Effects of Intracoronary Verapamil Administration in a Sheep Model of Acute Myocardial Ischemia and Reperfusion

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We evaluated the efficacy of intracoronary administration of verapamil hydrochloride in reducing myocardial injury during acute ischemia and reperfusion. Ischemia was induced by 30% and 45% reductions of circumflex arterial blood flow for successive 2-hour periods. A reperfusion period (1 hour and 45 minutes) followed ischemia upon deflation of a pneumatic occluder. Verapamil (30 μg/kg) was slowly injected into the circumflex artery as a bolus 15 minutes after each blood flow reduction step. To prevent verapamil-induced decreases in heart rate, ventricular pacing was established at 170 beats/min before a baseline period and maintained throughout the protocol. Creatine kinase activities (international units per milligram protein) measured in samples obtained from posterior papillary muscles were 15 ± 1 (mean ± SEM) and 10 ± 2 for animals receiving verapamil or its saline vehicle, respectively (p<0.05). Quantitative morphometry was performed on left ventricular myocardium after staining with p-nitro blue tetrazolium. Intracoronary administration of verapamil reduced the extent of left ventricular infarction, as disclosed by positive tetrazolium staining of the tissue, from 34 ± 4% of the left ventricle in vehicle-treated animals to 21 ± 4% of the left ventricle in verapamil-treated animals (p<0.05). We conclude that intracoronary administration of verapamil reduced the extent of myocardial infarction acutely, independent of increases in blood flow through the circumflex coronary artery or decreases in heart rate. Administration of verapamil was not associated with decreases in ventricular afterload, the pressure-rate index, cardiac output, or the maximum rate of pressure development in the left ventricle. Verapamil treatment of animals subjected to ischemia was not associated with sustained elevations of left atrial pressure to values above those measured in animals receiving the vehicle. (Circulation Research 1988;62;1138–1146)

Considerable attention has recently been focused on the evaluation of experimental models used to study the protective effects of pharmacological interventions during myocardial ischemia.1–3 In coronary ligation protocols using dogs, variability in the degree of collateral perfusion of the ischemic myocardium can lead to substantial differences in the magnitude of the ischemic stimuli among experimental groups.1–3 Moreover, in models that use total occlusion or fixed stenosis of a coronary artery, collateral–dependent perfusion of the ischemic tissue can be greatly influenced by changes in coronary perfusion pressure.6,7

We have developed a model of acute myocardial ischemia and reperfusion in sheep, a species in which coronary collateral vessels are not found.8 Schaper made this definitive assessment by perfusion of the ovine myocardium with radiopaque barium sulfate gelatin. The absence of any evidence of coronary collaterals in sheep demonstrable by this technique confirmed an earlier assessment by Vastesaeger et al.9 Myocardial ischemia is induced in this model with measured reductions of circumflex arterial blood flow. The maintenance of measured reductions of blood flow in the circumflex artery, in conjunction with the absence of collateral vessels, establishes an ischemic stimulus that is not influenced by changes in either blood flow through the stenosed coronary artery or collateral perfusion of the ischemic myocardium.

We chose to examine the effects of intracoronary administration of verapamil because earlier studies suggested that it may reduce myocardial ischemic injury. In vitro studies demonstrated that verapamil reduced lactate dehydrogenase release from ischemic rat hearts10 and promoted preservation of both mitochondrial function and active tension development in rabbit hearts subjected to ischemia and reperfusion.11 Several in vivo experimental studies,12–16 as well as a clinical study,17 have reported that verapamil treatment is associated with reduction of ischemic necrosis, as assessed by enzymatic or histological methods.

The protective efficacy of verapamil in myocardial ischemia has been questioned, however, by the implications of two studies with a dog model of coronary occlusion.18,19 In those experiments, intravenous verapamil administration did not reduce myocardial necrosis determined histologically after either 3 hours or 3 days of circumflex artery occlusion. In our experiments, we used controlled reductions in circumflex...
arterial blood flow to induce ischemia. We assessed ischemic injury to the left ventricle with quantitative morphometry after staining the tissue with p-nitro blue tetrazolium. The morphometric data indicate that after 4 hours of ischemia and 1 hour and 45 minutes of reperfusion intracoronary verapamil administration reduces infarction of the left ventricle. The reduction of ischemic injury that was demonstrated in these experiments was not contingent upon changes in heart rate, ventricular afterload, or myocardial oxygen demand, as reflected by the pressure-rate index.

