Verapamil Prevents Slowing of Transmural Conduction and Suppresses Arrhythmias in an Isolated Guinea Pig Ventricular Model of Ischemia and Reperfusion

Gui-Rong Li and Gregory R. Ferrier

Transmembrane electrical activity was recorded from endocardium and epicardium of isolated segments of guinea pig right ventricular free walls. An electrocardiogram was recorded by electrodes at opposite ends of the tissue bath. Endocardium was stimulated. Tissues were exposed to “ischemic” conditions (e.g., acidosis, hyperkalemia, hypoxia, and lactate) for 15 minutes and then were reperfused with “normal” Tyrode’s solution. Arrhythmias with characteristics of transmural reentry occurred in ischemic conditions and early reperfusion in 30% and 70% of 20 control hearts, respectively. Arrhythmias were associated with prolongation of transmural conduction time (CT) and abbreviation of endocardial effective refractory period. Verapamil significantly suppressed reperfusion arrhythmias at 0.1-1.0 μM but not at 3.0 μM. Verapamil also significantly decreased the incidence of arrhythmias during ischemic conditions at 0.5 μM but significantly promoted ischemic arrhythmias at 3.0 μM. Action potential duration and effective refractory period were not altered by verapamil during ischemic conditions or reperfusion. However, at 0.1-1.0 μM, verapamil prevented or attenuated prolongation of transmural CT by ischemic conditions and reperfusion. Transmural CT was further prolonged at 3 μM verapamil. In epicardial slices, 1 μM verapamil shortened CT transverse to fiber orientation during reperfusion but had no effect on longitudinal CT. Our results indicate that verapamil may suppress arrhythmias through differential effects on CT transverse and longitudinal to fiber orientation in anisotropic ventricular tissues and thus by specifically improving transmural conduction. (Circulation Research 1992;70:651–659)

KEY WORDS • verapamil • reperfusion • transmural reentry • cardiac arrhythmia • transmural conduction

A burst of potentially life-threatening cardiac arrhythmias can be induced by reperfusion of ischemic myocardium.1–6 The mechanisms involved in induction of reperfusion arrhythmias are not clear. However, several recent studies in in situ hearts and isolated tissues have suggested that both reentry and oscillatory afterpotentials (delayed afterdepolarizations) may be involved in the generation of these arrhythmias.7–10

Verapamil, a slow channel inhibitor, has been demonstrated to be effective in preventing arrhythmias in experimental ischemia.11–15 However, contradictory observations have been reported with respect to verapamil’s efficacy in preventing reperfusion arrhythmias.16–20 The mechanisms by which verapamil might suppress reperfusion arrhythmias are even less clear. Verapamil was found to suppress reperfusion-induced oscillatory afterpotentials and depolarization-induced automaticity but failed to prevent depolarization and conduction block in an isolated Purkinje tissue model of ischemia and reperfusion.21 Little is known about the effects of verapamil on the electrophysiological responses to reperfusion of cardiac tissues that may underlie induction of reentry.

Recently, we have developed an isolated guinea pig ventricular tissue model in which ischemia and reperfusion induce rapid sustained or nonsustained tachycardia, trigeminy, and closely coupled premature beats. This preparation allows recording of transmembrane electrical activity from endocardium and epicardium with standard microelectrode techniques, during the occurrence of arrhythmias.22–25 The characteristics of the arrhythmias and their responses to quinidine and lidocaine strongly suggest transmural reentry as the underlying mechanism.22–25 Confirmation of this mechanism will require high-resolution mapping. However, this model clearly induces an electrophysiological substrate that would be expected to induce or support transmural reentry and allows correlation of antiarhythmic efficacy with the cellular electrophysiological actions of drugs under the same pathophysiological conditions that induce arrhythmias.

The goals of the present investigation were to evaluate the potential efficacy of verapamil against reperfus-
sion-induced arrhythmias in this model and to determine the electrophysiological actions that accompany antiarrhythmic efficacy. Because changes in transmural conduction and refractory periods are prominent responses of the isolated guinea pig ventricular segments to ischemic conditions and reperfusion, the study focuses on possible antiarrhythmic actions on these electrophysiological parameters.

