Prevention of Transcoronary Macromolecular Leakage after Ischemia-Reperfusion by the Calcium Entry Blocker Nisoldipine

Direct Observations in Isolated Rat Hearts

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SUMMARY. Coronary microvascular damage appears to play a role in reperfusion injury after myocardial ischemia. This study was designed to afford direct viewing of the effects of myocardial ischemia-reperfusion on the coronary microcirculation and to determine whether pretreatment with the calcium blocker nisoldipine would attenuate any microvascular damage during reperfusion. Four groups of isolated rat hearts were perfused with a solution that contained red cells and fluorescent albumin, but was essentially free of platelets and leukocytes. Group I served as a nonischemic control. Group II hearts were subjected to 30 minutes of no-flow ischemia followed by reperfusion. Group III hearts were pretreated with nisoldipine (1 μg/min) for 5 minutes before ischemia, and group IV hearts were treated with nitroglycerin (93 μg/min) before and after ischemia to mimic the vasodilation caused by nisoldipine. Perfused coronary capillarity and transcoronary extravasation of plasma albumin were measured by direct visualization techniques before and after ischemia. For group I, there was no significant change in coronary resistance, perfused capillarity, or transcoronary extravasation with time. For both groups II and IV, ischemia-reperfusion caused no increase in coronary resistance, but a significant decrease in perfused capillarity and a marked increase in transcoronary extravasation of fluorescent albumin (P < 0.05). The nisoldipine group (group III) demonstrated a similar decrease in perfused capillarity but no increase in protein extravasation during reperfusion. These results indicate that, in the heart, platelets and/or leukocytes are not absolutely necessary to induce either the no-reflow phenomenon or the permeability damage observed during reperfusion after ischemia. The protective effect of treatment with nisoldipine appeared to be independent of vasodilation. We speculate that this calcium blocker reduced endothelial uptake of calcium during reperfusion, preventing endothelial deformation and formation of interendothelial gaps. (Circ Res 58: 127-136, 1986)
the various effects of I/R on the coronary microcirculation may increase and/or decrease radiotracer exchange, it was difficult to determine the exact nature of the microvascular damage from whole-organ radiotracer experiments. Accordingly, we developed a model to view directly the effects of I/R on coronary microvascular perfusion and vascular permeability to macromolecules. Such experiments may help sort the multiple pathophysiological events occurring at the level of the exchange vessels. The second aim of this study was to determine whether pretreatment with the calcium blocker nisoldipine attenuated any microvascular damage from I/R. The rationale for this experiment was based on the earlier work of Hladovec (1979), who reported that calcium blockers minimized endothelial desquamation caused by citrate. He suggested that a calcium-mediated mechanism governed the endothelial alterations that occur at low pH. Because pH is reduced also during global ischemia, we reasoned that alterations in vascular permeability after ischemia may be mediated also by calcium, in which case treatment with a calcium blocker might have some beneficial effects. We found that pretreatment with nisoldipine did prevent the increase in coronary permeability to macromolecules observed during reperfusion. The first report of this work was made at the 67th Annual FASEB Meeting (McDonagh and Roberts, 1983).

**Methods**

**Preparation of the Isolated Perfused Heart**

Details of the techniques for preparing the isolated hearts for observation under fluorescence microscopy were reported previously (McDonagh, 1983a; McDonagh et al., 1984a). Adult Sprague-Dawley rats (400–600 g) were anesthetized with sodium pentobarbitol (35 mg/kg, ip). In each, the trachea was intubated, and respiration was maintained with a Harvard small-animal respirator. A midsternal thoracotomy was performed, and the aorta ligated. The perfused heart then was carefully isolated from the thorax and placed on a special Lucite microscope stage. This excision technique eliminated any surgically induced ischemia. The pump was adjusted to achieve a similar coronary perfusion rate for all hearts studied. Coronary flow was then kept constant for the remainder of the experiments.

