Calcium Entry Blockade Prevents Leakage of Macromolecules Induced by Ischemia-Reperfusion in Skeletal Muscle

Jeffrey Paul, Alex Y. Bekker, and Walter N. Durán

Calcium kinetics and its intracellular mobilization are important in all biological processes. We used verapamil to examine the effect of calcium entry blockade on microvascular transport of macromolecules in ischemia-reperfusion injury. The rat cremaster muscle was splayed, placed in a Lucite intravital chamber, and suffused with bicarbonate buffer. The clearance of fluorescein isothiocyanate–conjugated dextran (FITC-dextran 150) was measured as an index of microvascular transport. After determination of baseline data (clearance of FITC-dextran 150, 3.0±0.5 μl/5 min/g), the muscle was made ischemic for 2 hours by clamping its vascular pedicle and subsequently was reperfused for 2 hours. Ischemia-reperfusion produced a marked increase in FITC-dextran clearance. After a peak of 12±2-fold increase observed in the first 15 minutes into reperfusion, FITC-dextran 150 clearance decreased in magnitude and stabilized at about sixfold above baseline. Verapamil did not change the baseline clearance values. Importantly, verapamil inhibited the ischemia-induced increase in clearance and maintained the values at or near the baseline values. We simultaneously determined the rate of release of 6-ketoprostaglandin F₁α (6-keto-PGF₁α) and thromboxane B₂ (TXB₂) into the suffusate. Verapamil decreased the baseline values of 6-keto-PGF₁α and increased those of TXB₂. Verapamil inhibited the ischemia-reperfusion–induced increase in 6-keto-PGF₁α but did not alter the effect of ischemia-reperfusion on TXB₂. Our main results demonstrate the effectiveness of verapamil in preventing microvascular alterations leading to increased leakage of macromolecules. (Circulation Research 1990;66:1636–1642)

Ca++ entry blockade contributes to preserving myocardial function after ischemia-reperfusion damage. Its mechanisms of protection include vasodilation, enhancement of collateral flow, and reduction of oxygen demand by stabilizing mitochondrial activity.1 Skeletal muscle, which has a metabolic rate lower than heart, can tolerate circulatory interruption for up to 6 hours without irreversible damage to the myocytes.2 However, microcirculatory derangements such as increased leakage of macromolecules and no reflow during reperfusion can occur in the rat cremaster muscle after only 30 minutes of ischemia.3 Calcium kinetics and mobilization of intracellular calcium are important in every biological process. Influx of extracellular calcium is partially required for the activation of blood-formed elements,4 and it also produces an increase in microvascular permeability to macromolecules.5 Inflammatory mediators (e.g., bradykinin and histamine), which increase macromolecular transport through the endothelial barrier, are dependent on an influx of Ca²⁺ ions into endothelial cells. Likewise, the early phase of ischemia-reperfusion syndrome is marked by an inflammatorylike response that includes microvascular leakage. Therefore, we tested the hypothesis that the increase in macromolecular clearance, induced by ischemia-reperfusion, may be partially explained by an influx of Ca²⁺ into endothelial cells; or alternatively, there is an influx of Ca²⁺ into tissues that regulates the endothelial barrier. We tested our hypothesis by administering verapamil, a calcium entry blocker.

Prostacyclin and thromboxane A₂ also have been implicated in modulating vascular permeability.6 The
levels of these prostanoids are transiently increased following ischemia-reperfusion in muscle. For this reason, we measured the release of prostacyclin and thromboxane in the suffusate bathing the muscle during ischemia-reperfusion and assessed the influence of calcium entry blockade on their release.

Materials and Methods
Preparation of Animals
Male Wistar-Furth rats (weight, 90–130 g) were anesthetized with sodium pentobarbital (45 mg/kg i.p.) and placed on a heating pad maintained at 37°C. A carotid artery was cannulated for monitoring blood pressure, and a jugular vein was cannulated for administering drugs, fluids, and supplements of anesthesia.

The right cremaster muscle was prepared for intravital microscopy according to Boric et al. The scrotum was opened longitudinally, and the adhering fascia was gently teased free of its muscular connections by blunt dissection. An incision in the muscle, parallel to the cremasteric artery, was made such that when the muscle was splayed open, the main artery was positioned in the center of the muscle sheet. Small vessels supplying the epididymis were ligated and divided. The testis was freed of its mesoepididymis and placed in the abdominal cavity. Small bleeding vessels along the cut edge were either ligated with 6-0 silk or gently cauterized.