We believe that this model offers the opportunity to investigate the efficacy of putative cardioprotective agents in a heart that is more sensitive to ischemic injury than that of the dog and at the same time less subject to variability in the magnitude of the ischemic stimulus, principally due to the lack of coronary collateral vessels.

Materials and Methods

Animal Preparation and Surgical Procedures

Adult sheep of either sex, weighing between 30 and 50 kg, were screened for hematocrit and white blood cell count before entrance into the study. If the hematocrit was less than 28% or if the white cell count was less than $3.5 \times 10^9$/mm$^3$, the sheep were excluded from the study. Animals were anesthetized intravenously with thiamylal sodium (20 mg/kg) and secured in the right lateral decubitus position. The animals were then ventilated with room air with a positive pressure respirator set to deliver a tidal volume of 10 ml/kg. Respiratory rate was adjusted to maintain arterial $P_{CO_2}$ between 35 and 40 mm Hg, and a positive end-expiratory pressure of 10 cm H$_2$O was used to reduce atelectasis. Supplemental injections of sodium pentobarbital (30–60 mg) were administered, as needed, to maintain a surgical plane of anesthesia.

Polyethylene catheters were placed in the right jugular vein and left carotid artery for administration of the anesthetic and measurement of arterial pressures, respectively. For cardiac output determinations, a flow-directed thermodilution catheter was positioned in the pulmonary artery through the right jugular vein. A Millar catheter-tip pressure transducer (Houston, Texas) was placed in the left ventricle through the left carotid artery for measurement of intraventricular pressure.

A thoracotomy was performed at the third left intercostal space, and the pericardium was incised to expose the heart. A polyethylene cannula was positioned in the left atrial appendage for measurement of pressure. Plunge electrodes were positioned in the apical region of the left ventricle for cardiac pacing as well as in the apical and basal posterior free walls for measurement of myocardial voltages. These electrodes were made with Teflon-coated steel wire (0.007 inches o.d.).

An epicardial incision was then made in the region of the atrioventricular sulcus, and epicardial connective tissue and fat were removed to expose the circumflex artery. An electromagnetic flow probe was placed about the circumflex artery approximately 3 cm distal to the coronary ostium, just distal to the first marginal branch of the circumflex. A pulmonary vascular occluder was affixed around the artery distal to the probe, and a 22-gauge catheter (I. V. Catheters, Becton Dickinson, Rutherford, New Jersey) was inserted into the artery distal to the occluder.

During the placement of this equipment, the artery was carefully inspected to ensure that no visibly discernible branches were located between the probe and the occluder or between the occluder and the intracoronary catheter. This provision ensured that arterial blood flow reductions and infusions of verapamil hydrochloride, or its saline vehicle (i.e., 0.9% NaCl), were made along a contiguous segment of the circumflex artery. Any animals that did not meet this anatomic criterion were excluded from the study.

Measured and Derived Variables

After completion of the surgical preparation, baseline values of measured and derived variables were recorded. Mean left atrial, arterial diastolic, and peak systolic arterial pressures were measured with Statham P23Db transducers (Los Angeles, California) positioned at the level of the left atrium. Pressure traces were recorded on a Grass model 7 direct-writing oscillographic recorder (Quincy, Massachusetts). Cardiac output was determined by the thermodilution technique with a Sorenson cardiac output computer (model 9350, Salt Lake City, Utah). The average of three consecutive determinations was recorded as the cardiac output. The maximum rate of pressure development in the left ventricle (LV $dP/dt_{max}$) was obtained by electronic differentiation of the pulsatile left ventricular pressure recording. Circumflex coronary arterial blood flow was measured with a Statham SP2202 blood flowmeter. Calibration curves for flow probes used in these experiments were obtained in vivo with timed collections of blood from the carotid artery. Elevations of the ST segment from the isoelectric point were measured for each electrode. The pressure-rate index was calculated as the product of the heart rate and the peak systolic pressure and divided by $10^3$.