Materials and Methods

Tissue Preparation

Male guinea pigs weighing 450–650 g were killed by stunning followed immediately by carotid exanguination. Hearts were quickly excised through a parasternal incision and rinsed in “normal” Tyrode’s solution (37°C) with the following millimolar composition: NaCl 129.0, KCl 4.0, NaH2PO4 0.9, NaHCO3 20.0, CaCl2 2.5, MgSO4 0.5, and dextrose 5.5. The pH was 7.4 when the solution was bubbled with a 95% O2–5% CO2 gas mixture. Segments of right ventricular free wall approximately 1 cm x 1 cm x 1.5 mm thick were shaved from left ventricles of guinea pigs. They were removed so the fiber orientation was parallel to the long axis of cut tissue and then placed in the tissue bath with the epicardial surface facing up28,29 and superfused with Tyrode’s solution.

Stimulus trains of 15 pulses, separated by 3-second pauses, were delivered to the endocardial surface in ventricular free-wall preparations or epicardial surface in epicardial preparation via bipolar silver electrodes. Stimuli, pulses with a duration of 5 msec and voltage 1.5 times diastolic threshold, were delivered at a basic cycle length of 500 msec.

Electrical Recording

Transmembrane potentials were recorded using standard microelectrode techniques. In right ventricular free-wall preparations, one microelectrode was used to record endocardial electrical activity at least 5 mm from the site of stimulation, and the other microelectrode was used to record transmembrane potential from an epicardial site. A high gain electrocardiogram (ECG) was recorded from the preparation by two Ag/AgCl wires placed in the tissue bath at opposite ends of the preparation. The ECG was amplified by a differential preamplifier (model P15D, Grass Instrument Co.).

In epicardial preparations, one microelectrode was impaled 4 mm from the site of stimulation in a direction transverse to fiber orientation, and the other electrode was impaled 4 mm from the site of stimulation in a direction longitudinal to fiber orientation. This permitted simultaneous measurement of conduction times perpendicular and parallel to fiber orientation. An ECG was recorded as described for right ventricular free-wall preparations.

Biological signals and a record of stimulation were displayed on an oscilloscope (model 5100, Tektronix) and were recorded on a microcomputer (80286 processor, Samsung) after analog-to-digital conversion (TL1-125, Axon Corp.) with a continuous data acquisition program (Axotape, Axon). Data were measured from on-line pen recordings (model 2220, Gould) or from Axotape recordings displayed on the computer.

Experimental Protocol

Preparations were equilibrated for 60 minutes in normal Tyrode’s solution. Tissues were then superfused for 15 minutes (10 minutes for epicardial preparations) with Tyrode’s solution modified to mimic ischemia. The “ischemic” Tyrode’s solution had the following millimolar composition: NaCl 123.0, KCl 8.0, NaH2PO4 0.9, NaHCO3 6.0, CaCl2 2.5, MgSO4 0.5, and sodium lactate 20.0. This solution was bubbled with a 90% N2–10% CO2 gas mixture. The solution had a pH of 6.8 at 37°C. Thus, the test solution mimicked hypoxia (PO2 48 mm Hg), acidosis, hyperkalemia, lactate accumulation, and substrate deprivation.10 After exposure to ischemic Tyrode’s solution, preparations were reperfused with normal Tyrode’s solution, and effects were monitored for 30 minutes. In experiments with verapamil, 0.05, 0.1, 0.5, 1.0, and 3.0 μM (±)-verapamil hydrochloride (Sigma Chemical Co., St. Louis, Mo.) was added to both the ischemic and reperfusion solutions.

