential. However, other factors probably also contribute to conduction slowing, including changes in excitability, cell coupling, or factors that further
depress the sodium current and hence alter the membrane responsiveness relationship.

In the present study, verapamil had a small but significant effect on the magnitude of ischemia-induced depolarization. This is consistent with the findings of Fleet et al., 32 who found that verapamil slowed the rate of rise in extracellular potassium in the central ischemic zone in pigs, and Clusin et al., 21 who demonstrated a reduction in TQ depression after diltiazem during ischemia in dogs. However, other investigators have shown no change in potassium release by verapamil in globally ischemic rabbit hearts 19 and no change in resting membrane potential by verapamil under conditions of combined hypoxia, hyperkalemia, and acidosis simulating ischemia in dog myocardium 44 or during ischemia in the isolated cat heart. 35

Bay K 8644 did not significantly affect resting potential either during normal perfusion or under ischemic conditions, although there was a trend toward greater depolarization at 8 to 10 min of ischemia. Calcium undoubtedly has multiple effects both intracellularly and extracellularly. Decreased extracellular calcium has been shown to decrease the rate of rise in extracellular potassium during global ischemia in the isolated rabbit septum. 40 Under nonischemic conditions, changes in extracellular calcium do not alter resting membrane potential in canine Purkinje fibers. 41 However, calcium ionophores, which act to increase calcium influx, have been shown to produce hyperpolarization of resting membrane potential. 42

Although verapamil had a minor effect on ischemia-induced depolarization, the observed preservation of action potential upstroke velocity cannot be explained on this basis alone. As illustrated in figure 4, verapamil altered membrane responsiveness such that at depolarized resting potentials during ischemia, \( V_{\text{max}} \) was significantly greater in the presence of verapamil. Bay K 8644 had the opposite effect such that at depolarized resting potentials, \( V_{\text{max}} \) was less than control. In this regard, the effect of verapamil is in agreement with the findings of Kimura et al., 34 who reported that although verapamil did not alter resting membrane potential under simulated ischemic conditions, there was a significant improvement in \( V_{\text{max}} \). El-Sherif and Lazzara 43 studied dog hearts in the late myocardial infarction period and found that some cells had relatively normal resting potentials but depressed upstrokes that improved in the presence of verapamil or D-600. It is noteworthy that cells of normal papillary muscles that are partially depolarized by high extracellular potassium exhibit depressed fast sodium–dependent upstrokes that do not improve with verapamil. 44 Ischemia causes greater depression of action potentials than that produced by similar levels of hyperkalemia in nonischemic tissue 45 and it is likely that agents that affect the activation state of calcium channels modulate those factors that depress \( V_{\text{max}} \) independent of membrane potential.

Although \( V_{\text{max}} \) is not merely a reflection of sodium conductance, one could speculate that increased intracellular calcium affects the kinetics of the fast sodium channels either directly or indirectly by altering other factors such as intracellular pH. 46 47

Calcium-channel activation or blockade also appears to affect determinants of conduction velocity not reflected in \( V_{\text{max}} \). As illustrated in figure 5, verapamil and Bay K 8644 shift the relationship of \( V_{\text{max}} \) to \( \Theta^2 \) in opposite directions. At more depressed levels of \( V_{\text{max}} \) during ischemia, corresponding \( \Theta^2 \)'s are greater than control in the presence of verapamil and less than control in the presence of Bay K 8644. This further suggests that changes in conduction velocity cannot be accounted for entirely by changes in sodium conductance. In this regard, Buchanan et al. 25 have demonstrated in papillary muscle that interventions that primarily alter sodium conductance, including tetrodotoxin, type I antiarrhythmics, and changes in the sodium gradient, affect \( V_{\text{max}} \) and conduction velocity as predicted by the relationship: \( V_{\text{max}} \propto \Theta^2 \). In contrast, certain other interventions that affect conduction velocity, such as changes in extracellular calcium or pH, change the slope of this relationship. 48

Pressler et al. 41 have demonstrated that increasing extracellular calcium produces an increase in internal longitudinal resistance that is blocked by lanthanum but not by verapamil. They interpreted their findings as suggesting that this effect is mediated by calcium influx due to sodium/calcium exchange (rather than calcium influx via "slow channels"). However, verapamil has been demonstrated to attenuate the increase in internal longitudinal resistance produced by simulated ischemic conditions. 49 This suggests that, during ischemia, additional factors may contribute to the increase in internal longitudinal resistance that can be affected by verapamil.

Calcium influx before and/or during ischemia might affect other electrophysiologic properties, such as membrane capacitance and threshold potential, by altering fixed surface changes. 50 In nonischemic preparations, increased extracellular calcium has been shown to alter membrane resistance and capacitance in conjunction with changes in excitability. 41

In summary, the results of the present study confirm the previous findings of others that calcium-channel
blockade attenuates ischemia-induced depression of action potentials and slowing of conduction. In addition, it was demonstrated that Bay K 8644, an agent that activates calcium channels, produces an exacerbation of these electrophysiologic effects of ischemia. These results are consistent with the hypothesis that these agents act by affecting calcium entry into myocardial cells. Analysis of the data suggests three separate components whereby the calcium-channel activation state can modulate the electrophysiologic response to ischemia: (1) small alterations in the magnitude of ischemia-induced depolarization of membrane potential that directly affect action potential upstroke velocity, (2) alterations in membrane responsiveness or the relation of $V_{max}$ to resting membrane potential, and (3) alterations in factors that change the relationship of $V_{max}$ to $q^2$, such as changes in excitability or cell-to-cell coupling.

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