The rat was mounted on a Lucite board in the prone position with the splayed cremaster held in position by five to eight ligatures. The remaining fascia was removed with the help of a dissecting microscope and fine forceps. Care was taken to keep the muscle moist while exposed to the atmosphere. The muscle was sealed in a tight chamber by applying a close-fitting transparent top. A small slit in the side of the chamber allowed the cremaster pedicle to exit the chamber while preventing any tissue or vascular damage. Bicarbonate buffer suffusate was pumped through the chamber at a rate of one chamber volume per minute (3 ml/min). The composition of the bicarbonate buffer was (mM/l) NaCl 131.9, KCl 4.7, CaCl2 2.0, MgSO4 1.2, and NaHCO3 18.0. The buffer was adjusted to pH 7.35, warmed to 35°C, and equilibrated with 95% N2-5% CO2 before entering the chamber. Care was taken in the design of the chamber to ensure adequate mixing without bubble accumulation.

The entire Lucite mount was transferred to a Nikon Optiphot microscope (Garden City, N.Y.) fitted for both epi-illumination and bright-field illumination. The fluorescent epi-illumination system was composed of a 100-W mercury DC lamp source, a fluorescein isothiocyanate excitation filter (488 nm), a dichroic mirror, and a 515-nm barrier filter. A Sony VO5600 videotape recorder (Park Ridge, N.J.), a Cohu 4410 SIT-TV camera (San Diego, Calif.), and a Sony TV monitor comprised the recording system.

Experimental Protocol
The animal was allowed to stabilize for 45 minutes after the surgical procedure. Fluorescein isothiocyanate–conjugated dextran with a molecular weight of 150,000 (FITC-dextran 150, Sigma Chemical Co., St. Louis, Mo.) was administered (100 mg/kg i.v.) as a 5% saline solution. Suffusate was collected in borosilicate glass vials at 5-minute intervals for evaluation of dextran clearance and eicosanoid concentration. After administration of the fluorochrome, 30 minutes of equilibration was allowed before beginning the control period (Figure 1).

Ischemia-Reperfusion Protocol
One hour of baseline suffusate collection was followed by 2 hours of ischemia, which was achieved by cross-clamping the cremasteric pedicle with an atraumatic microvascular clamp. Verification of ischemia and reperfusion was obtained by intravital microscopy. After the ischemic period, circulation was reinstated for 2 hours by removal of the clamp. Suffusate flow was also stopped during ischemia and restarted during reperfusion. In separate time control studies, we established that the preparation remains stable at least 4 hours (Table 1).

In the treated animals (n=5), verapamil (2.0 mg/kg) was given as a 0.5-ml infusion over 10 minutes. This dose is effective in reducing ischemia-induced cardiac arrhythmias with transient effects on blood pressure and heart rate. We verified in pilot exper-
imments that this dose of verapamil causes hypotension for 10 minutes and that blood pressure returns to normal values within 30 minutes. Therefore, ve-
rapamil was given 1 hour before inducing ischemia to ensure normal values of mean arterial pressure during the performance of the experimental protocol. In the untreated animals (n = 5), 0.2 ml saline was injected instead of a verapamil solution. This volume had no effect on macromolecular clearance or on systemic hemodynamics.

Evaluation of Clearance

Carotid blood samples were taken every hour to measure the plasma concentration of FITC-dextran 150. Hematocrit tubes (20 µl) were filled in duplicate and centrifuged. The plasma portion was transferred to a vial and diluted to 10 ml with saline. The concentration of FITC-dextran 150 in the plasma and suffusate was ascertained by using a Perkin-Elmer LS-3 spectrofluorometer (Norwalk, Conn.). A standard curve was constructed with known concentrations of FITC-dextran 150 ranging from 5.0 ng/ml to 2.0 µg/ml (see also Gawlowski and Durán).10

The total extravasation of FITC-dextran 150 per 5 minutes and per gram wet tissue weight was evaluated for each suffusate sample. Figure 2 shows that plasma FITC-dextran 150 concentration fell linearly as a function of time over a period of 6 hours. The regression coefficient was 0.98; therefore, plasma concentrations were estimated for each collection by interpolation between two determinations. Our observations on plasma concentration decay confirm and extend those published earlier by Boric et al.8