The incidence of arrhythmias, action potential durations (at 90% repolarization), endocardial effective refractory period (ERP), and conduction times from the stimulus to each recording site were measured during control, ischemic, and reperfusion periods. ERP was measured by interpolation of an extra stimulus after the last regular stimulus of the train. The stimulus strength was two times diastolic threshold. Initially, the stimulus was delivered during the refractory period of the last regular driven beat. To determine the ERP test interval was then gradually lengthened by increments of 5–10 msec until the test stimulus induced an action potential that propagated to a recording electrode. When the control ERP had been established, with experience it was possible to determine the ERP at specific test times through the protocol with interpolation of test beats in only two or three sequential trains. Thus, the time required for each determination was 21–31 seconds. The speed of determination was important because of the rapid changes in duration of ERP encountered early in reperfusion. To facilitate consideration of the interplay of conduction times and ERP, the conduction time reported corresponds to the same train that defined ERP.

Because ischemic conditions can alter electrophysiological actions of antiarrhythmic drugs,30 the effects of verapamil on electrophysiological parameters listed above were also determined in six preparations not exposed to ischemic conditions and reperfusion. In these experiments cumulative dose–response relations were determined by increasing the concentration of verapamil stepwise from 0.05 to 3.0 μM at 20-minute intervals.

The number of preparations in which arrhythmias appeared during ischemic conditions or reperfusion were recorded separately, both in the absence and
presence of verapamil. Arrhythmias included early premature beats (coupling interval shorter than 250 msec), nonsustained tachycardia (more than three beats), and sustained tachycardia (30 seconds or greater). Differences were determined using the $\chi^2$ test. Other data were compiled as mean±SEM. Differences were determined with paired or unpaired test. Differences with a value of $p<0.05$ were considered statistically significant. Corrections for unequal numbers of replicates (missing data) were made when necessary by failure of conduction.

**Results**

**Incidence of Arrhythmias**

Figure 1 shows traces recorded from a representative experiment in which a right ventricular free-wall preparation was exposed to ischemic conditions followed by reperfusion. Panel A was recorded during the preischemic control period. Each endocardial stimulus initiated an impulse that propagated to both endocardial (10-msec) and epicardial (23-msec) recording sites. The ECG showed a biphasic waveform that corresponded in time to activation of endocardium and epicardium and with quiescence during diastole. Transmural conduction block developed with exposure to ischemic conditions. Conduction block persisted into early reperfusion as shown in panel B, which was recorded after 0.5 minute of reperfusion. Arrhythmias were never observed during complete transmural conduction block. Recovery of conduction was associated with prolonged transmural conduction times (49 msec) and the appearance of bigeminy (panels B and C). After 1 minute of reperfusion (panel D), driven beats were followed by bursts of rapid nonsustained tachycardia or closely coupled premature beats. Continuous ECG activity lasted for the complete duration of each burst of ectopic activity but terminated abruptly with cessation of the tachycardias or bigeminal complexes. With continued reperfusion, transmural conduction times gradually shortened (35 msec) and arrhythmias disappeared (panels E and F). Reperfusion arrhythmias lasted from several seconds to more than 15 minutes in different preparations. Arrhythmias were induced by reperfusion in 14 (70%) of 20 control preparations (Figure 2). In preparations exhibiting reperfusion arrhythmias, 64% of arrhythmias were rapid sustained or nonsustained tachycardia; the remainder were closely coupled premature beats. Rapid tachycardia or closely coupled premature beats also occurred in six (30%) of 20 preparations during ischemic conditions when transmural conduction was depressed but had not failed (Figure 2).

Figure 2 also shows the effects of increasing concentrations of verapamil on the incidence of arrhythmias during ischemia and reperfusion. As the concentration of verapamil increased from 0.05 to 0.5 $\mu$M, the incidence of ischemic arrhythmias decreased. Suppression of arrhythmias during ischemic conditions was statistically significant for 0.5 $\mu$M verapamil. At 1.0 $\mu$M, verapamil was less protective, and at 3.0 $\mu$M, verapamil
TABLE 1. Effects of Verapamil on Conduction Time, Effective Refractory Period, and Action Potential Duration in Guinea Pig Right Ventricular Free Wall Under Normoxic Conditions