Experimental Protocol

Heart rate was established before the baseline period and maintained throughout the protocol in each experimental group at about 170 beats/min with ventricular pacing. In preliminary experiments, it was determined that the heart rates of anesthetized sheep range from about 145 to 165 beats/min. A paced heart rate of 170 beats/min was, therefore, adequate to achieve overdrive suppression of sinus rhythm. Constant current (2.5 mA) cardiac pacing, with a stimulus duration of 0.6 msec, consistently achieved ventricular capture while producing a minimal artifact on the ECG trace. Experimental protocols were begun after establishing a steady-state baseline period of mean circumflex arterial blood flow. A steady-state baseline was defined as a 30-minute period during which circumflex arterial blood flow varied by not more than 10%. This baseline was made in each animal before the induction
of ischemia. Circumflex arterial blood flow was then reduced in the ischemia protocol in two stages to minimize the incidence of arrhythmias. Preliminary experiments indicated that the coronary blood flow reduction protocol described below produced consistent myocardial ischemic injury without inducing ventricular fibrillation. Thus, all animals that were instrumented were included in the study.

Changes in circumflex arterial blood flow are depicted in Figure 1. Pressure was increased in the occluder to cause initial blood flow reductions approximating 30% of the baseline value, and these flow reductions were maintained for 2 hours. This was followed by a second 2-hour period of ischemia during which arterial blood flow was reduced by about 45% of the baseline value. A period of reperfusion (1 hour and 45 minutes) was begun upon deflation of the occluder. Circumflex coronary arterial blood flow was unregulated during the reperfusion period in the myocardial ischemia (MI) groups, as it was throughout the protocol in the saline- and verapamil-treated control groups. Hemodynamic measurements and myocardial ST-segment voltages were recorded 15 minutes after the beginning of the sham-ischemia protocol. Hemodynamic measurements and myocardial ST-segment voltages were recorded 15 minutes after the induction of ischemia and at 30-minute intervals thereafter. In experiments during which coronary arterial blood flow was not reduced, hemodynamic measurements and myocardial voltages were recorded 15 minutes after the beginning of the sham-ischemia protocol.

Animals that were subjected to myocardial ischemia (MI + saline, n = 6 and MI + verapamil, n = 7) received intracoronary infusions of 0.9% NaCl or verapamil (30 μg/kg, in 0.9% NaCl). Verapamil, or an equivalent volume of saline, was administered as a bolus through the intracoronary catheter 15 minutes after each coronary arterial blood flow reduction step. Drug and vehicle infusions were administered at a volume infusion rate of 4 ml/min, with each infusion lasting about 2 minutes. Animals that were not subjected to myocardial ischemia (saline control, n = 6 and verapamil control, n = 6) received intracoronary infusions of 0.9% NaCl or verapamil (30 μg/kg, in 0.9% NaCl) at times during the protocol that corresponded to the infusions administered to the MI groups. Surgical and instrumentation procedures were identical among the four groups. The occluder was placed on the circumflex artery of animals in the control groups, although it was not inflated to reduce blood flow.

Assessment of Myocardial Injury

At the conclusion of the experiment, the heart was excised; the left ventricle was dissected free of the great vessels, atria, and right ventricle and was immersed in iced 0.9% NaCl. The left ventricle was cut into 10 slices, each of about 7 mm thickness. The tissue was then incubated in 0.1% p-nitro blue tetrazolium in Sorenson's buffer.20 The sliced, stained ventricular slice from each experiment was stored in 10% buffered formalin before photography.

For morphometry of the stained left ventricle, photographs of the heart slices were projected so that the images were superimposed on a point-counting grid that was 8 inches in diameter and contained 25 equidistant points.21 The slides were projected so that the image of each ventricular slice filled as much of the grid as possible.