Clearance (Cl) was calculated from the equation Cl = (S/P)(F/W), where S and P are the concentrations of FITC-dextran 150 in the suffusate and plasma, respectively, and F and W are sulfusate flow rate and tissue wet weight. Cumulative clearance values for the respective experimental periods were assessed by adding the individual clearances over the appropriate time interval.8

### Table 1. Stability of Clearance of Fluorescein Isothiocyanate-Conjugated Dextran 150 as a Function of Time

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Clearance (µl/5 min/g)</th>
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<tbody>
<tr>
<td>0.0</td>
<td>3.05±1.50</td>
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<tr>
<td>0.5</td>
<td>2.86±0.91</td>
</tr>
<tr>
<td>1.0</td>
<td>3.73±0.98</td>
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<tr>
<td>1.5</td>
<td>3.77±0.90</td>
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<tr>
<td>2.0</td>
<td>4.29±0.76</td>
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<tr>
<td>2.5</td>
<td>3.65±0.94</td>
</tr>
<tr>
<td>3.0</td>
<td>6.84±1.81</td>
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<tr>
<td>3.5</td>
<td>4.57±1.37</td>
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<tr>
<td>4.0</td>
<td>4.51±0.96</td>
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</tbody>
</table>

Values are mean±SEM. The number of observations is five for all time periods.

Analysis of Eicosanoids

Selected sulfusate samples were analyzed by radioimmunoassay for 6-ketoprostaglandin F1α (6-keto-PGF1α), the stable metabolite of prostacyclin, and for thromboxane B2 (TXB2), the stable metabolite of thromboxane A2. Lipid extractions were obtained by first acidifying 5 ml of sulfusate to pH 3.5 with 4N formic acid and then extracting twice with equal volumes of ethyl acetate. The pooled solvent layers were evaporated to dryness under N2 and stored in 0.5 ml of radioimmunoassay buffer at −20°C.

Radioimmunoassays for TXB2 and 6-keto-PGF1α were performed by using the method described by Reingold et al.11 The lower limits of detection, defined as a 10% shift in labeled ligand binding, were 10 pg for 6-keto-PGF1α and 5 pg for TXB2. Interassay and intra-assay variances were less than 5% and 10%, respectively. The measured mass of prostanooids was expressed subsequently in terms of picograms per milliliter per minute to reflect the rate of their release into the suffusate.

Statistical Analysis

Clearance data were calculated as microliters per 5 minutes per gram tissue wet weight. Group data are reported as mean±SEM. Clearances were measured before ischemia for baseline values, and they were also obtained continuously during reperfusion. The data from the control and verapamil-treated groups were compared in terms of the ratio of postischemic and preischemic values for every 15 minutes of reperfusion. A two-way analysis of variance was performed by categorizing the observations into 1) time of reperfusion and 2) treatment. Testing for least significant difference among the individual blocks was performed only if the F value was significant at 0.05.12

Results

Intravital Microscopy

The microcirculation exhibited vigorous flow during the stabilization and equilibration periods. Cross-

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**FIGURE 2. Decay of fluorescein isothiocyanate-conjugated dextran 150 (FITC-DEX 150) plasma concentration as a function of time. The graph illustrates a representative experiment.**
clamping of the cremasteric vascular pedicle completely arrested the circulation. Upon reperfusion, blood flow began immediately. In the control animals, large aggregates of red blood cells and other elements that had dislodged were either washed into collecting venules or plugged small vessels further downstream. Large areas of no-reflow were seen after 2 hours of reperfusion, with aggregates clearly visible within the microscopic field. FITC-dextran 150 remained within the vessels during the preischemic period. Ischemia-reperfusion induced extravasation of fluorescent macromolecules, which was manifested by a generalized fluorescent field. This effect persisted for the 2 hours of reperfusion.

In all the animals tested, neither injection of the fluorochrome nor cross-clamping the cremasteric pedicle produced sustained changes in mean arterial pressure (98±5 mm Hg). Injection of verapamil (2.0 mg/kg i.v.) produced a maximal transient decrease of approximately 60% in mean arterial pressure. This hypotensive phase lasted for only 10 minutes. Mean arterial blood pressure returned to baseline values by 30 minutes and remained there for the duration of the experiment.