The coronary perfusate employed in these studies was modified from that described earlier by Bergmann et al. (1979). It consisted of Krebs solution with 2 g/100 ml albumin [Sigma, fraction V, bovine serum albumin (BSA)] and washed human red cells (20% hematocrit). The potassium in the perfusate was elevated to 40 mM to arrest the heart. This red cell-containing perfusate was referred to as K(2)RBC.

**Direct Visualization of the Coronary Microcirculation**

Figure 1 is a schematic representation of the isolated heart perfusion circuit and the intravital microscope system that permits direct viewing of the left ventricular epicardial microcirculation. After 10 minutes of perfusion with the K(2)RBC solution, the hearts were perfused with a K(2)RBC solution containing albumin labeled with fluorescein isothiocyanate (FITC-BSA). This solution was referred to as K(2*)RBC. Details of the labeling procedure were reported previously (McDonagh and Williams, 1984). Blood gases, total osmotic pressures, and colloid osmotic pressures were the same for both solutions. After the K(2*)RBC perfusate reached the heart, the epicardial microcirculation was illuminated with a 100-W mercury light source and viewed with a fluorescence microscope (Zeiss). The microscope image was viewed continually with a low-light level television camera (Cohu SIT model 4410), displayed on a video monitor (RCA TC1110), and recorded on videotape (Sony 2611).

To examine the effects of ischemia-reperfusion on transcoronary extravasation of the labeled plasma albumin...
(FITC-BSA), we selected six to eight coronary capillary-venular fields before ischemia and videotaped them at 245X (specimen to monitor). Our selection criteria stipulated that each coronary capillary-venular field (1) be on the free wall of the left ventricle (McDonagh et al., 1984a), (2) be reasonably easy to identify, (3) contain a superficial venule that was at least 125 μm in diameter, and (4) be adjacent to a coronary capillary field with few collecting venules. After 10 and 20 minutes of reperfusion, the same fields were relocated and videotaped. Transcoronary extravasation of the fluorescent protein in each field was assessed by playing back the videotape through a video densitometer (Vista Electronics model 307). The development and rationale of this procedure were described in detail previously (McDonagh, 1983a; McDonagh et al., 1984a). Briefly, a 70-μm-square densitometer window was positioned initially over a venule (120-250 μm in diameter), and an intensity reading was taken (Fig. 2). The same window was then positioned over an adjacent capillary field, and another intensity reading was taken. The intensity reading over the venule was called I, and the reading over the capillary field was called O. Because the intravascular concentration of FITC-BSA was held constant during each experiment and among preparations, the I reading did not change throughout each experiment and served as a reference for the O reading (see McDonagh, 1983a, Fig. 2). The O:I ratio was then calculated and served as a comparative index of the leakage of fluorescent albumin (FITC-BSA) from the vascular space into the tissue space.

To examine the effects of ischemia-reperfusion on coronary microvascular perfusion, three to five capillary fields were videotaped at 520X before ischemia and at two times during reperfusion after ischemia. Each capillary field was brought into clear focus, and the camera was rotated to align the image of the capillaries vertically. The red cell-perfused capillaries crossing a 200-μm raster line were then counted (McDonagh et al., 1984a; Clemens et al., 1985).

Other Physiological Measures

Coronary blood flow was determined from the calibrated roller pump and confirmed by a timed perfusate collection in a graduated cylinder. Coronary perfusion pressure was measured continually via a sideline with a pressure transducer (Statham P23Db) and a recorder (Gould model 2400). The pressure transducer was referenced to the height of the heart on the microscope stage. Coronary vascular resistance was calculated as the coronary perfusion pressure divided by coronary flow per gram. Blood gas measurements were made periodically throughout each experiment (Radiometer). The pH was maintained at 7.4; the PCO₂ from 35–55 mm Hg, and the PO₂ from 400–550 mm Hg. At the conclusion of the experiments, the hearts were immediately weighed and the cardiac water content was determined as a wet weight-to-dry weight ratio (W:D) (McDonagh and Laks, 1982).