The number of points superimposed on ventricular myocardium was counted. Points falling on endocardial or epicardial fat or connective tissue were omitted, as were those points in the ventricular lumen. Myocardial muscle is stained deep blue by p-nitro blue tetrazolium according to the activity of a dehydrogenase enzyme system.22 Ischemia-induced depletion of coenzymes in this system is indicated by tissue that remains unstained after incubation with p-nitro blue tetrazolium. It has been established23,24 that the assessment of ischemic injury by differential staining with tetrazolium salts is a reliable indicator of myocardial necrosis when compared with histological examination of ischemic tissue. Unstained ventricular myocardium was defined as a positive indication of ischemic necrosis for these experiments. Data were then expressed as the percentage of total points counted for each heart overlying unstained, or injured, myocardium. Point counting was performed by two individuals who were
unaware of the protocol to which a sample heart had been subjected. The scores of these individuals were then averaged.

A plot was made of the cumulative percentage of points counted (cumulative, for a given heart, with respect to the ventricular slices considered in sequence from apex to base) that were superimposed on unstained myocardium. The total number of all points counted (stained and unstained) necessary to achieve a plateau in this plot was used to define the number of points representative of a statistically valid sample for each heart.

Tissue samples weighing about 300 mg were obtained from the anterior and posterior papillary muscles after dissection of the left ventricle. Samples obtained from the anterior and posterior papillary muscles were considered to represent nonischemic and ischemic myocardium, respectively, in sheep subjected to coronary blood flow reductions. The samples were processed according to standard methods. A commercially prepared (Sigma Chemical, St. Louis, Missouri) creatine kinase assay reagent was used to measure enzyme activity, and two determinations of creatine kinase activity in a control preparation (Sigma) were made at the time of each experimental assay. Creatine kinase activity was normalized to the soluble protein content of the myocardial tissue homogenate and expressed as international units of activity per milligram of homogeneous soluble protein.

Statistical Analyses

One-way analysis of variance and a Dunnett's test for multiple comparisons of experimental values to the baseline value were performed to test for changes in hemodynamic variables within an experimental group over time. Two-way analysis of variance and a t test for unpaired data were used to evaluate differences between groups. One-way analysis of variance and a t test for unpaired data were used to evaluate creatine kinase enzyme activities and morphometric data. The Mann-Whitney U test was used in cases of unequal variances. A probability of less than 5% was considered indicative of a statistically significant difference.

Results

Induction of Myocardial Ischemia

Changes in the ST segment of the ECG are shown in Figure 2. Significant increases in the ST segment occurred in both MI groups within 15 minutes of coronary blood flow reduction, and these voltage changes were sustained for the duration of the period of ischemia in both groups. Measured changes in the ST segment voltages did not differ between the two MI groups or the two control groups. Intracoronary administration of verapamil did not improve the electrical dysfunction of ischemic myocardium as indicated by measured changes in the ST segment nor did the drug affect the ST-segment voltages measured in the posterior free wall of animals not subjected to myocardial ischemia.

Myocardial ischemia induces changes in the cardiac action potential that underlie the observed changes in the local ECG, and these changes are a function of both the magnitude and time course of the perfusion deficit. The regional heterogeneity in both the severity of ischemia and its electrical manifestations that have been reported in total occlusion protocols might be more pronounced in a partial occlusion protocol. The fact that local voltages were measured in a partial occlusion protocol may account for the observed variability in the ST-segment changes in the MI groups.

Hemodynamic Response of Sheep to Intracoronary Verapamil Administration

Hemodynamic variables for the saline and verapamil control groups are presented in Table 1. Data are
presented for the baseline period, the first determination after each verapamil or saline bolus, and the first and last determinations of the reperfusion period. Intracoronary administration of verapamil was not associated with a reduction of myocardial oxygen demand in animals not subjected to myocardial ischemia, as reflected by the pressure-rate index. Arterial diastolic pressures were transiently reduced in the verapamil control group just after each drug bolus. Verapamil administration had no effect on the cardiac output in animals not subjected to myocardial ischemia, and although there were trends toward increased left atrial pressures with each infusion, no significant increases in left atrial pressure were recorded. Each administration of verapamil produced a transient depression of the LV $dP/dt_{max}$, although only the value of the LV $dP/dt_{max}$ recorded after the second bolus represented a significant decline from the baseline value.