**Clearance of FITC-Dextran 150**

Plasma concentration of FITC-dextran 150 after a single intravenous administration decreased in a linear fashion. In our experiments, the average rate of fluorochrome removal from plasma was also linear, with an average rate of 0.18 mg/hr (Figure 2).

In a separate set of experiments, we tested the stability of the preparation with regard to macromolecular transport. To this end, the clearance of FITC-dextran 150 was determined continuously for 4 hours. Table 1 shows that cremasteric muscles had relatively constant values of FITC-dextran 150 with a mean±SEM value of 4.14±0.17 μl/5 min/g for all the observations.

After the equilibration period, the baseline clearance of FITC-dextran 150 was 3.07±0.53 μl/5 min/g tissue (Table 2). The average clearance after 1 hour from verapamil injection was 3.27±0.95 μl/5 min/g. Therefore, administration of verapamil did not significantly increase the clearance of FITC-dextran 150. Because the injection of verapamil transiently decreased mean blood pressure, steady-state FITC-dextran 150 clearances were calculated only after the arterial pressure had returned to and stabilized at normal values.

The time course of typical experiments is illustrated in Figure 3. The statistical analysis of the effect of verapamil on macromolecular leakage is displayed in Table 2. After 2 hours of ischemia, the macromolecular clearances of control animals increased immediately at reperfusion, with a maximal average increase of 11.9±2-fold observed within the first 15 minutes of reperfusion (Figure 3, Table 2). Subsequently, clearance values decreased in magnitude and remained at approximately sixfold above baseline for the remainder of the experiment. The clearance of the verapamil-treated rats increased by only 2.9±0.65-fold during the first 15 minutes of reperfusion over preischemic values. For the next 105 minutes, verapamil-treated rats showed no significant increases in clearance above baseline levels. By 120 minutes of reperfusion, the average increase in clearance was only 2.1±0.31-fold over preischemic values (Table 2). Although this value was statistically above baseline, it was well below the average clearances at 120 minutes of rats in the ischemia-reperfusion group (2.1±0.31 versus 6.7±0.21, p=0.01).

**Release of Eicosanoids Into Suffusate**

Based on their temporal relation to changes in clearance of FITC-dextran 150, selected suffusate samples were chosen for 6-keto-PGF1α and TxB2 analysis. For the control animals, three samples were collected during the preischemic period to determine the baseline rate of eicosanoids released into the suffusate. For the verapamil-treated group, samples were collected before drug administration and 1 hour after drug administration just before induction of

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**TABLE 2. Protection Against Increased Leakage of Fluorescein Isothiocyanate-Conjugated Dextran 150 by Verapamil After Ischemia-Reperfusion**

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<thead>
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<th>Table 2. Protection Against Increased Leakage of Fluorescein Isothiocyanate-Conjugated Dextran 150 by Verapamil After Ischemia-Reperfusion</th>
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<tr>
<td><strong>Baseline (μl/5 min/g)</strong></td>
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<tr>
<td><strong>Reperfusion (min)</strong></td>
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<td>15</td>
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Except for baseline, data are presented as the ratio of postischemic-to-preischemic clearance values (mean±SEM). The number of observations is five for all time periods.

*Verapamil group compared to baseline.
†Compared to control group.

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**FIGURE 3. Effect of verapamil on clearance of fluorescein isothiocyanate-conjugated dextran 150 ischemia-reperfusion. The graph illustrates single representative experiments.**
ischemia. The value obtained before administration of verapamil was used as the baseline measurement to evaluate both the direct effects of the calcium entry blocking agent on basal release of eicosanoids and in response to ischemia-reperfusion (Figures 4 and 5).

The baseline rate of 6-keto-PGF₁₅ₒᵤᵦ released into the suffusate was 1.35±0.52 pg/ml/min. Ischemiareperfusion produced a fourfold increase in the rate of release of 6-keto-PGF₁₅ₒᵤᵦ. This maximal increase was observed at the first 5-minute collection of suffusate after reperfusion (Figure 4). Within 60 minutes of reperfusion, the rate of tissue release of 6-keto-PGF₁₅ₒᵤᵦ had returned to preischemia levels.

The calculated baseline rate of TXB₂ released into the suffusate during the control period was 0.38±0.05 pg/ml/min. The increase in TXB₂ induced by ischemia-reperfusion paralleled that of 6-keto-PGF₁₅ₒᵤᵦ. The release of TXB₂ increased by 2.6-fold within 5 minutes of reperfusion (Figure 5) and returned to preischemia values by 30 minutes of reperfusion.

