Ischemic Preconditioning Attenuates Hypoperfusion after Retinal Ischemia in Rats

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PURPOSE. Retinal blood flow (RBF) was measured in rats to test the hypotheses that hypoperfusion follows severe ischemia in the retina and that ischemic preconditioning (IPC) attenuates this change in blood flow.

METHODS. Male Sprague-Dawley rats were anesthetized with halothane and mechanically ventilated by tracheostomy to maintain normocarbia and normoxia. Retinal ischemia was induced for 0, 5, 30, 60, 75, or 90 minutes. RBF was measured 60 and 150 minutes after the end of ischemia using the radioactive microsphere blood flow method, and electroretinography was performed during the first 120 minutes after ischemia to quantitate the extent of functional recovery. Additional groups received IPC (5 minutes of ischemia) 24 hours before 30, 60, or 75 minutes of ischemia.

RESULTS. Control (0 minutes' ischemia) RBF was 22 ± 3 ml/100 g per minute (mean ± SE). At 60 minutes after 5, 30, 60, 75, or 90 minutes of ischemia, RBF was 15 ± 2 (NS), 11 ± 1, 8 ± 2, 8 ± 1, and 10 ± 1 ml/100 g per minute, respectively (significance, P < 0.05 versus control). At 150 minutes after 5, 30, 60, 75, or 90 minutes of ischemia, RBF was 18 ± 3 (NS), 13 ± 1, 12 ± 2, 12 ± 1, and 11 ± 1 ml/100 g per minute respectively (significance, P < 0.05 versus control). With prior IPC, RBF after 30 and 60 minutes of ischemia was 21 ± 1 and 19 ± 3 ml/100 g per minute (both NS compared with control; P < 0.05 compared with 30 or 60 minutes of ischemia without IPC). When ischemia was 75 minutes in duration, IPC did not prevent postischemic hypoperfusion. The extent of recovery of the electroretinogram b wave was inversely related to the length of ischemia.

CONCLUSIONS. Postischemic hypoperfusion is present in the rat retina 60 minutes after ischemia, does not resolve by 150 minutes after ischemia, and is attenuated by IPC when ischemia is 60 minutes or less in duration. Maintenance of postischemic perfusion in the retina may be one of the mechanisms involved in the neuroprotection afforded by IPC. (Invest Ophthalmol Vis Sci. 1999;40: 2925–2931)

In previous studies, it has been shown that the degree of damage after retinal ischemia is related to the duration of ischemia. The pathophysiology of retinal ischemic damage has been studied in a variety of different animal models such as the rat, rabbit, cat, and monkey. These studies have shown the involvement in ischemic damage of factors such as adenosine, nitric oxide, excitatory amino acids, oxygen free radicals, and altered gene expression. Recently, it was found that the retina is capable of being rendered tolerant to ischemia by ischemic preconditioning (IPC). IPC provides a unique model for examining the pathophysiology of retinal ischemia, yet apart from demonstration of the essential role of adenosine in this phenomenon, little is known about the mechanisms responsible for IPC. In this study we examined whether IPC affects blood flow after ischemia.

The effects of the duration of ischemia on blood flow and on permanent retinal damage seem to be related to the species tested and the model used to produce ischemia. In some species, such as monkeys, permanent injury is seen only after prolonged ischemia, whereas in others, such as rats, shorter periods are required to produce a similar degree of damage. We have studied retinal blood flow (RBF) after ischemia in the cat. After 60 minutes of ischemia produced by elevation of the intraocular pressure to values exceeding systemic arterial blood pressure, significant postischemic hyperemia was found within the first 15 minutes after the restoration of circulation. No subsequent decreases in flow compared with preischemic baseline were found as late as 4 hours after the end of ischemia. In the rhesus monkey, Hayreh and Weingeist showed that retinal vessels remained narrowed in the follow-up period weeks after prolonged (>105 minutes) clamping of the central retinal artery. Retinal damage after ischemia also seemed to be related to the duration of ischemia, because more than 105 minutes of ischemia invariably resulted in irreversible injury. In the rat retina, Hughes found severe thinning of the inner retinal layers and histologic evidence suggesting regional areas of nonperfusion after ischemia that lasted 60 minutes or longer. Whether such alterations in perfusion are related to the extent of postischemic recovery has not been studied in the rat, nor has blood flow been quanti-
tated in this increasingly used model of ischemia and reperfusion. The findings of these previous studies suggest a hypothesis that retinal ischemic damage could be caused, at least in part, by postischemic hyperperfusion that limits recovery. We tested this hypothesis by measuring blood flow and electrical function in the rat retina after varying periods of ischemia.