**Experimental Protocol**

The original study involved three groups: baseline control hearts, which were not subjected to ischemia (group I), hearts pretreated with either vehicle or saline and then subjected to 30 minutes of global ischemia followed by reperfusion (group II), and hearts treated with the calcium entry blocker nisoldipine (Miles Labs), 1.0 μg/min, for 5 minutes before ischemia (group III). We found that pretreatment with nisoldipine caused coronary vasodilation (decreased perfusion pressure at constant flow) that extended throughout the reperfusion period. In an effort to control for the vasodilation induced by nisoldipine, we examined another coronary vasodilator, nitroglycerin, not known to be a specific calcium entry blocker (Antonacrio, 1984). Accordingly, a fourth group of hearts (group IV) was subjected to I/R, but treated with nitroglycerin (93 μg/min, Abbott), which mimicked the decrease in perfusion pressure induced by nisoldipine.

In each experiment, the capillary-venular fields were videotaped for O:I measurements before ischemia (control) and after 10 and 20 minutes of reperfusion (R₄₀ and R₅₀). The capillary fields were videotaped for perfused capillary density measurements immediately after the O:I measurements, that is, control and at R₄₀ and R₅₀. The ischemic hearts were treated with either vehicle, nisoldipine, or nitroglycerin for 5 minutes immediately before the 30-minute period of global ischemia. During the ischemic period, the hearts were covered with saline-soaked cushions.
gauze and kept normothermic (34–36°C) with a high-intensity lamp. At the end of the ischemic period, the hearts were reperfused for 35 minutes. The total length of all experiments was 105 minutes.

**Data Analysis**

Statistical data analysis was performed with the Statistical Package for the Social Sciences (Nie et al., 1975). Comparisons among groups were made by analysis of variance. Comparisons within groups before and after ischemia were made with a paired t-test, and all results were expressed as mean ± SEM.

**Results**

The four groups in this study were tested with a constant coronary flow protocol; that is, coronary flows were kept the same before and after ischemia. Table 1 summarizes the mean values of coronary flow, perfusion pressure, and coronary vascular resistance for all four groups before ischemia and at two times during reperfusion. Analysis of variance indicated that the preischemia coronary flows and initial pressures were not different for the four groups. The initial coronary resistance values were also similar, and for the nonischemic control group, Group I, the mean perfusion pressure at the end of the experiment, was only 5% greater than the initial value, indicating a stable coronary resistance in this model. For group II, pretreatment with the dilute polyethylene glycol vehicle, prior to ischemia, caused no change in perfusion pressure. Early during reperfusion, the coronary resistance was significantly decreased (—36%), demonstrating a hyperemic response. The resistance increased thereafter, and by the end of the experiment ("Late reperfusion" in Table 1) was less than 1% greater than its preischemic value.

In the nisoldipine group (group III), there was a 50% decrease in vascular resistance during pretreatment. Early during reperfusion, the vascular resistance fell 61% below the control value. Vasodilation was sustained throughout the entire reperfusion period. At the end of the experiment, the resistance was 27% less than the preischemic value. For group IV, we found that pretreatment with 93 μg/min nitroglycerin was required to produce a vasodilation similar to that observed with 1 μg/min nisoldipine. Furthermore, the dilation dissipated rapidly when nitroglycerin infusion was discontinued. With some practice, however, we were able to maintain vasodilation throughout reperfusion by infusing nitroglycerin continually. As Table 1 indicates, after 35 minutes of reperfusion, the degree of vasodilation was similar for both the nisoldipine and nitroglycerin groups (27% vs. 28% below control, respectively).