Administration of verapamil decreased the baseline values of 6-keto-PGF₁₅ₒᵤᵦ and increased TXB₂. One hour after verapamil, the average rate of release of 6-keto-PGF₁₅ₒᵤᵦ was 0.38±0.07 of the baseline values (Figure 4). On the other hand, the average rate of release of TXB₂ in the suffusate increased by 2.8±0.96-fold after verapamil injection (Figure 5).

Pretreatment with verapamil inhibited the rise in 6-keto-PGF₁₅ₒᵤᵦ produced by ischemia-reperfusion. By 60 minutes of reperfusion, the rate of release of 6-keto-PGF₁₅ₒᵤᵦ had returned to predrug and preischemia values (Figure 4).

TXB₂ release, which was elevated by the administration of verapamil, did not increase further after ischemia-reperfusion. The time course of the rate of TXB₂ released into the suffusate was similar to that observed in the nontreated ischemia-reperfusion experiments (Figure 5).

**Discussion**

Our main results support the hypothesis that calcium entry blockade, as produced by verapamil, protects against increases in microvascular leakage of macromolecules induced by ischemia-reperfusion injury. Pretreatment with verapamil maintained clearance of FITC-dextran 150 at or near preischemic levels during the reperfusion period.

**Experimental Model**

The exteriorized cremaster preparation was used because this model allows us to simultaneously measure extravasation of macromolecules, to evaluate microvascular alterations with intravital microscopy, and to collect samples for analysis of eicosanoids. The model also provides easy access to a common vascular pedicle and thus ensures the complete arrest of the microcirculation and the reproducibility of ischemia-reperfusion injury.

We analyzed suffusate samples as an index of the rate of synthesis/release of prostacyclin and thromboxane A₂. The suffusate is contiguous with the interstitium. Thus, changes in eicosanoid levels in the suffusate reflect alterations in the entry rate of these compounds into that compartment. An advantage of the sampling technique is that it does not interfere with the circulation of the muscle. For our purposes, these determinations are adequate and satisfy our objective of determining simultaneously the release of eicosanoids and changes in leakage of macromolecules in the control and verapamil-treated rats.

**Release of Eicosanoids**

Our data did not show any clear correlation between eicosanoid level and macromolecular leakage in response to ischemia-reperfusion in either the control or verapamil-treated rats. Our results on basal and posts ischemic levels of 6-keto-PGF₁₅ₒᵤᵦ and TXB₂ do not support an important role for these two
cicosanoids in modulating microvascular perme- selectivity after circulatory interruption. However, other investigators have proposed that prostacyclin and thromboxane modulate endothelial permeability in tissue cultures.6

Intravenous verapamil affected baseline release of 6-keto-PGF1α and TXB2 as well as their production in response to ischemia-reperfusion. Our observations are in general agreement with reports in which verapamil altered basal eicosanoid levels in different experimental models. Intravenous administration of verapamil to dogs decreased 6-keto-PGF1α and slightly elevated TXB2 plasma levels.13 In cardiac myocyte cultures, verapamil produced a biphasic dose response on prostaglandin I2 synthesis with doses of 10−7 M causing the greatest stimulation. At doses greater than and less than 10−7 M, verapamil had either a submaximal or an inhibitory effect.14 Shunting of eicosanoid precursors or sensitivity of various tissues to calcium entry blockade may explain the divergent effect of intravenous verapamil on thromboxane and prostacyclin levels.15

Clearance of FITC-Dextran 150

Our control studies confirm previous work from our laboratory3,16,17 demonstrating that ischemia increases the clearance of macromolecules, which is immediately evident at reperfusion and lasts for at least 2 hours. Our control studies are in agreement with the observations that ischemia-reperfusion in the dog gracilis muscle decreases the osmotic reflection coefficient, as estimated by the isogravimetric technique.18 In a separate study, we showed that the large initial peak of macromolecular clearance, observed at reperfusion, represents mainly washout of FITC-dextran 150 accumulated during the ischemic period.19 Therefore, we have based our conclusions about alterations of the microvascular barrier on the sustained elevated clearances observed after the first 15 minutes of reperfusion.