We sought to examine in this study whether protection against ischemic damage by IPC is related, at least in part, to the preservation of postischemic perfusion. In particular, we investigated the hypothesis that IPC prevents postischemic hyperperfusion. To test this hypothesis, rats were subjected to varying durations of ischemia, preceded 24 hours earlier by a 5-minute period of ischemia. This protocol was shown previously to result in complete histologic and functional protection of the rat retina despite ischemia that lasted 60 minutes.1

METHODS

Surgical Preparation

Procedures in this investigation conformed to the ARVO Resolution on the Use of Animals in Ophthalmic and Vision Research and were approved by our Animal Care and Radiation Safety Committees. Rats (220–330 g) were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Animals were allowed free access to food and water before the experiments. Anesthesia was induced by mask inhalation of 5% halothane (Halocarbon, River Edge, NJ) in oxygen. Once unconscious, animals were injected intramuscularly with a mixture of 80 to 125 mg/kg ketamine (Parke-Davis, Morris Plains, NJ), and 5 to 9 mg/kg xylazine (Miles, Shawnee Mission, KS). Adequacy of anesthesia was tested by tail-clamping with a hemostat, and additional ketamine and xylazine or halothane was administered as necessary to prevent response to surgical stimulation. Temperature was maintained at 36°C to 37°C with a warming blanket. To provide additional analgesia, areas of skin incision were subcutaneously infiltrated with 0.25% bupivacaine (Abbott Laboratories, North Chicago, IL). After a tracheostomy, the trachea was cannulated using PE-240 tubing (Intramedic, Becton-Dickinson, Parsippany, NJ).

Ventilation was controlled using a rodent respirator (model 681; Harvard Apparatus, South Natick, MA), set to deliver a tidal volume of 3.5 to 4.5 ml per breath at a respiratory rate of 45 to 60 per minute. Oxygen saturation and heart rate were measured from a hind paw or the tail using a pulse oximeter (Biox 3740, Ohmeda, Louisville, CO). Fraction of inspired oxygen (Fio2) was adjusted using an air-oxygen mixture to maintain arterial oxygen saturation (SaO2) of more than 92%. The femoral artery was cannulated with PE-10 tubing which was then advanced until its tip was in the midabdominal aorta. To prevent thrombosis in the catheter and in the retinal circulation during the ensuing period of ischemia, 100 U/kg heparin was injected directly into the ipsilateral femoral vein by 30-gauge needle, and direct pressure was applied to achieve hemostasis. The femoral vein was ligated to prevent the return of nonoxygenated blood into the circulation. The arterial catheter was flushed intermittently using normal saline containing heparin (10 U/ml, Solopak, Elk Grove Village, IL), and the mean arterial blood pressure was continuously monitored throughout the experiment (Series 7000 monitor, Marquette Electronics, Milwaukee, WI). Subsequent anesthesia was maintained with 0.5% to 1.25% halothane, adjusted to maintain blood pressure and heart rate within 10% to 20% of baseline values. However, halothane concentration was held constant at approximately 0.7% during blood flow measurement to avoid any influence of varying halothane concentration on the results. Maintenance fluids (1.0–1.5 ml/h) were provided through the arterial cannula with heparinized normal saline. Arterial blood gas tensions (pH, PaO2, PaCO2) and hematocrit were measured with a portable clinical analyzer (I-stat; Sensor Devices, Waukesha, WI), and respiration was adjusted to maintain PaO2 at 30 to 40 mm Hg (normocarbia), PaCO2 at 70 to 130 mm Hg (normoxia), and pH at 7.4 to 7.5. Hematocrit (%) was measured from the same blood sample to rule out a possible influence of hemodilution on the blood flow results.