The measurements of red-cell capillarity are summarized in Table 2. The preischemia values were similar for the four groups and analysis of variance indicated that there were no significant differences among the control capillarity measurements. Table 2 indicates that roughly seven red cell perfused capillaries were observed crossing the 200-μm raster line. The depth of focus for these determinations was 25.3 μm; thus the red cell-perfused capillarity for the arrested rat heart was approximately 1400 capillaries/mm² or roughly 50% of the total capillarity (McDonagh et al., 1985). For group I, there was no significant change in perfused capillarity throughout the experiments. For group II, we measured a statistically significant decrease in perfused capillarity both after 15 and after 25 minutes of reperfusion (P < 0.05). The decreased capillarity

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Condition</th>
<th>n†</th>
<th>Coronary flow (ml/min per g)</th>
<th>Perfusion pressure (mm Hg)</th>
<th>Coronary resistance (mm Hg/ml per min per g)</th>
<th>Change‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>No ischemia</td>
<td>6</td>
<td>1.7 ± 0.1</td>
<td>90 ± 3</td>
<td>55 ± 4</td>
<td>+5</td>
</tr>
<tr>
<td>II</td>
<td>Ischemia-reperfusion,</td>
<td>9</td>
<td>1.8 ± 0.1</td>
<td>95 ± 5</td>
<td>55 ± 5</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>pretreat. with vehi-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Ischemia-reperfusion,</td>
<td>7</td>
<td>1.8 ± 0.1</td>
<td>86 ± 8</td>
<td>49 ± 6</td>
<td>-27</td>
</tr>
<tr>
<td></td>
<td>pretreat. with nisol-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Ischemia-reperfusion,</td>
<td>6</td>
<td>1.6 ± 0.1</td>
<td>90 ± 11</td>
<td>57 ± 7</td>
<td>-28</td>
</tr>
<tr>
<td></td>
<td>treat. with nitro-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>glycerin</td>
<td></td>
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</tbody>
</table>

* Preischemia = 20 minutes of perfusion; Early reperfusion = within 3 minutes after reperfusion was begun; Late reperfusion = after 35 minutes of reperfusion.

† Number of hearts studied.

‡ Change in coronary resistance late during reperfusion compared to preischemic control value.
TABLE 2
Left Ventricular Epicardial Perfused Capillary before and after Ischemia

<table>
<thead>
<tr>
<th>Group</th>
<th>n*</th>
<th>Preischemia control</th>
<th>Reperfusion</th>
<th>% Change</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No ischemia (I)</td>
<td>6</td>
<td>6.9 ± 0.3</td>
<td>6.1 ± 0.5</td>
<td>6.5 ± 0.5</td>
<td>-6</td>
</tr>
<tr>
<td>Ischemia-reperfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (II)</td>
<td>9</td>
<td>6.1 ± 0.3</td>
<td>4.4 ± 0.4</td>
<td>4.6 ± 0.5</td>
<td>-25</td>
</tr>
<tr>
<td>Nisoldipine (III)</td>
<td>7</td>
<td>6.5 ± 0.3</td>
<td>4.3 ± 0.6</td>
<td>4.9 ± 0.4</td>
<td>-25</td>
</tr>
<tr>
<td>Nitroglycerin (IV)</td>
<td>6</td>
<td>7.4 ± 0.3</td>
<td>5.0 ± 0.7</td>
<td>5.0 ± 1.0</td>
<td>-32</td>
</tr>
</tbody>
</table>

* Number of hearts studied; 3–5 fields per heart.
† Change after 25 minutes of reperfusion (R25) compared to preischemia value.
‡ Result of paired t-test comparing control to R25.

During reperfusion indicates that a "no-reflow" response occurred; however, the degree of no-reflow was not as pronounced as it was earlier when we reperfused globally ischemic rat hearts with whole blood (McDonagh et al., 1985). For group III, the red cell-perfused capillarities measured after both 15 and 25 minutes of reperfusion were also significantly decreased (P < 0.05) from the preischemic value. The 25% decrease in capillarity after 25 minutes of reperfusion was the same for both groups II and III, indicating that pharmacological coronary vasodilation did not attenuate the no-reflow response to I/R. For the nitroglycerin-treated hearts, also, we observed a significant decrease in perfused capillarity which was similar to that measured for groups II and III.

