Effects of Hypothermia and Aging on Postischemic Reperfusion in Rat Eyes

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Abstract: The acute changes in choroidal blood flow during postischemic reperfusion were investigated by using laser Doppler flowmetry in young (4 months) and aged (more than 18 months) Wistar rats under normothermic and hypothermic conditions. Choroidal blood flow was measured by using a laser Doppler probe attached to the scleral surface before, during, and after temporary ischemia produced by an elevation of intraocular pressure up to 80 mmHg. Body temperature was maintained either from 38 to 39°C (normothermia) or from 30 to 33°C (hypothermia). Under the normothermic condition, postischemic reperfusion showed hyperperfusion dominantly in all groups (117.1±4.9% of the baseline value after 10 min of ischemia, 208.6±16.1% after 30 min, and 176.6±17.1% after 50 min). Exposure to hypothermia attenuated the postischemic hyperperfusion (101.9±11.7% after 10 min of ischemia, 152.9±11.2% after 30 min, and 107.8±19.9% after 50 min). In aged rats, the response of choroidal blood flow during reperfusion was variable. The no-reflow phenomenon was observed in 1 of 5 rats, marked hyperperfusion (238 and 177%) in 2 rats, and a small magnitude (127 and 115%) of hyperperfusion in the other 2 rats, whereas marked hyperperfusion was observed in all rats of the young group after 30 min of ischemia. These results suggest that hyperperfusion is dominant during the acute phase of postischemic reperfusion in young rats under normothermia. Hypothermia attenuates the postischemic hyperperfusion of the choroidal blood flow. The circulatory response during postischemic reperfusion becomes variable with age. [Japanese Journal of Physiology, 48, 9–15, 1998]

Key words: aging, choroidal blood flow, hypothermia, laser Doppler flowmetry, postischemic reperfusion.

Postischemic reperfusion has been extensively investigated in many organs such as the brain and heart. In the brain, hyperperfusion followed by delayed hypoperfusion is commonly observed during the reperfusion period after ischemia [1]. The hyperperfusion is often accompanied by extravasation of albumin and causes serious tissue damage in the gerbil brain [2]. The no-reflow phenomenon, in which the tissue remains ischemic even after circulation is restored, is also observed in the gerbil brain [2]. On the other hand, little information is in the literature concerning postischemic reperfusion in eye tissues. Roth and Pietrzyk [3] showed hyperperfusion after 1 h of retinal ischemia in cats. Neither the delayed hypoperfusion nor the no-reflow phenomenon was demonstrated in their study. Recently, we showed that even 10 min of ischemia caused slight hyperperfusion in rat choroidal blood flow (ChBF) [4]. No previous studies have investigated the relationship between the period of ischemia and the pattern of reperfusion in eye tissues. Thus the first purpose of the present study is to compare acute changes in ChBF during reperfusion after 10, 30, and 50 min of ischemia in rats. We hypothesized that a longer period of ischemia causes greater hyperperfusion.

Karibe et al. [5] reported that mild hypothermia reduced postischemic hyperperfusion, delayed hypoperfusion, and neuronal damage in the rat brain.
Faberowski et al. [6] demonstrated histologically that hypothermia protected the retina from ischemic injury. We suggested that hypothermia attenuated mild hyperperfusion following 10 min of ischemia in rat eyes [4]. It is of interest to examine whether hypothermia also decreases more severe hyperperfusion following a longer period of ischemia. The second purpose of this study is to compare the effect of hypothermia (30–33°C) on the hyperperfusion following 10, 30, and 50 min of ischemia in rat eyes.

Rowland et al. [7] found that heart tissue is more tolerant to ischemia-reperfusion injury in newborn rats than in adults. Kirsch et al. [8] reported that piglets (2 weeks) showed improved neurological recovery after transient ischemia in comparison with older pigs (6 to 8 months). Delayed hyperperfusion occurred in all cerebral regions except in the white matter in adult pigs, whereas it was observed only in the brain stem and caudate nucleus in the piglets. These findings suggest that tissue injuries because of ischemia-reperfusion are more severe in aged animals. Little is mentioned in the literature, however, about the effect of aging on postischemic reperfusion in eye tissues. The third purpose of the present study is to compare the changes in ChBF during postischemic reperfusion between young (4 months) and aged (more than 18 months) rats. Since ocular ischemia occurs more often in old patients than in young, these data will provide important information for the treatment of ischemic disease of the eye.