Ischemia Induction

Local anesthesia was achieved by placing a drop of 0.5% proparacaine (Allergan, Humacao, Puerto Rico) on the cornea. A sterile 24 g silk suture was placed under direct vision behind the globe of each eye around the optic nerve and blood vessels and looped through a short piece of PE-200 tubing. In control rats receiving no ischemia, the sutures were placed but not tightened. In experimental groups the suture was tightened bilaterally by advancing the tubing toward the eye while pulling on the sutures and then clamping it to maintain its position. Ischemia and hypoperfusion were verified by the presence of retinal pallor observed during fundoscopic examination and by the absence of the b wave on the electroretinogram (ERG). Because both the central retinal and posterior ciliary arteries are occluded, this method of ischemia produces panocular ischemia, but the retinal effects were the focus of this study. After the target duration of ischemia was completed, the sutures were removed. Reperfusion of the retina and absence of retinal hemorrhage were verified by fundoscopy. The corneas were intermittently irrigated throughout the experiment with balanced salt solution (Alcon, Fort Worth, TX) to prevent exposure keratopathy.

Blood Flow Measurement

Approximately 30 minutes before blood flow measurement, the left ventricle was cannulated after hemisternotomy with a 27-gauge, 0.5-in., butterfly needle. The tip was verified to be correctly positioned by direct measurement of left ventricular pressure. Animals were allowed to stabilize for approximately 20 minutes, and normal saline was injected in 0.5-ml increments into the left ventricle to maintain mean blood pressure of more than 65 mm Hg.

Nb-95-labeled 15-μm diameter microspheres (NEN Life Science Products, Boston, MA) were dissolved in normal saline containing 0.01% Tween. The vial was shaken and sonicated in warm water to prevent microsphere aggregation and to achieve a uniform concentration before each use. Radioactivity was determined in a reference standard for each vial using a gamma counter (Minaxi Autogamma 5000 series, Packard Instruments, Downers Grove, IL), and the concentration of microspheres therein was determined by manual counting on a hemocytometer (Hauser Scientific, Horsham, PA). The specific activity of a known volume of microsphere solution was determined, and the count per million (cpm) per microsphere was calculated. Microspheres (1–2 million in 1 ml total volume) were injected into the left ventricle over 15 seconds and the catheter flushed immediately thereafter with 1.0 ml normal
TABLE 1. Experimental Design

<table>
<thead>
<tr>
<th>Minutes of Ischemia</th>
<th>0 (Control)</th>
<th>5</th>
<th>30</th>
<th>60</th>
<th>75</th>
<th>90</th>
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<td>10</td>
<td>7</td>
<td>5</td>
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<td>Blood flow 240 minutes after ischemia</td>
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<td>3</td>
<td>5</td>
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<td>3</td>
</tr>
<tr>
<td>Blood flow during ischemia</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Blood flow 150 minutes after ischemia (simultaneous ERG)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Blood flow 60 minutes after ischemia with prior 5-minute period of PC</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>3</td>
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<td>Sham PC blood flow 60 minutes after ischemia</td>
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<td>5</td>
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<td>5</td>
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Data are the number of animals in each group. PC, preconditioning.