![Figure 3](http://circres.ahajournals.org/issue)
Figure 3 summarizes the results of the transcoronary protein extravasation measurements. The preischemia control O:I values were similar for the four groups and similar to those of our earlier experiments with K(2)RBC perfusate (0.45 ± 0.02, McDonagh, 1983a). In the nonischemic control group (panel A, Fig. 3), we observed some extravasation of FITC-BSA with time during perfusion; however, a paired t-test indicated that the final O:I value was not statistically different from the initial value. In the vehicle-treated group (panel B, Fig. 3), we did not observe extravasation of FITC-BSA during the no-flow ischemic period. Upon reperfusion, however, a diffuse extravasation was observed. After 10 minutes of reperfusion (R10), resolution of the microvessels was reduced markedly as the fluorescent plasma protein accumulated in the tissue space. At this time, the O:I ratio was 48% larger than the preischemia value (P < 0.05). Extravasation continued with time and, after 20 minutes of reperfusion (R20), the O:I was 68% larger than the preischemia value (P < 0.05). At this time, it was occasionally difficult to find some of the capillary-venular fields chosen before ischemia and to make some of the perfused capillarity readings. In the nitroglycerin group (Fig. 3, panel C) we also observed marked protein extravasation during reperfusion. After 20 minutes of reperfusion the O:I increased 60% compared to its preischemia value (P < 0.05). In striking contrast to the marked extravasation observed for the other groups subjected to ischemia-reperfusion, Figure 3 (panel D) indicates that no significant FITC-BSA leakage occurred in the group pretreated with nisoldipine. The resolution of microvessels was excellent for most fields throughout reperfusion.

Figure 4 illustrates the results of the cardiac water analysis. For reference, the left ventricular wet weight-to-dry weight ratio (W:D) ratio for freshly excised rat hearts is given also (hatched box). Comparing the W:D results for the control nonischemic hearts to the freshly excised hearts makes it apparent that isolation and 105 minutes of perfusion with the K(2)RBC solution caused some myocardial water accumulation. This degree of fluid accumulation is much less, however, than that reported for isolated hearts perfused with an asanguinous solution [W:D = 6.7 (Bergmann et al., 1979); W:D = 6.1 (Tilton et al., 1983)]. Thirty minutes of ischemia followed by 35 minutes of reperfusion caused a significant increase in myocardial water content for both the untreated and the nisoldipine-treated groups compared to the no-ischemia group (P < 0.05); however, the hearts treated with nitroglycerin before ischemia and throughout reperfusion did not demonstrate an increase in cardiac water content. These results suggest that the coronary vasodilators nisoldipine and nitroglycerin had very different effects on transcoronary fluid exchange.

**Discussion**

In this study, we viewed directly the effects of ischemia followed by reperfusion on the coronary microcirculation. We were interested in observing the interrelationship of changes in vascular permeability and the no-reflow phenomena. Furthermore, we wanted to test the hypothesis that the increase in vascular permeability may be attenuated by modulating cellular calcium influx. We found that 30 minutes of no-flow ischemia followed by reperfusion caused a marked increase in coronary permeability to macromolecules, and that this increase was prevented by pretreatment with the calcium entry blocker nisoldipine.