<table>
<thead>
<tr>
<th>Endocardium</th>
<th>Verapamil (μM)</th>
<th>Control</th>
<th>0.05</th>
<th>0.1</th>
<th>0.5</th>
<th>1.0</th>
<th>3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT (msec)</td>
<td>11±1</td>
<td>11±1</td>
<td>11±1</td>
<td>11±1</td>
<td>11±1</td>
<td>11±1</td>
<td>11±1</td>
</tr>
<tr>
<td>ERP (msec)</td>
<td>107±6</td>
<td>108±4</td>
<td>109±5</td>
<td>111±6</td>
<td>110±4</td>
<td>112±5</td>
<td></td>
</tr>
<tr>
<td>APD90 (msec)</td>
<td>115±6</td>
<td>115±6</td>
<td>119±6</td>
<td>119±7</td>
<td>119±6</td>
<td>121±5</td>
<td></td>
</tr>
<tr>
<td>Epicardium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT (msec)</td>
<td>22±1</td>
<td>22±1</td>
<td>22±2</td>
<td>22±2</td>
<td>22±1</td>
<td>22±1</td>
<td>22±1</td>
</tr>
<tr>
<td>ERP (msec)</td>
<td>100±4</td>
<td>101±4</td>
<td>104±6</td>
<td>105±6</td>
<td>106±6</td>
<td>106±6</td>
<td></td>
</tr>
<tr>
<td>APD90 (msec)</td>
<td>100±7</td>
<td>101±8</td>
<td>102±8</td>
<td>105±9</td>
<td>107±8</td>
<td>107±7</td>
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</tr>
</tbody>
</table>

Values are mean±SEM for six free-wall preparations. CT, conduction time; ERP, effective refractory period; APD90, action potential duration at 90% repolarization.

caused a significant increase in the incidence of arrhythmias \((p<0.05\) versus control).

Verapamil also exerted a biphasic effect on reperfusion arrhythmias. Verapamil at 0.05 μM slightly reduced the incidence of reperfusion arrhythmias \((p>0.05\). Higher concentrations of verapamil (0.1, 0.5, and 1.0 μM) significantly reduced the incidence of arrhythmias. However, the highest concentration of verapamil tested (3.0 μM) failed to significantly protect against reperfusion arrhythmias.

Action Potential Duration and Effective Refractory Period

Our previous study demonstrated that arrhythmias occurred in this model when transmural conduction time was prolonged and endocardial action potential duration at 90% repolarization \((\text{APD}_{90})\) was abbreviated.\(^{23}\) Abbreviation of the APD should permit reexcitation of the endocardium at a shorter interval than possible under control conditions and could be an important substrate for reentry.

It is widely accepted that prolongation of APD and ERPs represents mechanisms of antiarrhythmic drug action exerted by certain antiarrhythmic drugs. However, these effects are not normally associated with verapamil under normoxic conditions. We confirmed that verapamil, at concentrations of 0.05 to 3.0 μM, did not significantly alter \(\text{APD}_{90}\) or ERP under normal physiological conditions in six preparations (Table 1).

Exposure of tissue to ischemic conditions might modify the effects of verapamil. Therefore, the effects of verapamil on endocardial \(\text{APD}_{90}\) and ERP were also measured during exposure to ischemic conditions and reperfusion. Figure 3A shows that in the absence of verapamil, the \(\text{APD}_{90}\) of endocardium shortened markedly during ischemia \((p<0.01\). Reperfusion was accompanied by gradual recovery of \(\text{APD}_{90}\). The effects of ischemic conditions and reperfusion on \(\text{APD}_{90}\) were not statistically altered by verapamil at any concentration tested.

Figure 3B shows the effects of ischemic conditions and reperfusion on the endocardial ERP in the absence and presence of verapamil. The ERP shortened markedly after exposure to ischemic conditions for 15 minutes \((p<0.01\) and gradually increased during the first 10 minutes of reperfusion. Abbreviation of ERP was less than abbreviation of APD. Thus, endocardial tissues exhibited postrepolarization refractoriness during ischemic conditions but not under control conditions. Verapamil did not significantly alter the ERP changes induced by ischemia or reperfusion at any concentration tested. Thus, the antiarrhythmic effect of verapamil could not be attributed to alteration of APD or ERP.