The only significant hemodynamic changes associated with infusion of verapamil into the circumflex artery of sheep in the control group were transient depressions of arterial diastolic pressure and the LV $dP/dt_{max}$. This indicates that, at least in this animal species, the negative inotropic effects of verapamil are minimal in nonischemic hearts at the dose used.

The data for sheep with myocardial ischemia are presented in Table 2 and correspond with the time periods in Table 1. There were no differences in the pressure-rate index values between the MI groups at any determination, nor was verapamil administration associated with a reduction of myocardial oxygen demand just after each saline or verapamil bolus, and the first and last determinations of the sham-reperfusion period.

TABLE 1. Hemodynamic Variables for Saline and Verapamil Control Sheep

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Experimental group</th>
<th>PRI</th>
<th>APd (mm Hg)</th>
<th>Q (l/min)</th>
<th>Pla (mm Hg)</th>
<th>$dP/dt_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Saline</td>
<td>16±1</td>
<td>84±4</td>
<td>2.60±0.28</td>
<td>10±1</td>
<td>11.9±1.4</td>
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<td></td>
<td>Verapamil</td>
<td>17±1</td>
<td>90±5</td>
<td>2.78±0.30</td>
<td>9±1</td>
<td>12.9±1.7</td>
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<td>45</td>
<td>Saline</td>
<td>15±1</td>
<td>80±3</td>
<td>2.68±0.23</td>
<td>12±2</td>
<td>12.1±1.5</td>
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<td></td>
<td>Verapamil</td>
<td>14±1</td>
<td>74±4*</td>
<td>2.62±0.34</td>
<td>11±2</td>
<td>9.2±1.4</td>
</tr>
<tr>
<td>165</td>
<td>Saline</td>
<td>15±1</td>
<td>79±4</td>
<td>2.73±0.26</td>
<td>10±1</td>
<td>10.8±0.8</td>
</tr>
<tr>
<td></td>
<td>Verapamil</td>
<td>14±1</td>
<td>72±5*</td>
<td>2.43±0.26</td>
<td>13±1</td>
<td>7.9±0.8†</td>
</tr>
<tr>
<td>255</td>
<td>Saline</td>
<td>15±0</td>
<td>79±4</td>
<td>2.85±0.28</td>
<td>12±1</td>
<td>11.3±1.3</td>
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<tr>
<td></td>
<td>Verapamil</td>
<td>17±1</td>
<td>89±4</td>
<td>3.23±0.14</td>
<td>11±1</td>
<td>15±0.9</td>
</tr>
<tr>
<td>345</td>
<td>Saline</td>
<td>14±1</td>
<td>72±4</td>
<td>2.74±0.26</td>
<td>13±1</td>
<td>10.4±1</td>
</tr>
<tr>
<td></td>
<td>Verapamil</td>
<td>16±1</td>
<td>86±6</td>
<td>3.25±0.30</td>
<td>10±1</td>
<td>14.2±1.2</td>
</tr>
</tbody>
</table>

Values presented are mean ± SEM. For all variables, $n=6$. Times subsequent to baseline correspond to the times just after each saline or verapamil bolus, and the first and last determinations of the sham-reperfusion period.