**Comments on the Experimental Model**

The model used in these studies was designed to combine the known stability of a red cell-perfused rat heart preparation (Bergmann et al., 1979;
McDonagh et al., 1984a) with the established effects of 30 minutes of global ischemia followed by reperfusion on rat heart function (Hearse et al., 1976; Edoute et al., 1983) and microvascular patency (Camilleri et al., 1976; McDonagh et al., 1985). The constancy of this model is seen in Table 1. The initial coronary blood flow rates and vascular resistances were quite similar among all groups studied. There was no significant change in either coronary resistance or red cell perfused capillarity in the group I hearts which were perfused continually during the 105-minute experimental period. The use of a Swank blood filter (10-μm cutoff) was essential in maintenance of a stable preparation because this filter was particularly effective in removing microemboli. Very small microemboli should be considered when blood is administered directly into the coronary arteries, as sometimes it is during cardiac surgery (McDonagh and Laks, 1982).

Effects of Ischemia-Reperfusion on Microvascular Perfusion

In the present study, all groups subjected to ischemia demonstrated a reduction in perfused capillarity during reperfusion. The decreases in perfused capillarity were similar (25–31%), regardless of whether or not coronary vasodilators were employed. At the conclusion of the experiments, we observed no significant increase in coronary resistance in any of the four groups. In fact, groups III and IV were vasodilated throughout reperfusion. It appears then that a significant reduction in perfused capillarity need not be accompanied by an increase in coronary vascular resistance.

Other investigators have reported a decrease in myocardial perfusion following a period of ischemia (Gavin et al., 1983; Uretzky et al., 1983), and several hypotheses have emerged to explain the no-reflow phenomenon. The cell-swelling hypothesis maintains that microvascular perfusion is compromised because myocyte swelling and edema compress the coronary microcirculation (Powers et al., 1984). Endothelial cell swelling may also narrow the lumen of microvessels (Armiger and Gavin, 1975). In our study, all groups subjected to I/R demonstrated a similar microvascular perfusion defect, including the nitroglycerin group which had no increase in cardiac water content. Moreover, in another recent study, we observed a striking no-reflow response to 30 minutes of global ischemia with no increase in myocyte cell size (McDonagh et al., 1985). These findings suggest that increased cardiac water and myocyte swelling are not necessary to induce a no-reflow response.

Several other investigators have reported a microvascular perfusion defect in isolated heart models (Alanen et al., 1980; Edoute et al., 1983; Tilton et al., 1983). Tilton et al. (1983) concluded that 30 minutes of no-flow ischemia followed by reperfusion led to a 'reduction in functional vascular cross-sectional area' caused perhaps by vasoconstriction. We agree that the functional cross-sectional area of the coronary microcirculation was reduced, but our results indicate that vasoconstriction was not the primary cause because coronary vasodilation did not attenuate this effect.

Another mechanism thought to compromise perfusion after no-flow ischemia is microvascular plugging by microthrombi. There is evidence that both platelets and leukocytes participate in this response (Feinberg et al., 1982; Schmid-Schoenbein and Engler, 1982). When our hearts were perfused with K(2) RBC solution, which was essentially leukocyte-free, we observed a 25% decrease in coronary capillarity. This decrease was much less than the 70% decrease observed recently when hearts were perfused with whole blood (McDonagh et al., 1985). We conclude that microthrombi are not absolutely required to induce the no-reflow phenomenon. However, the degree of no-reflow was worse when leukocytes and platelets were present in the perfusate.