Electroretinography

To quantitate posts ischemic recovery after varying durations of ischemia, electroretinography was performed in animals in which blood flow was measured 2.5 hours after the end of ischemia. The ERG was recorded as we have previously described.1,20 In brief, animals were dark adapted overnight, and surgical preparation and ERG recordings were performed under dim red light. Pupillary dilation was maintained with topical 0.5% tropicamide (Alcon) and Cycloprolidr (0.2% cyclopentolate and 1% phenylephrine HCl; Alcon). Platinum needle electroencephalogram electrodes (Grass, Providence, RI) were bent and placed in contact longitudinally with the corneal surfaces bilaterally to avoid contact between the sharp needle tip and the cornea. A reference electrode was clipped on the tongue, and a grounding electrode was placed subcutaneously in the animal’s back.

Corneal electrical responses to 10 μs white-light flashes delivered by a Ganzfeld stimulator (Nicolet, Madison, WI) were recorded (Spirit 486 System; Nicolet). Electroretinogram b wave data were obtained before ischemia (baseline), during ischemia, and at 30, 60, 90, and 120 minutes after ischemia ended. Data at each time point were collected by averaging the results of three flashes delivered at least 2 minutes apart. Flashes at a single maximal intensity were used because our previous studies had indicated the accuracy and adequacy of this approach.1,20 The ERG b wave amplitude at each time point after ischemia was measured and reported as a percentage of the baseline, nonischemic b wave amplitude.

 IPC

To study the effects of IPC on postischemic blood flow, several groups of animals received 5 minutes of retinal ischemia 24 hours before the more prolonged ischemia and blood flow study. The preconditioning ischemia was performed using ketamine-xylazine anesthesia, as previously described.1

Study Protocol

The experimental design is summarized in Table 1. Control animals received sham retinal ischemia (sutures were placed but not tightened), and microspheres were injected for blood flow measurement 60 minutes later. To validate the appropriateness of this group as a suitable control for rats in which blood flow measurements were performed at later time points (150 minutes or longer after ischemia ended) or after longer periods of ischemia, blood flow was measured at 240 minutes after sham ischemia in three additional animals. Based on our previous studies,16 with an estimated SE = 10% of the mean and expected Δ mean of approximately 50%, three to five rats per group would suffice to demonstrate statistical significance.

Blood flow was measured 60 minutes after ischemia in five groups of animals subjected to 5, 30, 60, 75, or 90 minutes of ischemia. In another five groups of animals, in which the ERG was recorded during the first 120 minutes after 5, 30, 60, 75, and 90 minutes of ischemia, blood flow was measured 150 minutes after ischemia. These time points for blood flow measurements (60 and 150 minutes after ischemia) were chosen for three reasons. First, they were within the period during which recovery was simultaneously measured using the ERG, comparable to that of our previous studies.1,7 Second, the blood flow measurements were performed at time points similar to those in our earlier investigation of postischemic blood flow in cats.16 Third, these measurements were made at times comparable to those made after cerebral ischemia.23
IPC was performed 24 hours before blood flow studies in three additional groups of animals. This timing was based on previous reports from our laboratory indicating that a 24-hour separation between 5 minutes IPC and 60 minutes of ischemia resulted in complete protection against ischemic injury.3,12 Blood flow was measured in these animals at 60 minutes after 30, 60, or 75 minutes of ischemia.

To rule out any possible effects of anesthesia itself during IPC on blood flow, 3 animals received sham IPC parallel to the 60 minutes’ ischemia-60 minutes later blood flow group. In an additional group of three animals, blood flow was measured during ischemia 15 to 20 minutes after onset. This group provided quantitative data on the completeness of ischemia.

Statistical Analysis

Data were analyzed using commercial software (Stata ver. 5.0; Stata, College Station, TX). Data for all experimental groups were compared with the control group with an unpaired t-test. RBF at 60 minutes after ischemia for each duration of ischemia was compared with the corresponding RBF at 150 minutes similarly. RBF in groups receiving IPC was compared with RBF in groups with the same duration of ischemia but without preconditioning using an unpaired t-test. P < 0.05 was considered statistically significant, and all values are reported as means ± SEM.