Some of these results (10 min of ischemia) have been presented at the 6th World Congress for Microcirculation.

METHODS

Animal preparation. Seventy-two male Wistar rats, 4 months old (n=64) and more than 18 months old (n=8), were used in this study. All procedures were reviewed and approved by the guidelines for Animal Experimentation at the Faculty of Medicine, Tottori University, Yonago, Japan, and conformed to Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences, published by the Physiological Society of Japan. The rats were anesthetized with ketamine hydrochloride (100 mg/kg, I.M., Sankyo, Tokyo) which was sufficient to maintain a satisfactory level of pain avoidance throughout the experiment; immobilized with pancuronium bromide (0.2 mg/kg, I.M., Orgnon Teknika, Oss, The Netherlands); and fixed at a stereotaxic animal frame. After tracheotomy, they were artificially ventilated with room air. The respirator (SN-480-7, Shinano, Tokyo) was adjusted at 80–100 cycles/min to maintain PaO₂ at 30–40 mmHg throughout the experiments. A canula was inserted into the left femoral artery of each rat for monitoring blood pressure by using a pressure transducer (TP-200T, Nihon Kohden, Tokyo) and for sampling arterial blood intermittently for blood gas analysis (CD-Oximeter, Instrumentation Laboratory, Barcelona, Spain). Animals in which these parameters changed significantly during the experiment were deleted from the study.

Measurement of ChBF. ChBF was measured continuously by means of a laser Doppler flowmeter (LDF) (ALF21, Advance, Tokyo) in the left eye. Although this method does not yield measurements of absolute value, it provides valuable relative measurements of blood flow [9]. We modified the procedures for laser Doppler flowmetry that have been reported elsewhere [10, 11]. Briefly, a small part of the bulbar conjunctive, approximately 2–3 mm posterior to the upper limbus of the left eye, was removed and a laser probe placed on the exposed sclera by using a micro-manipulator. A traction thread was sutured at the upper eyelid and the superior rectus muscle to secure the eyeball in a suitable position for ChBF measurement. The tension of the traction thread was maintained at a minimal level. Care was taken to avoid the probe being placed on a visible vessel. The laser probe operates in the infrared region at 780 nm. Spontaneous eye movement was not observed throughout the experiment. An artificial aqueous equivalent (Opeguard MA, Senju, Japan) was dropped periodically to keep the scleral surface moist. Blood flow can be calculated as the product of blood flow velocity and blood volume. Tissue blood volume correlates linearly with the integrated intensity of the power spectrum of scattered light [9]. A 27-gauge needle was inserted into the anterior chamber through the cornea and connected via a three-way stopcock to a reservoir filled with the artificial aqueous equivalent and to a pressure transducer. Intraocular pressure (IOP) was altered by changing the height of the reservoir.

Ischemia-reperfusion protocol. Rats were divided into 10 groups, as shown in Table 1. After the baseline value of ChBF was measured, IOP was elevated from 15 to 80 mmHg to produce ischemia in the eye tissues for 10 min (Groups 1 and 4), for 30 min (Groups 2, 5, and 9), or 50 min (Groups 3 and 6), then returned to 15 mmHg to allow reperfusion. In the control groups (Groups 7, 8, and 10), IOP was maintained at 15 mmHg throughout the experiment. The baseline value of 15 mmHg was chosen because IOP is supposed to be 10–20 mmHg in normal persons, and normal IOP in male Wistar rats is about 18 mmHg [12].
The IOP during the ischemia period was set at 80 mmHg, since it is the maximum level clinically observed in patients with acute glaucoma. Body temperature was monitored by a thermistor (D226, Takara, Tokyo) inserted into the rectum and maintained either at 38–39°C (normothermia; Groups 1, 2, 3, 7, 9, and 10) or at 30–33°C (hypothermia; Groups 4, 5, 6, and 8) by using a heating pad or a cooling pad. The present study was focused on the acute change in ChBF immediately after ischemia. Preliminary studies showed a gradual decrease in ChBF after 90 min from the beginning of the control experiment without ischemia. A similar decrease in ChBF has been also shown by Roth and Pietrzyk [3]. Therefore we finished the experiments within 1 h after the onset of ischemia, during which no significant decrease in ChBF was observed. Thus delayed hypoperfusion was not investigated in this study.