TABLE 2. Hemodynamic Variables for Sheep With Ischemic Myocardium

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Experimental group</th>
<th>PRI</th>
<th>APd (mm Hg)</th>
<th>Q (l/min)</th>
<th>Pla (mm Hg)</th>
<th>$dP/dt_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Saline + MI</td>
<td>17±1</td>
<td>90±5</td>
<td>3.42±0.25</td>
<td>9±2</td>
<td>15.0±1.6</td>
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<tr>
<td></td>
<td>Verapamil + MI</td>
<td>15±1</td>
<td>81±3</td>
<td>2.96±0.18</td>
<td>12±1</td>
<td>12.9±1.5</td>
</tr>
<tr>
<td>45</td>
<td>Saline + MI</td>
<td>15±1</td>
<td>84±5</td>
<td>2.86±0.27</td>
<td>11±1</td>
<td>11.3±1.1</td>
</tr>
<tr>
<td></td>
<td>Verapamil + MI</td>
<td>15±1</td>
<td>74±3</td>
<td>2.70±0.17</td>
<td>15±0*</td>
<td>10.4±1.1</td>
</tr>
<tr>
<td>165</td>
<td>Saline + MI</td>
<td>15±1</td>
<td>82±3</td>
<td>2.69±0.20</td>
<td>14±1</td>
<td>10.0±0.6</td>
</tr>
<tr>
<td></td>
<td>Verapamil + MI</td>
<td>14±1</td>
<td>73±3</td>
<td>2.82±0.23</td>
<td>17±1</td>
<td>11.1±1.1</td>
</tr>
<tr>
<td>255</td>
<td>Saline + MI</td>
<td>16±1</td>
<td>86±3</td>
<td>3.39±0.45</td>
<td>15±1</td>
<td>11.7±0.8</td>
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<tr>
<td></td>
<td>Verapamil + MI</td>
<td>16±0</td>
<td>81±1</td>
<td>3.02±0.23</td>
<td>16±1</td>
<td>11.8±0.7</td>
</tr>
<tr>
<td>345</td>
<td>Saline + MI</td>
<td>16±0</td>
<td>85±2</td>
<td>3.26±0.32</td>
<td>14±1</td>
<td>11.7±0.8</td>
</tr>
<tr>
<td></td>
<td>Verapamil + MI</td>
<td>15±0</td>
<td>78±2*</td>
<td>3.23±0.27</td>
<td>16±1</td>
<td>12.0±0.7</td>
</tr>
</tbody>
</table>

Values presented are mean ± SEM. For Saline + MI, $n=6$ and for Verapamil + MI, $n=7$. Times subsequent to baseline correspond to the times just after each saline or verapamil bolus and the first and last determinations of the reperfusion period.

*Significant difference ($p<0.05$) between baseline and subsequent determinations. †Significant difference ($p<0.05$) between saline- and verapamil-treated control groups.
FIGURE 3. Myocardial creatine kinase (CK) activities. Bar heights represent mean values of enzyme activity measured in samples obtained from the anterior papillary muscle (open bars) and posterior papillary muscle (solid bars) of sheep. Anterior and posterior papillary muscles were selected as sample locations to represent nonischemic and ischemic myocardial tissue, respectively. Statistical significance of comparisons of CK activities measured in posterior papillary muscle samples is shown within the brackets.

associated with depression of the pressure-rate index within the MI + verapamil group over time. Although the pressure rate index is valuable as a gross assessment of changes in the myocardial oxygen demand of the whole heart, it is quite possible that intracoronary administration of this drug caused local changes in myocardial contractile energy expenditure. The variables measured in this study would not reflect such local changes.

Arterial diastolic pressures were not different between the MI groups during the period of ischemia. Although values of this variable were lower in the MI + verapamil group than in the MI + saline group for the last three measurements of the reperfusion period, it is unlikely that ventricular afterload reduction at this time contributed to a reduction in myocardial injury since values of circumflex arterial blood flow approximating those of the baseline period were measured in both MI groups at this time.

Neither the induction of myocardial ischemia, nor verapamil treatment of sheep subjected to ischemia were associated with reductions in cardiac output. The coronary blood flow reductions used in these experiments caused increases in the left atrial pressure of both MI groups. There were only three determinations, however, at which left atrial pressure was higher in the MI + verapamil group than in the MI + saline group. Verapamil administration was not associated with depression of the LV dP/dt\text{max} of MI animals to values below those measured in the sheep receiving saline. These data indicate that intracoronary administration of verapamil did not cause sustained depression of cardiac function in animals subjected to ischemia.

Myocardial creatine kinase activities measured in samples obtained from the anterior and posterior papillary muscles are depicted in Figure 3. Myocardial ischemia caused significant reductions of creatine kinase activity measured in the posterior papillary muscle samples from animals receiving only the vehicle. Verapamil administration was associated with preservation of creatine kinase activity in the posterior papillary muscle samples from sheep subjected to circumflex arterial blood flow reductions.