Transcoronary Exchange of Fluid and Macromolecules

Compared to the nonischemic control group, we observed marked transcoronary leakage of FITC-BSA in both the untreated and nitroglycerin-treated groups during reperfusion. This observation was supported by the results of the O:I measurements given in Figure 3. In contrast, for the nisoldipine group, we did not observe significant transcoronary leakage of FITC-BSA during reperfusion. When leakage was observed, it was diffuse and was not confined to postcapillary venules. The permeability response was different from the more localized 'leaky spots' of fluorescence observed in other preparations (Svensjo et al., 1979). It was similar to the leakage observed earlier when isolated hearts were perfused with an asanguinous, protein-free solution (McDonagh, 1983a). Since leaky spots were not present in our studies, the O:I measure was employed (McDonagh, 1983a; McDonagh et al., 1984a). It may be argued that the observed differences in the O:I values during reperfusion were not actually due to differences in transcoronary protein extravasation. Perhaps nisoldipine absorbed or somehow interfered with the FITC fluorescence. During the pretreatment periods with either nisoldipine or nitroglycerin, we neither observed nor measured a change in fluorescence. Furthermore, no changes were induced by nisoldipine in our earlier studies with this agent (McDonagh et al., 1984a). All four groups of hearts were perfused at the same flow rate and at the same FITC-BSA delivery rate. The duration of exposure to the exciting light was the same for the four groups; thus, differences in fluorescence background or differential bleaching do not explain the observed differences seen in Figure 3. Furthermore, the degree of tissue hydration
does not appear to play a role, since the cardiac water contents of the vehicle and nisoldipine groups were the same (Fig. 4), yet the (O:I) values during reperfusion were quite different (Fig. 3).

The differences in the observed transcoronary leakage of fluorescent protein for the four groups was more likely due to differences in transcoronary protein convection and/or diffusion. The relationship between transvascular protein flow (J) and the convective and diffusive driving forces has been expressed by Taylor and Granger (1984) as:

\[ J = J_c (1 - \sigma) C_v + PSAC \]  

(1)

The first term on the righthand side of Equation 1 indicates that protein exchange from blood to tissue will occur along with fluid movement (J_c) provided that the protein reflection coefficient (\( \sigma \)) is less than unity. Because the cardiac fluid content was elevated in the untreated group, fluid convection may have contributed to the protein extravasation observed in that group during reperfusion. However, the cardiac water content did not increase in the nitroglycerin group, yet we did observe a marked increase in protein extravasation. Since the degree of extravasation was quite similar for these two groups (Fig. 3, b and c), increased fluid convection was not a requisite for increased transcoronary protein extravasation after I/R. The other determinant of transvascular protein exchange is diffusion, the second term in Equation 1. The diffusive protein flux is a function of vascular permeability (P), the surface area available for exchange (S), and the transcoronary protein gradient (\( \Delta C \)). Since the flow rates and the perfused capillarities were quite similar for all groups during reperfusion, it is doubtful that differences in FITC-BSA extravasation were due to different transcoronary protein gradients or different exchanging surface areas. From this analysis and from our previous findings with radiolabeled albumin (McDonagh and Laks, 1982) we conclude that ischemia-reperfusion caused an increase in coronary microvascular permeability to macromolecules.

Nisoldipine prevented the permeability increase after I/R but did not prevent fluid accumulation. Nitroglycerin treatment had the opposite effects: increased protein extravasation without edema. We did not measure microvascular pressures directly, but suspect that these two coronary dilators had opposite effects on capillary hydrostatic pressure (P_c). There is evidence that nitroglycerin exerts a relatively greater effect on the resistance of the postcapillary venous microcirculation (R_v) and that nisoldipine exerts a relatively greater effect on the resistance of the precapillary arteriolar microcirculation (R_a) (Antonacci, 1984). If so, then nitroglycerin treatment may have decreased the post to precapillary resistance ratio (R_v/R_a) which would decrease the P_c (Folkow and Neil, 1971) and promote fluid reabsorption from tissue to blood (Gore and McDonagh, 1980). Conversely, nisoldipine treatment may have caused an increase in R_v/R_a, increasing P_c, which would promote fluid filtration and an increase in cardiac water content. To test the hypothesis that nisoldipine and nitroglycerin had opposite effects on transcoronary fluid exchange, we perfused two other groups of hearts continually for 105 minutes. After 70 minutes of perfusion, 50% vasodilation was induced by either continuous infusion of nisoldipine (1 \( \mu \)g/min) or continuous infusion of nitroglycerin (93 \( \mu \)g/min). At the conclusion of these experiments, we measured the cardiac water contents and found that W:D ratio for the nitroglycerin group (5.2 ± 0.1, n = 5) was significantly less than the W:D for the nisoldipine group (5.5 ± 0.1, n = 5) (\( P < 0.05 \)). These results support the idea that nitroglycerin treatment had a modest effect favoring fluid reabsorption, and that nisoldipine treatment favored fluid filtration. These findings help explain the apparent uncoupling of protein movement and fluid convection in the two groups treated with vasodilators.