**Data analysis.** The output of LDF recording was averaged for 30 s before ischemia (baseline value), during ischemia (at 1 and 9 min in Groups 1 and 4; at 1, 9, and 29 min in Groups 2, 5, and 9; and at 1, 9, and 49 min in Groups 3 and 6 after the onset of ischemia), and after ischemia (at 1 and 9 min in Groups 1, 3, 4, and 6; and at 1, 9, and 29 min in Groups 2, 5, and 9 after the end of ischemia). In the present study, the relative ChBF was expressed as a percentage of the baseline value in each rat. Data shown in the text and figures are expressed as means±standard error of the mean. Results were statistically analyzed either by Student's t-test or by an ANOVA with repeated measures followed by a post hoc Scheffe’s test. The difference in means was considered significant when p<0.05.

**RESULTS**

No significant changes were observed in mean arterial pressure and \( P_a_{\text{CO}_2} \) before ischemia versus after ischemia (within 5 min before the end of the experiment) in any experimental group (Table 2). Exposure to hypothermia did not affect these parameters. There were also no significant differences in the parameters between young and aged rats. In the control experiments (Groups 7 and 8), ChBF did not change throughout the experiments (Figs. 1–3).

**Relationship between duration of ischemia and pattern of reperfusion**

Under normothermia (Groups 1, 2, and 3, \( n=24 \)), ChBF decreased to 46.4±3.2% of the baseline value during exposure to 80 mmHg of IOP. In these groups, ChBF increased above the baseline level at 1 min of the reperfusion period and returned nearly to the baseline by 9 min after the end of ischemia. The no-reflow phenomenon was not observed in any rat. In Group 1, ChBF increased to 117.1±4.9% at 1 min of the reperfusion period (\( p<0.01 \), Fig. 1a). More remarkable hyperperfusion was recorded in Group 2 (208.6±16.1%, Fig. 2a) and in Group 3 (176.6±17.1%, Fig. 3a). The magnitude of hyperperfusion was greater in Group 2 (\( p<0.05 \)) and in Group 3 (\( p<0.05 \)) than in Group 1, but no significant difference was found between Group 2 and Group 3.

**Effect of hypothermia**

Under hypothermia (Groups 4, 5, and 6, \( n=24 \)), ChBF decreased to 42.5±2.9% of the baseline value during the ischemic period. In Group 4, hyperperfu-

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**Table 1. Animal groups and their experimental conditions.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Age</th>
<th>Ischemic period</th>
<th>Body temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>4 months 10 min</td>
<td>Normothermia</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>4 months 30 min</td>
<td>Normothermia</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>4 months 50 min</td>
<td>Normothermia</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>4 months 10 min</td>
<td>Hypothermia</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>4 months 30 min</td>
<td>Hypothermia</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>4 months 50 min</td>
<td>Hypothermia</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>4 months</td>
<td>Normothermia</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>4 months</td>
<td>Normothermia</td>
</tr>
<tr>
<td>9</td>
<td>5 &gt;18 months 30 min</td>
<td>Normothermia</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3 &gt;18 months</td>
<td>Control</td>
<td>Normothermia</td>
</tr>
</tbody>
</table>

\( n \): number of rats.

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**Table 2. Mean arterial pressure and \( P_a_{\text{CO}_2} \) tension before and after ischemia.**

<table>
<thead>
<tr>
<th></th>
<th>( P_a_{\text{CO}_2} ) (mmHg)</th>
<th>Mean arterial pressure (mmHg)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Normothermia rats</td>
<td>32</td>
<td>115.7±2.0</td>
</tr>
<tr>
<td>Hypothermia rats</td>
<td>32</td>
<td>115.1±2.0</td>
</tr>
<tr>
<td>Aged rats</td>
<td>8</td>
<td>120.9±4.4</td>
</tr>
</tbody>
</table>

\( n \): number of rats. Normothermia rats: Groups 1, 2, 3, and 7; hypothermia rats: Groups 4, 5, 6, and 8; aged rats: Groups 9 and 10. NS: not significant (vs. before). Values are expressed as means±standard error of the mean.