The results of morphometric analysis of the staining of left ventricular myocardium with p-nitro blue tetrazolium are depicted in Figure 4. Verapamil treatment reduced necrosis such that the percentage of left ventricle that was infarcted in the MI + saline group was

FIGURE 4. Results of morphometric analysis of left ventricular myocardium stained with p-nitro blue tetrazolium for four groups of sheep in this experimental series. Data are expressed as percentage of points counted that were considered positive as an indication of ischemic injury. Determinations are shown for each heart as well as the mean value for each group. Vertical bars represent standard errors of the mean. Statistical significance of comparisons of group means is indicated within the brackets.
34 ± 4%, whereas the figure for the MI + verapamil group was 21 ± 4%. Staining in the two control groups suggested that the placement of pacing and ECG electrodes was associated with some degree of myocardial injury. Similar placement procedures were used for pacing and ECG electrodes among the experimental groups.

Flow probes, occluders, and intracoronary catheters were consistently positioned just distal to the first marginal branch of the circumflex artery in these experiments. Measured baseline circumflex arterial blood flows were not different between the MI groups (MI + saline: 63 ± 6 ml/min, n = 6; MI + verapamil: 49 ± 5 ml/min, n = 7). Comparable blood flow reductions were maintained in both MI groups during the period of ischemia. In our laboratory, we measured mean pressure in the circumflex coronary artery, distal to an occluding clamp, in two sheep. Mean pressure values in these animals were between 6 and 12 mm Hg. These pressure values were stable during a 2-minute measurement period, and the animals had normal mean arterial pressures. These coronary arterial pressures are not suggestive of substantive collateral coronary perfusion. Given the absence of collateral vessels in sheep, the only source of blood flow to the capillary bed of the circumflex artery should be through the main trunk of the artery, through which flow was measured continuously. Thus, the ischemic stimuli administered to the MI groups in these experiments should be commensurate.

**Discussion**

Two studies have provided evidence suggesting that verapamil does not reduce the development of necrosis during myocardial ischemia. Several salient differences exist between the models used in those reports and the experiments described in this study. Circumflex arterial blood flow reductions used in the experiments with the sheep heart approximated 30% and 45% of baseline values for successive 2-hour periods. This represents a less severe degree of blood flow reduction than that occurring in the dog heart after ligation of a coronary artery. Maintenance of a higher level of perfusion during ischemia may have promoted the manifestation of a protective effect of verapamil administration. It may also be the case that this drug is more potent at reducing necrosis in moderately ischemic myocardium than in severely hypoperfused tissue. This possibility has been suggested previously.

The use of intracoronary drug administration, particularly given the maintenance of some blood flow through the stenosed coronary artery, may also have contributed to more effective drug delivery to ischemic cardiac myocytes in the present study, than in studies in which verapamil was administered intravenously after coronary ligation. Intravenous administration of verapamil in experimental studies has also been associated with increases in heart rate and decreases in arterial blood pressure. Increased myocardial oxygen demand, associated with elevations of heart rate in such studies, could tend to offset the protective effect of the drug. Maintenance of heart rate in the present experiments reduced this source of variability in myocardial oxygen demand. Reductions of arterial blood pressure, particularly in models in which perfusion of the ischemic tissue is collateral-dependent, would not only tend to exacerbate ischemia but would also reduce drug delivery to hypoperfused tissue. The use of an intracoronary catheter, especially for the administration of vasoactive compounds, maximizes delivery of the drug to the heart at concentrations that minimize changes in systemic arterial pressure.

Given the increasing prevalence of coronary thrombolysis and angioplasty in the treatment of myocardial ischemia, it is appropriate to develop models that describe the effects of intracoronary administration of pharmacological agents. Coronary angioplasty procedures are performed to improve perfusion to a region of jeopardized myocardium distal to a critical stenosis. Information obtained experimentally with intracoronary drug administration offers the advantage of the proximity of the intracoronary catheters to the site of evolving infarction in the clinical setting.

This study demonstrates that, at least in the sheep heart, a dose of verapamil capable of reducing ischemic injury could be infused directly into the hypoperfused coronary artery without causing a sustained cardiodepressant effect. Clinical intracoronary administration of verapamil should be viewed critically, however, due to the possibility of induction of atrioventricular nodal block.