Conduction Time

The effects of verapamil on conduction times to both endocardial and epicardial sites were assessed both in preparations exposed to normoxic conditions and in preparations subjected to ischemic conditions and reperfusion. Verapamil at 0.05–3.0 μM had no effect on endocardial or transmural conduction times in the preparations not exposed to ischemic conditions and reperfusion (Table 1).
Figure 4A shows the effects of ischemic conditions and reperfusion on endocardial conduction times in the absence and presence of verapamil. After exposure to ischemic conditions for 15 minutes, conduction time to the endocardial site increased only slightly (approximately 20%). This change was not statistically significant. Verapamil at 0.05–3.0 μM had no significant effect on endocardial conduction times during ischemic conditions or reperfusion.

The effects of verapamil on transmural conduction times were more complex. Figure 4B (filled circles) shows conduction times measured from the endocardial stimulus to the upstroke of the action potential at the epicardial recording site. Ischemic conditions significantly depressed transmural conduction. Transmural conduction time increased gradually from 25±1 msec during control (ischemia, 0 minutes) to 52±4 msec (108% increase) at 15 minutes of ischemic conditions (p<0.01). Transmural conduction remained prolonged (50±4 msec) at 2 minutes of reperfusion and only subsequently showed progressive recovery toward control values.

Verapamil had marked effects on transmural conduction times during ischemic conditions and reperfusion but not under control conditions (Figure 4B). These effects were dose dependent and biphasic. At low to intermediate concentrations (open symbols), verapamil attenuated prolongation of conduction during ischemic conditions and reperfusion. This effect was maximal at 0.5 μM and declined at higher concentrations. The highest concentration tested (3.0 μM, filled triangles) showed no significant improvement of transmural conduction times, and in fact, a trend toward prolongation of conduction time was seen in late ischemia and early reperfusion. Improvement of transmural conduction by verapamil was statistically significant in early reperfusion for 0.1, 0.5, and 1.0 μM verapamil and was significant throughout ischemic conditions and reperfusion for 0.5 μM verapamil.

Reperfusion arrhythmias usually appeared within the first 2 minutes of reperfusion. Figure 5 shows dose–response curves for the effects of verapamil on transmural conduction times during the period bracketed by the end of ischemic conditions and 2 minutes of reperfusion. Conduction times in the absence of drug are indicated by the points identified as control. The broken line corresponds to the control conduction time at 15 minutes of ischemic conditions. Increasing concentrations of verapamil were associated with a dose-depen-
dent improvement in conduction during the bracketed period at concentrations from 0.05 to 1.0 \( \mu M \). This dose–response relation closely matches the antiarrhythmic effect shown in Figure 2. In addition, the protective effects on transmural conduction and reperfusion arrhythmias both disappeared at 3.0 \( \mu M \).

Verapamil also reduced the incidence of the transmural conduction block during ischemic conditions and reperfusion. During the pres ischemic period, all preparations showed one-to-one propagation to epicardium of activity initiated by endocardial stimulation. In the absence of verapamil, ischemia was associated with a gradual increase in the percentage of preparations showing complete or near-complete transmural conduction block (Figure 6). By 15 minutes of ischemic conditions, approximately 45% (nine of 20) preparations showed failure of propagation of activity to the epicardium. Significant conduction block persisted for the first 2 minutes of reperfusion but gradually disappeared with further reperfusion. In the presence of all concentrations of verapamil (0.05–3.0 \( \mu M \)), the incidence of conduction block was decreased, especially at 2 minutes of reperfusion (Figure 6).