RESULTS

Figure 1 contains a representative series of ERG tracings obtained in one animal at baseline during 30 minutes of ischemia and at 30, 60, 90, and 120 minutes after ischemia, (i.e., during reperfusion). Increasing the duration of ischemia resulted in decreasing recovery of the ERG b wave amplitude throughout the first 120 minutes after ischemia (Fig. 2). The recovery of the ERG b wave amplitude in the group receiving only 5 minutes of ischemia was rapid and complete, reaching 108% of baseline amplitude 30 minutes after ischemia ended and remaining slightly higher than 100% of baseline amplitude throughout the first 120 minutes after ischemia. In the group receiving 30 minutes of ischemia, the b wave amplitude recovered to 51% of baseline 30 minutes after ischemia and increased to 74% of baseline by 120 minutes after ischemia. After 60 minutes of ischemia, the b wave amplitude recovery was 25% of baseline at 30 minutes after ischemia and plateaued at 38% of baseline at 90 and 120 minutes after ischemia. After a more prolonged ischemia duration of 75 minutes, recovery of the ERG b wave amplitude was limited to 11% at 120 minutes after ischemia. Because there was minimal recovery of the ERG b wave after 75 minutes of ischemia, ERG measurements were not made in animals receiving 90 minutes of ischemia.

The number of microspheres impacted in the combined retinas of each animal ranged from 39 ± 4 (RBF measured at 75 minutes after 60 minutes of ischemia) to 190 ± 34 (control). The number of microspheres collected in the reference arterial blood sample was at least 5200 in all the animals. The baseline, nonischemic RBF (control) was 22 ± 3 ml/100 g per minute, whereas RBF measured at 240 minutes after sham ischemia was 27 ± 6 (NS versus control). It was therefore reasonable to assume that the RBF was constant in these animals throughout the whole period up to 240 minutes after sham ischemia. Our original control group could therefore be used as a valid control for groups in which RBF measurement was made at 150 minutes after ischemia as well as at 60 minutes after ischemia. RBF measured during 30 minutes of ischemia was 3.4 ± 1.0 ml/100 g per minute (approximately 15% of control; P < 0.0001 versus control), and choroidal-scleral blood flow (CoBF) was 5 ± 3 ml/100 g per minute compared with 145 ± 26 ml/100 g per minute in control animals (5% of control; P < 0.0004). RBF at 60 minutes after 5-, 30-, 60-, 75-, and 90-minute periods of ischemia (and compared with control) was 15 ± 2 (NS), 11 ± 1 (P < 0.004), 8 ± 2 (P < 0.002), 8 ± 1 (P < 0.001), and 10 ± 1 ml/100 g per minute (P < 0.003), respectively (Fig. 3), indicating clearly that postischemic RBF was decreased from baseline when ischemia was 30 minutes or more in duration. There were no significant differences in RBF between the 60-, 75-, and 90-minute ischemia groups. Despite increasing periods of ischemia, there was no evidence of decreased choroidal blood flow. Compared with a baseline value of 145 ± 26
ml/100 g per minute; hyperperfusion when ischemia lasted 90 minutes (412 ml/100 g per minute). Also showing significant hyperperfusion when ischemia exceeded 60 minutes, the large variability generally did not enable us to demonstrate statistically significant differences.

At 150 minutes after each duration of ischemia, there was a trend for recovery of the RBF toward that in control eyes, but there was no significant difference between RBF at 60 minutes after ischemia and 150 minutes after ischemia for any duration of ischemia. RBF at 150 minutes after 5, 30, 60, 75, and 90 minutes of ischemia was 18 ± 3 (NS), 13 ± 1 (P < 0.02), 12 ± 5 (P < 0.04), 12 ± 2 (P < 0.01), and 11 ± 1 (P < 0.009) ml/100 g per minute, respectively, compared with control (Fig. 3). The trend in CoBF 150 minutes after ischemia was similar to that at 60 minutes after ischemia, also showing significant hyperperfusion when ischemia lasted 90 minutes (412 ± 115 ml/100 g per minute; P < 0.04 versus control).