Clearance of macromolecules is dependent on microvascular surface area for transport, permeselectivity of the barrier, convection, and diffusion. Changes in arteriolar tone per se do not cause parallel changes in clearance.10,20 For example, although bradykinin, adenosine, and papavarine are potent vasodilators, only bradykinin increases macromolecular clearance at low doses,19 whereas platelet activating factor, a potent topical vasoconstrictor, increases macromolecular clearance.20 Because vascular tone is not immediately well correlated with clearance, and surface area was probably not significantly affected in our preparation, we believe that alterations in permeselectivity of the microvascular barrier is the most likely mechanism responsible for the sustained rise in macromolecular clearance following ischemia-reperfusion.

Possible Mechanisms of Verapamil Protection

Myocyte damage due to ischemia-reperfusion is associated with an increase in sarcolemmal calcium influx,21,22 with most of the calcium accumulating in the mitochondria. Calcium entry blockers probably protect by retarding the influx of calcium into the mitochondria, thereby maintaining oxidative phosphorylation and preserving a cellular supply of ATP.21 Full protection by verapamil is not obtained most likely because some of the calcium influx is by nonspecific pathways.21

Increases in microvascular permeability are thought to result from the release of a variety of inflammatory mediators, including histamine, bradykinin, platelet-activating factor, phospholipase-derived products, proteases, and free radicals. The origin of these substances appears to be the blood-formed elements.23 Activation of cellular processes that produce the aforementioned inflammatory mediators is partially controlled by the influx of extracellular calcium. Verapamil, a classic calcium entry blocker of voltage-sensitive channels, also blocks leukotriene and superoxide production from granulocytes,4,24 histamine-induced vascular leakage,5,25 platelet aggregation,26,27 and platelet activating factor–induced edema.28 Proteases involved in superoxide and elastase release from granulocytes also require calcium.

Ischemia-reperfusion damage appears to be associated with a calcium influx into all affected cells. Benefits, therefore, may be derived from blocking the entry of calcium to these cells. Several studies have demonstrated that intravenous verapamil partially reduces calcium influx into 1) vascular smooth muscle to inhibit catecholamine-induced vasospasm,29 2) leukocytes to inhibit free radical and leukotriene synthesis, 3) red blood cells to inhibit crenation, 4) platelets to inhibit degranulation after reperfusion, 5) myocytes to reduce uncoupling of oxidative phosphorylation and to maintain cellular ATP, and 6) all cells containing calcium-dependent proteases and phospholipases to inhibit free radicals and lyso phosphatide formation. Calcium entry blockade also has been shown to ameliorate vascular leakage in the isolated, perfused arrested heart model.30 Interestingly, in the latter study, the perfusate was free of leukocytes and platelets, suggesting that the cause of the increased microvascular leakage permeability after ischemia-reperfusion may be by direct alteration of the endothelium. These observations support our hypothesis that the prevention of loss of endothelial permselectivity to macromolecules after ischemia-reperfusion is related to the ability of verapamil to block calcium entry to both microvascular and blood-formed elements.

Several agents have been shown to partially ameliorate the microvascular damage induced by ischemia-reperfusion. Substances that scavenge oxygen-derived free radicals are helpful in preventing reductions in the osmotic reflection coefficient in dog gracilis muscle.18 Monoclonal antibodies to neutrophil surface-adhesion glycoproteins prevent the adhesion of neutrophils to venular walls and also partially reduce the microvascular damage associated with ischemia-reperfusion.31 Glucocorticoids, be-
cause of their anti-inflammatory effects, have some therapeutic value in the cremaster and the hamster cheek pouch models of macromolecular leakage after circulatory interruption. In comparison with these agents, our data show that blockade of calcium channels affords greater protection against microvascular derangement following ischemia-reperfusion in skeletal muscle.

The initial microcirculatory responses after ischemia-reperfusion in skeletal muscle represent inflammatory processes. The same events are seen after circulatory interruption in many other organs, including heart, bowel, and lung. Most inflammatory processes involving microvascular and blood-formed elements are initiated by the influx of extracellular calcium. Therefore, calcium entry blockade, alone or as a component of anti-inflammatory therapy, may prove to be useful in the treatment of ischemia-reperfusion injury in skeletal muscle and probably in other organs, including heart.

References

Key Words • ischemia-reperfusion injury • skeletal muscle • calcium entry blockade • microvascular permeability • verapamil • prostacyclin • thromboxane A2
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