**Effects of Verapamil on Anisotropic Conduction in Ventricular Epicardium**

Ischemia and reperfusion caused marked slowing of transmural conduction at a time when conduction over the endocardial surface was not significantly affected. Transmural conduction is necessarily perpendicular to fiber orientation, whereas endocardial spread of activity can occur through a branching Purkinje network and therefore is largely parallel to fiber orientation. It is possible that the differential effects of ischemia and reperfusion, and the different effects of verapamil on endocardial conduction and transmural conduction are related to anisotropic conduction properties. Alternatively, the differential effects could be related to the greater severity of ischemic conditions expected in intramural tissues compared with superficial tissues.

Isolated epicardial slices permit simultaneous measurement of conduction transverse and longitudinal to fiber orientation, while eliminating possible differential effects related to intramural versus surface conditions. Figure 7 shows the effects of ischemic conditions and reperfusion on conduction transverse and longitudinal to fiber orientation. The duration of ischemic conditions was shortened to 10 minutes because depression of epicardial excitability encountered with longer exposure interfered with stimulation. In the absence of verapamil, conduction times longitudinal to fiber orientation increased only slightly. This change was not statistically significant. However, conduction times transverse to fiber orientation increased significantly from 22±1 to 30±3 msec by 10 minutes of ischemic conditions (\( p<0.01 \)).

The effects of verapamil were determined at the concentrations that caused the greatest protection and no protection against reperfusion arrhythmias. Verapamil at 1.0 and 3.0 \( \mu M \) did not affect conduction longitudinal to fiber orientation during either ischemia or reperfusion. However, 1.0 \( \mu M \) verapamil significantly facilitated the conduction transverse to fiber orientation at 2 and 5 minutes of reperfusion (\( p<0.05 \)). In contrast, 3.0 \( \mu M \) verapamil significantly lengthened the conduction times by 5 and 10 minutes of ischemic conditions (\( p<0.05 \)) and failed to significantly alter conduction in reperfusion. Thus, the effects of verapamil on conduction transverse to fiber orientation were similar to those on transmural
conduction, in which 1.0 μM verapamil improved conduction in early reperfusion but 3.0 μM did not.

Discussion

The present study demonstrates that verapamil has a biphasic effect on reperfusion arrhythmias in our in vitro model. Low (0.05 μM) and high (3.0 μM) concentrations of verapamil were not antiarrhythmic. Intermediate concentrations (0.1–1.0 μM) exerted a significant protective effect against reperfusion arrhythmias. The effective antiarrhythmic blood concentration of verapamil is about 125–400 ng/ml, but 90% of this agent is bound to plasma protein in humans; therefore, only 12.5–40 ng/ml verapamil can freely exchange with extravascular tissue. Verapamil’s metabolite norverapamil also appears in plasma at concentrations similar to its parent compound. If norverapamil also exerts similar efficacy to verapamil, the total free concentrations of verapamil plus norverapamil would be 25–80 ng/ml. The verapamil concentrations used in this study correspond to 25–1,473 ng/ml. The lowest concentration of verapamil, 0.05 μM (25 ng/ml), showed a slight antiarrhythmic action. Statistically significant suppression of reperfusion arrhythmias occurred only at intermediate concentrations of 0.1–1 μM (49–490 ng/ml). The highest concentration (3 μM = 1,473 ng/ml) did not protect against reperfusion arrhythmias and significantly promoted arrhythmias during ischemic conditions. The concentrations of verapamil that were antiarrhythmic in this study tend to be higher but overlap the therapeutic range of free verapamil in humans. The difference between the therapeutic range in humans and the effective range in our isolated ventricular model may be related to differences in species or duration of exposure to drug. Also, consideration of only unbound fractions of drug does not take into account possible buffering effects of the large protein-bound store in vivo and the ability of this store to replete unbound drug levels that have been reduced by tissue uptake.

The biphasic effect of verapamil on reperfusion arrhythmias in this study offers a possible explanation for conflicting observations about the efficacy of verapamil in suppressing reperfusion arrhythmias. Verapamil suppressed ischemia and reperfusion arrhythmias over only a limited range of concentrations. Both higher and lower concentrations would not possess significant antiarrhythmic action, and the higher concentration may be proarrhythmic during ischemic conditions.