The degree of ischemic injury indicated by the morphometric and biochemical data resulting from 4 hours of partial occlusion suggests that the sheep heart is more sensitive to blood flow reductions than are the hearts of species more commonly used in experimental studies. This may be a consequence of the absence of collateral vessels in these animals. Although the vast majority of acute myocardial ischemic injury in humans may be due to total occlusion of a coronary artery, clinical manifestations of both acute ischemia and cardiac failure have been documented in patients with partial lumen obstruction. The sheep heart provides a sensitive model for evaluating the cardio-protective activity of agents during ischemia induced by partial occlusion of a coronary artery. This model should also be less subject to changes in perfusion of ischemic tissue that may occur subsequent to coronary occlusion in species richly endowed with collateral vessels.

Most experimental data evaluating measures directed at preservation of ischemic myocardium have been obtained in models that use both total occlusion of a coronary artery and intravenous administration of pharmacological agents. In our experiments, we evaluated the protective efficacy of intracoronary administration of verapamil during controlled reductions of coronary blood flow. Perfusion of ischemic myocardium in a dog model after coronary occlusion occurs by virtue of collateral blood vessels. Agents that improve perfusion of ischemic tissue by dilation of coronary collateral and conductance vessels can directly
affect the magnitude of the ischemic stimulus in that experimental model. Moreover, it has been suggested that there is a need for experimental models of acute myocardial ischemia that are independent of drug-induced changes in tissue perfusion.

Although we did not measure regional myocardial blood flow in these experiments, the absence of coronary collateral vessels in sheep in conjunction with measured circumflex arterial blood flow reductions strongly suggests that increased blood flow to hypoperfused myocardium was not responsible for the reduction of ischemic injury observed in verapamil-treated animals. There exists the possibility that verapamil may redistribute blood flow to the endocar-dium within the ischemic zone. Such a redistribution of blood flow would tend to reduce infarction. Although the methods used in this study could not distinguish such an effect, several investigators have reported that verapamil has minimal effects on blood flow within ischemic myocardium. The reduction of necrosis demonstrated after intracoronary verapamil administration was also independent of reductions in heart rate, the pressure-rate index, and ventricular afterload.

Other investigators have reported that verapamil exhibited a selectivity for ischemic myocardial tissue in the manifestation of its negative inotropic activity. Our protocol would not necessarily disclose modest regional changes in contractility that might have been induced by verapamil. It is conceivable, therefore, that regional reduction of energetically expensive contractile activity could have contributed to the reduction of myocardial injury we observed after verapamil administration. It is important to note, at the same time, that intracoronary administration of this drug did not cause any physiologically pertinent depression of cardiac function.

These experimental data must be viewed in the context of the model used. Our data were obtained using a measured-coronary blood flow reduction to induce ischemia for a 4-hour period. It cannot be concluded that intracoronary verapamil administration would preserve ischemic tissue irrespective of the time course of hypoperfusion. The data do indicate that, as revealed by biochemical assays, drug administration did at least delay the onset of necrosis during the protocol. The increasing clinical emphasis placed on the rapid reperfusion of the patient with acute myocardial ischemia provides a real temporal context for the knowledge obtained through acute protocols.

A number of studies have presented information supporting several possible mechanisms of action for the observed reduction of ischemic injury after verapamil treatment. The selective depressant effect of the drug on ischemic cardiac muscle has already been mentioned. Cellular and metabolic effects of verapamil may greatly contribute to its cardioprotective action. Ischemia-induced loss of cellular calcium homeostasis is intimately interrelated with sarcolem-mal integrity, the viability of cellular organelles, and the maintenance of cellular energy stores. In this regard, the capacities of verapamil to reduce ATP depletion after hypoxemia, preserve mitochondrial function after ischemia, and reduce tissue acidosis during ischemia may be responsible for our observations. Schaper has suggested that the degree of collateral perfusion of human myocardium may, in different individuals, span the range from almost none, as is the case in rats, pigs, and sheep, to that of moderate collateral flow, as is found in the dog and cat, and that of extensive collateral flow, as is found in the guinea pig. This model of acute ischemia in the sheep heart in comparison to the dog model may more closely simulate the onset of acute ischemia in individuals in whom sufficient time has not elapsed to elicit the development of collateral vessels.

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Effects of intracoronary verapamil administration in a sheep model of acute myocardial ischemia and reperfusion.

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