**Maintenance of Microvascular Integrity**

Studies of myocardial protection from ischemic damage have focused primarily on the myocyte (Bittl and Shine, 1983; Jolly et al., 1984; McDonagh et al., 1984b); however, other studies have reported microvascular protection with propranolol (Kloner et al., 1977; Haack et al., 1981), mannitol (Willerson et al., 1972; Fabiani, 1976; Powell et al., 1976; Powers et al., 1984), and allopurinol (Fabiani, 1976), but not with hyaluronidase (Tilton et al., 1985). Microvascular protection is considered to be important because, when severe microvascular damage occurs, a permeability edema may develop, and myocardial enzymes are free to diffuse and wash out of the heart. Furthermore, the no-reflow response compromises myocardial delivery of nutrients and protective agents (McDonagh, 1983b). The protective effects of calcium entry blockers on cardiac function and ultrastructure are well established (Nayler et al., 1972; Sherman et al., 1981); however, to our knowledge, our initial report on nisoldipine was the first to demonstrate directly a protective effect on microvascular permeability to macromolecules (McDonagh and Roberts, 1983). These results were substantiated later by Tilton et al. (1984), who used the calcium blocker diltiazem. They found in untreated hearts that the blood-to-tissue albumin exchange parameter (K_v), which is a function of both convection and diffusion, increased 8-fold after ischemia-reperfusion. Their calcium-blocker pretreatment protocol was remarkably similar to that described in our earlier report (McDonagh and Roberts, 1983). Pretreatment with diltiazem for 5 minutes before inducing ischemia attenuated the increase in K_v with reperfusion.

The effect of nisoldipine on transcoronary protein extravasation was apparently not caused by the vasodilatory properties of this calcium entry blocker.
because all groups subjected to ischemia demonstrated vasodilation during reperfusion. Some effect of calcium blockade on vascular permeability was probably responsible for the effects that we observed.

Recently, Mayhan and Joyner (1984) reported that, in the hamster cheek pouch preparation, the calcium blocker verapamil attenuated the vascular permeability increase induced by histamine. Since the effect of histamine on vascular permeability is due to endothelial cell deformation in postcapillary venules and formation of interendothelial gaps (Majno and Palade, 1961), the results of Mayhan and Joyner (1984) suggest that endothelial deformation and vascular permeability are mediated by calcium. Calcium may also modulate vascular permeability after ischemia-reperfusion. Constantinides and Robinson (1969) found that ischemic conditions, anoxia and decreased pH, caused openings between arterial endothelium and speculated that the "opening of junctions was caused by contractions of the endothelial cells." Cell membrane permeability increases during ischemia, and perhaps calcium accumulates in endothelium as it does in cardiac myocytes (Vanhoutte, 1981). This pathological calcium influx may cause endothelial deformation and formation of interendothelial gaps. If so, then nisoldipine treatment may have attenuated endothelial calcium accumulation and cell deformation. This possible action of calcium is supported by our recent finding that transcoronary protein extravasation was increased markedly when isolated hearts were treated with the calcium ionophore A23187 (Gaither and McDonagh, 1985). Based on the earlier work of Hladovec (1979), Vanhoutte (1981) suggested that, during ischemia, calcium blockers may play a role in maintenance of microvascular integrity. The results of our study support this suggestion.

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INDEX TERMS: Coronary microcirculation • Vascular permeability • Myocardial protection • Calcium entry blocker • Nisoldipine
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