Neither ERP nor APD₀ was significantly affected by any concentration of verapamil in preparations under normal conditions or preparations exposed to ischemic conditions and reperfusion. It is clear that the antiarrhythmic effect of verapamil in this model cannot be related to prolongation of ERP or APD₀.

We have characterized reperfusion arrhythmias induced by this model in a separate study. The arrhythmias exhibit characteristics that are most compatible with transmural reentry as the underlying mechanism. Rapid tachycardias or bigeminal and trigeminal complexes were initiated by driven beats and always exhibited an alternating pattern of endocardial and epicardial activation. Arrhythmias appeared only when transmural conduction time was prolonged and the sum of orthograde and apparent retrograde conduction times exceeded the duration of the endocardial ERP. Epicardial excitation was obligatory for the appearance of arrhythmias. Rapid tachycardias and bigeminy and trigeminy never occurred in the presence of transmural conduction block and could be terminated by preexcitation of the epicardium by stimuli delivered to the epicardium. Antiarrhythmic concentrations of both quinidine and lidocaine exerted effects that would be expected to interrupt transmural reentry. At low concentrations quinidine prevented prolongation of transmural conduction, much like verapamil in the present study. The apparent round-trip conduction time (endocardial, epicardial, and endocardial activation) was shorter than the duration of the endocardial ERP, and arrhythmias terminated. A higher concentration failed to shorten transmural conduction and failed to terminate arrhythmias. Lidocaine, on the other hand, had no effect on transmural conduction at antiarrhythmic concentrations. Lidocaine markedly depressed excitability of the epicardium and prevented activation of the epicardium by activity initiated in the endocardium. As noted above, activation of epicardium is essential for the occurrence of rapid arrhythmias and closely coupled premature beats in this model, and failure of epicardial activation was associated with suppression of reperfusion arrhythmias by lidocaine.

Although definitive evidence confirming that reperfusion arrhythmias are caused by transmural reentry in this model will require detailed mapping studies, one still may consider how the observed electrophysiological effects would be expected to affect transmural reentry. In the absence of verapamil, the endocardial ERP was approximately 80 msec at 2 minutes of reperfusion. The transmural conduction time at this time in reperfusion was approximately 50 msec. If retrograde conduction is approximately 10% longer than orthograde conduction, as determined in a previous study, the round-trip conduction time would be 105 msec. Thus the round-trip conduction time would exceed the endocardial ERP, and reactivation of the endocardium would be possible. The most protective concentrations of verapamil shortened conduction time at 2 minutes of reperfusion to approximately 33 msec. Estimated round-trip conduction would then be approximately 69 msec, which is equal to or slightly shorter than the ERP measured at that time. Thus, the probability that endocardium could be reactivated would be decreased. These figures are means, and the actual success or failure of activation would vary with the individual preparation. At the highest concentration tested, verapamil actually prolonged conduction slightly at 2 minutes of reperfusion, and one would predict that transmural reentry would not be prevented. Indeed, this concentration of verapamil failed to suppress reperfusion arrhythmias. These effects are very similar to those of low and intermediate concentrations of quinidine.

Verapamil, at a concentration of 3.0 μM, significantly increased the incidence of arrhythmias observed during ischemic conditions (Figure 2). This effect may reflect decreased incidence of transmural conduction block observed in response to verapamil (Figure 6). As noted above, arrhythmias never occurred when endocardial activity failed to reach the epicardium. Ischemic conditions were associated with a progressive increase in complete transmural conduction block. The high inci-
idence of transmural conduction block most likely explains the lower incidence of arrhythmias during ischemic conditions as compared with reperfusion. Successful transmural conduction returned rapidly upon reperfusion; however, conduction times remained prolonged, ERP was still abbreviated, and transmural reentry was possible. At low and intermediate concentrations, verapamil decreased the incidence of conduction block during both ischemic conditions and reperfusion. This action by itself would be predicted to increase the incidence of arrhythmias. However, verapamil also decreased transmural conduction time, and the incidence of arrhythmias decreased. At 3 μM, verapamil decreased the incidence of transmural conduction block but did not improve transmural conduction time. In fact, transmural conduction time was slightly increased, and the incidence of arrhythmias significantly increased (Figures 2, 4B, and 6).