Consistent with our previous findings, 24 hours after 5 minutes of ischemia (i.e., IPC), there was no evident damage to the eyes.1 IPC 24 hours before ischemia dramatically attenuated retinal postischemic hyperperfusion after either 30 or 60 minutes of ischemia but not after 75 minutes of ischemia (Fig. 4). After 30 minutes of ischemia, the RBF was improved from 11 ± 1 ml/100 g per minute without IPC to 21 ± 1 ml/100 g per minute with prior IPC (P < 0.001). After 60 minutes of ischemia the improvement in RBF was from 8 ± 2 ml/100 g per minute without IPC to 19 ± 3 ml/100 g per minute with IPC (P < 0.02). In both instances, the RBF with IPC was not significantly different from control blood flow (22 ± 3 ml/100 g per minute). After 75 minutes of ischemia, RBF was 9 ± 1 ml/100 g per minute with IPC, unchanged from RBF of 8 ± 1 ml/100 g per minute without IPC. In three animals receiving sham IPC 24 hours before 60 minutes of ischemia, RBF at 60 minutes after ischemia was 9 ± 2 ml/100 g per minute (NS versus the blood flow measurement at 60 minutes after 60 minutes of ischemia), indicating that sham IPC and anesthesia 24 hours previously could not themselves be responsible for the blood flow-preserving effects of IPC.

In preliminary experiments, the acute injection of microspheres was not found to produce significant alterations in blood pressure. There were no significant differences in PaO2, oxygen saturation, or hematocrit between any groups in the study. Left versus right cerebral cortical blood flow did not differ within any group, verifying that equal distribution of microspheres was achieved during experiments.16,22 Given a relatively low mean arterial pressure (MAP) of the control group (66 ± 5 mm Hg), several of the experimental groups were found to have a MAP significantly different from control. Thus, a resistance factor was also determined (MAP/RBF) to correct for any possible effect of variations in MAP on the RBF. Groups found to have significant retinal hypoperfusion also had a significant increase in resistance. Therefore, the low MAP of the control group did not affect our results.

### Discussion

Consistent with our earlier findings in rats, increasing the duration of ischemia resulted in diminishing postischemic recovery of electrical function.2 After a brief period of ischemia of 5 minutes, there was complete recovery of electrical function, and no significant decrease in RBF. However, RBF after ischemia clearly demonstrated a pattern of profound delayed postischemic retinal hypoperfusion when the duration of ischemia exceeded 30 minutes. Nonetheless, beyond 60 minutes of ischemia, despite increasingly impaired or absent recovery of the b wave, there was no further decrease in postischemic RBF. Another finding of the study was that IPC was completely effective in preventing delayed postischemic retinal hypoperfusion after periods of ischemia as long as 60 minutes. These new findings suggest that IPC may protect the retina against prolonged ischemia, in part, by a hemodynamic-based mechanism.

The results differed from our previous examination of RBF after ischemia in cats16 and from the findings after central retinal artery occlusion in rhesus monkeys,13,14 indicating that
the effects of ischemia on blood flow are, to some degree, species dependent. Other reasons for varying findings from earlier investigations in other species may be differences in the duration of and the method used to produce ischemia. In monkeys, retinal damage consistently appeared only when the duration of ischemia exceeded 105 minutes. In addition, damage to the vasculature (narrowed vessels), still evident weeks later, was also only found after prolonged ischemia. In studies of both rats and cats, the method used to produce ischemia resulted in panocular ischemia. This more complete ischemia could have resulted in a greater degree of vascular damage compared with that in the monkey study, and may have led to injury to the outer retina and retinal pigment epithelium. Thus, shorter periods of ischemia in rats resulted in a greater degree of damage compared with results of ischemia of similar duration in monkeys. In the cat, the absence of hypoperfusion could be explained by use of only a single duration of ischemia (60 minutes), differing anesthetic technique, or the presence of collateral circulation. The cat retina also appears more resistant to ischemic damage in comparison to the rat retina.