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Fig. 1. (a) Changes in choroidal blood flow (ChBF) during and after 10 min of ischemia in young rats under normothermia (△, Group 1, n=8) and in control rats (□, Group 7, n=8). *Significantly different from the baseline value (p<0.01). (b) Changes in ChBF during and after 10 min of ischemia in young rats under hypothermia (△, Group 4, n=8) and in control rats (□, Group 8, n=8). ChBF before ischemia was taken as 100%.

Fig. 2. (a) Changes in choroidal blood flow (ChBF) during and after 30 min of ischemia in young rats under normothermia (△, Group 2, n=8) and in control rats (□, Group 7, n=8). *Significantly different from the baseline value (p<0.01). (b) Changes in ChBF during and after 30 min of ischemia in young rats under hypothermia (△, Group 5, n=8) and in control rats (□, Group 8, n=8). ChBF before ischemia was taken as 100%. *Significantly different from the baseline value (p<0.01).

sion (117–158%) was observed in 3 of 8 rats after ischemia, and ChBF returned nearly to baseline levels (88–96%) in 4 rats but recovered poorly (46%) in 1 rat. The average value of ChBF at 1 min after ischemia was 101.9±11.7% (Fig. 1b). In Group 5, hyperperfusion was observed in all rats (Fig. 2b). The magnitude of hyperperfusion (152.9±11.2%) was smaller (p<0.05) than in the normothermic group (Group 2). Hypothermia markedly suppressed the hyperperfusion in the rats exposed to 50 min ischemia (Fig. 3b). ChBF at 1 min after ischemia ranged from 78 to 136% of the baseline value. The average value was 107.8±7.1%, which was significantly (p<0.05) smaller than the average value in Group 3 (176.6±17.1%).

Effect of aging

In aged rats (Group 9), the response of ChBF during reperfusion was variable. A no-reflow pattern was observed in 1 rat in which ChBF did not return to the baseline by 30 min after the end of ischemia (Fig. 4). Marked hyperperfusion (238 and 177%) occurred in 2 of 5 rats, and a small magnitude (127 and 115%) of hyperperfusion was recorded in the other 2 rats. Throughout the control experiments (Group 10), ChBF did not change (data not shown).

DISCUSSION

The present results show that hyperperfusion is dominant during the early stage of postischemic reperfusion (less than 1 h after the cessation of ischemia) in the eyes, which is consistent with the result reported by Roth and Pietrzyk [3]. The no-reflow phenomenon was not observed in young rats under normothermia. Hyperperfusion itself is a physiological response to
the preceded ischemia. However, it is often accompanied by extravasation of albumin [2], edema formation [5], and increased production of hydroxyl radicals [13], which cause tissue damage. Traupe et al. [1] demonstrated by using the hydrogen clearance technique that 90% of cats showed hyperperfusion after 60 min of cerebral ischemia, whereas it occurred in only 50 and 40% after 15 and 30 min of ischemia. They suggested that the temporal resolution of the technique and the short duration of the hyperperfusion might be why hyperperfusion was not detected in all cases. Thus in the present study we used laser Doppler flowmetry, which can measure blood flow continuously. The results show that hyperperfusion occurs even after 10 min of ischemia in rat eyes. Since the magnitude of hyperperfusion was significantly greater in Group 2 and Group 3 than in Group 1, our hypothesis, that a longer period of ischemia causes greater hyperperfusion, seems to be correct.

Laser Doppler flowmetry has been used to assess microcirculatory blood flow in multiple tissues, including the choroid [10, 11]. Since the laser probe is placed on the exposed sclera from the outside, the sclera is situated between the laser source and the choroidal tissue. However, the laser probe that operated in the infrared region at 780 nm can detect a good signal from choroidal tissue underneath the sclera [10]. The laser beam may also reach the retinal vascular beds. Riva et al. [14] measured ocular blood flow with laser Doppler flowmetry, in which the laser beam was delivered through a fundus camera, and discussed that the contribution of retinal blood flow to the obtained value should be about 1/8 that of ChBF. Blood flow measurement using a radioactive microsphere showed that ChBF is about 100 times that of retinal blood flow in cats [15] and in pigs [16]. Therefore the output obtained with the technique used in the present study is supposed to represent mainly ChBF. Arterial blood to the choroid is supplied from the long posterior ciliary artery, several anterior ciliary arteries, and a variable number of short posterior ciliary arteries [17]. The choroidal capillaries are arranged in a single layer restricted to the inner portion of the choroid [17]. This arrangement enables the capillaries to supply nutrition to the outer retina. The ChBF is important not only to supply an adequate amount of oxygen to the choroidal tissue and the outer retina, but also to protect the eye tissues from thermal damage under extreme conditions such as snowstorm, sauna bath, and observation of very bright objects [18]. Thus the laser Doppler flowmetry used in the present study provides useful information from the viewpoint of tissue protection and of nutrition.