Reports on effects of verapamil on myocardial conduction are contradictory.14,35–39 Kupersmith et al.35 examined the effects of verapamil during acute myocardial ischemia and reported that verapamil slowed intraventricular conduction, but only in ischemic myocardium. Several other researchers reported that verapamil reduced conduction delay induced by myocardial ischemia (and reperfusion).14,36–39 These contradictory observations could reflect different plasma concentrations of verapamil, as demonstrated in the present study. Further, our study indicates that the effect observed would depend on the tissue monitored and the recording method, since effects on conduction would be expected only for transmural conduction or epicardial spread of activation perpendicular to fiber orientation, and only during ischemic conditions or early reperfusion. Endocardial conduction was not significantly affected by verapamil during normoxic conditions, ischemic conditions, or reperfusion.

One might propose that the absence of effects on endocardial conduction are only apparent and simply represent a less sensitive system to detect changes in endocardial conduction because of the shorter control conduction times. We believe that this is unlikely because even the effects of ischemic conditions in the absence of drug are not proportional. As shown in Figure 4, transmural conduction increased more than 100% in response to ischemic conditions, whereas endocardial conduction increased less than 20%. In addition, the experiments on epicardial slices demonstrated marked differences in effects of verapamil on conduction transverse and longitudinal to fiber orientation despite the fact that distances between stimulating and recording electrodes were identical for both directions.

Both the occurrence of arrhythmias and the efficacy of verapamil in the present study appear to be closely associated with effects exerted specifically on transmural conduction. The mechanisms underlying these observations are not completely clear. However, several of the present and published observations suggest that the relation between conduction and fiber orientation may play an important role. Conduction velocity is much slower transverse to fiber orientation than longitudinal.50–53 This difference can be related to differences in axial resistance in the two directions.46,41 Elevation of intracellular calcium levels has been demonstrated to increase intracellular resistance, presumably by an action on gap junctions.44,45 Ischemia and reperfusion of mammalian myocardium are associated with intracellular calcium overload.54–57 The resulting electrical uncoupling of cells results in slowing of conduction in either muscle or Purkinje fibers.58,59 Thus, marked slowing of transmural conduction in ischemia and reperfusion may represent the effects of calcium overload superimposed on the intrinsically high axial resistance perpendicular to fiber orientation. In addition, elevation of extracellular potassium decreases the margin of safety for propagation transverse to fiber orientation more than for propagation longitudinal to fiber orientation.50 This effect might reflect a reduction in sarcolemmal resistance in the presence of high axial resistance. Thus, elevation of both intracellular calcium and extracellular potassium can contribute to selective depression of transmural conduction.

The ability of verapamil to attenuate prolongation of conduction time perpendicular to fiber orientation can be explained by the reduction of intracellular calcium overload during ischemic conditions and reperfusion. Verapamil pretreatment has been demonstrated to decrease myocardial intracellular calcium overload induced by ischemia and/or reperfusion.51,52 In addition, verapamil also has been shown to reduce the decline in the ATP-generating and O₂-utilizing capacity of the mitochondria in response to ischemia or ischemia plus reperfusion.41

The mechanism by which high concentrations of verapamil prolonged transmural conduction time is even less certain. The (+) isomer of (±)-verapamil can inhibit the fast sodium current.53 The importance of this action in the effect of verapamil may increase with increasing concentration of drug and with increased sensitivity of the myocardium to sodium channel blockade under ischemic conditions.30 However, it still is not clear why this should affect transmural conduction more than endocardial conduction. One might hypothesize that epicardium is more sensitive to Na⁺ channel blockade, as has been demonstrated for tetrodotoxin51 and lidocaine,52 and that slowing of conduction occurs in this tissue. However, as shown in the present experiments with epicardial slices, slowing of epicardial conduction also occurred only with conduction perpendicular to fiber orientation.

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