Retinal hypoperfusion to a remarkably similar degree as in our study has recently been described in newborn pigs subjected to a profound ischemic insult consisting of a combination of severe hypoxia, hypotension, bradycardia, and acidosis. However, the mechanisms responsible for these effects in the retina are not yet known. Postischemic hypoperfusion is believed to represent a functional abnormality of the vasculature. Depletion of or abnormal reactivity to essential vasoactive factors, such as nitric oxide or adenosine, or leukocyte adherence to the damaged blood vessel wall is a potential pathogenic alteration. Changes in nitric oxide synthase gene expression and abnormal leukocyte dynamics both have recently been described after transient retinal ischemia in the rat, suggesting that altered vascular reactivity is in fact a significant factor in postischemic retinal vascular dysfunction.

Another mechanism that is likely to contribute to hypoperfusion is postischemic edema, which can directly compress the retinal blood vessels. The greatest degree of flow impairment with edema would be in the central portion of the macular area where ganglion cell density and swelling are maximal. Clinically, such edema would manifest as the cherry-red spot. Increasing duration of ischemia would be expected to result in worsening edema and therefore a greater compromise of postischemic circulation.

IPC with 5 minutes of retinal ischemia 24 hours before prolonged ischemia in the rat was completely effective in preventing the functional and histologic damage resulting from 60 minutes of ischemia. Little is known at present about the mechanism of this effect except that stimulation of adenosine receptors is necessary to confer this protection. In the present study, after IPC, hypoperfusion was dramatically reduced compared with that in subjects not receiving this pretreatment, although the mechanism of this effect remains to be determined. However, the effect of IPC was limited by the duration of ischemia. After 75 minutes of ischemia there was no improvement of RBF with IPC. This indicates that after prolonged ischemia, other mechanisms not countered by IPC’s protective effects become more significant contributors to postischemic retinal dysfunction.

Although for unclear reasons the MAP in the control group was lower than in any experimental group, it is not likely to have affected our findings to any significant degree, for two reasons. First, the MAP in the control group (66 ± 5 mm Hg) was within a range in which autoregulation should maintain constant RBF regardless of systemic arterial pressure. Secondly, in the unlikely event that the lower MAP of the control group had an effect on the RBF, it would have caused a tendency toward decreased RBF. Had the MAP of the control group been higher (similar to that in the experimental groups), the control RBF could only have been higher and the findings of postischemic retinal hypoperfusion would have been attenuated even further.

A large number of microspheres must be injected to measure flow in the retina of a small animal such as the rat. There were no significant hemodynamic effects after the single injection of microspheres used in this study. It is commonly believed that a minimum of 400 microspheres must be impacted in the organ under study for accurate measurements (<20% error) of regional blood flow, although impaction of this many spheres is not achievable in the retina of the rat. Nonetheless, accurate blood flow measurements are in fact achievable in the retina or in other organs of rats with far fewer numbers of impacted spheres. In particular, Nose et al. showed that if the numbers of microspheres in the reference blood sample and tissue of interest were higher than 400 and 49, respectively, the 95% confidence intervals could be determined for a single measurement, and provided that differences between the compared groups were large enough, less than 400 spheres sufficed to demonstrate a statistical difference.

In conclusion, the present study demonstrated progressive loss of recovery of electrical function after increasing duration of retinal ischemia in the rat. Accompanying these findings was severe postischemic retinal hypoperfusion when duration of ischemia exceeded 30 minutes. The hypoperfusion was completely attenuated by prior IPC when ischemia was 60 minutes or less in duration. Although these findings cannot yet be directly applied to the treatment of retinal vascular occlusion in humans, the study suggests yet another mechanism for the intriguing phenomenon of IPC in the in vivo retina.

References