**Fig. 3.** (a) Changes in choroidal blood flow (ChBF) during and after 50 min of ischemia in young rats under normothermia (△, Group 3, n=8) and in control rats (○, Group 7, n=8). ChBF before ischemia was taken as 100%. *Significantly different from the baseline value (p<0.01). (b) Changes in choroidal blood flow (ChBF) during and after 50 min of ischemia in young rats under hypothermia (△, Group 6, n=8) and in control rats (○, Group 8, n=8). ChBF before ischemia was taken as 100%.

**Fig. 4.** Changes in choroidal blood flow (ChBF) during and after ischemia under normothermic condition in 5 aged rats (Group 9). Postischemic reperfusion was variable among the individual rats.
The perfusion pressure for choroidal circulation is determined as the mean arterial pressure in the ophthalmic artery minus the IOP. Therefore a reduction of the arterial pressure or elevation of IOP decreases the perfusion pressure, which decreases choroidal blood flow. In the present study, ischemia was produced by elevating intraocular pressure from 15 to 80 mmHg. Although choroidal autoregulation is modestly effective when the IOP is increased up to 40 mmHg [19], the elevation of IOP to 80 mmHg obviously decreased perfusion pressure to beyond the autoregulation range. Choroidal ischemia is commonly produced by acute glaucoma (IOP-induced ischemia) and vascular occlusion (hypotension-induced ischemia). The elevation of IOP to 80 mmHg produced incomplete ischemia in rats. Complete ischemia does not usually happen in glaucoma, since IOP does not clinically exceed mean arterial pressure. On the contrary, a complete cessation of ChBF may occur theoretically when the feeding artery is occluded. The efficacy of choroidal autoregulation is different between IOP-induced ischemia and arterial hypotension-induced ischemia [19]. Thus the animal model in the present study is useful solely as a model for ischemia associated with acute glaucoma, not as an occlusion model.

The mechanism of postischemic hyperperfusion is still controversial. Many factors have been listed, including loss of autoregulation [20], neurogenic factor [21], release of superoxide [22] or nitric oxide [23], and metabolic factors such as an accumulation of lactate [24, 25] and adenosine [26]. Roth [15] also demonstrated that adenosine blockade partially inhibited postischemic hyperperfusion in the retina of cat. Karibe et al. [5] reported that hypothermia reduced postischemic hyperperfusion and neuronal damage in the rat brain. Hypothermia decreases adenosine formation [27], reduces glutamate release [28, 29], inhibits protease activation [30], and decreases hydroxyl radical production [13]. Thus we hypothesized that hypothermia attenuates the hyperperfusion after ischemia also in choroidal tissue through these mechanisms. Although these factors were not evaluated in the present study, the results supported this hypothesis, since the postischemic hyperperfusion was greater in normothermic rats (Groups 2 and 3) than in hypothermic rats (Groups 5 and 6). Further study will be needed to clarify the importance of each factor. Whatever the mechanism is, however, hypothermia may have clinical benefit to patients exposed to reperfusion after an elevation of IOP.

It has been known that young animals have a better neurological tolerance to transient ischemia than older animals do [31, 32]. Kirsh et al. [8] demonstrated that piglets show quicker recovery of cerebral blood flow, cerebral oxygen consumption, and somatosensory-evoked potentials after global ischemia than older pigs do. A newborn rat myocardium is more tolerant to a global ischemia followed by reperfusion compared with the adult myocardium, and this improved recovery is associated with decreased oxygen radicals, increased catalase activity, and augmented stress protein expression [7]. Although these reports demonstrated the changes that occurred during the period of matura-

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