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Nitric Oxide Synthase Expression in Ischemic Rat Retinas

Miwako Kobayashi,* Toshihiko Kuroiwa,†
Reiko Shimokawa,† Riki Okeda† and Takashi Tokoro*

*Department of Ophthalmology, Faculty of Medicine, †Department of Neuropathology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan

Purpose: To investigate the expression of nitric oxide synthase (NOS) in the ischemic retina.

Methods: Retinal ischemia was induced in rats by bilateral common carotid artery occlusion (BCCAO) for various lengths of time. Using the retina after BCCAO, expression of neuronal NOS (nNOS) and inducible NOS (iNOS) and identification of their positive cells were studied by histological and immunohistochemical examinations.

Results: Histological examinations revealed significant reduction in the thickness of the inner plexiform layer and the outer plexiform layer of the retina. Expression of nNOS was detected in retinal ganglion cells, amacrine cells, and Müller cells after BCCAO. The expression of nNOS and iNOS detected in Müller cells became stronger and persisted long after BCCAO.

Conclusions: In the ischemic retina, Müller cells and retinal ganglion cells expressed nNOS and iNOS. These phenomena may be involved in the ischemic damage to the retina. **Jpn J Ophthalmol 2000;44:235–244** © 2000 Japanese Ophthalmological Society

Key Words: Bilateral common carotid arteries occlusion, inducible nitric oxide synthase, ischemic retina, neuronal nitric oxide synthase, rat.

Introduction

Ocular ischemic syndrome is induced by occlusion or stenosis of the carotid artery and causes ischemic changes of the eye, particularly in the retina, resulting in visual impairment. Common causes of ocular ischemic syndrome include sclerosis, inflammation, and injury of the carotid artery.[1–3] The mechanisms by which retinal ischemia causes tissue damage to the retina are still unknown.

In the brain, however, recent studies using animal models of cerebral ischemia indicate significant involvement of nitric oxide (NO) in the neuronal damage to the central nervous system.[4–7]

Nitric oxide is produced when L-arginine is changed to L-citrulline by nitric oxide synthase (NOS).[8,9] Nitric oxide has a variety of biological activities, which include relaxation of the blood vessels,[10] cytotoxic activities,[11] and functions as a neurotransmitter in the central and peripheral nervous systems.[12,13] Because of the very short half-life of NO, its production is evaluated by measuring NOS using immunohistochemical techniques or in situ hybridization.[12,14–17] High expression of NOS was demonstrated in the ischemic brain, particularly in the hippocampus. The hippocampus is known to be highly susceptible to ischemia and to exhibit degenerative changes.[18] Furthermore, administration of NOS inhibitor protected the degenerative changes of the hippocampus in animals with brain ischemia.[19] These data on the brain from previous studies suggest a significant involvement of NO in tissue damage to the ischemic brain.[4–7] Therefore, it is suggested that NO also plays a role in the tissue damage to the ischemic retina.[20–22] However, no studies have investigated the distribution of NOS in the ischemic retina.

The present study was aimed at investigating the expression of NOS isoforms, neuronal NOS (nNOS)

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Correspondence and reprint requests to: Miwako KOBAYASHI, MD, Department of Ophthalmology, Yokohama Sakae Kyosai Hospital, 132-2 Katsura-cho Sakae-ku, Yokohama-shi 247-8581, Japan

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and inducible NOS (iNOS), in the retina of a rat model of ocular ischemic syndrome with cerebral chronic hypoperfusion.[23–26]

Materials and Methods

Animals

An inbred strain of Wistar rats (males, 7 weeks old) were purchased from Sankyo Lab Service (Shizuoka). The animals were kept in a room on a 12-hour light–dark cycle. All investigations were conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement on the Use of Animals in Ophthalmic and Vision Research.

Surgical Procedure

After inducing deep anesthesia in the experimental animals with intramuscular injection of ketamine (250 mg/kg), the bilateral common carotid arteries were exposed via a midventral incision and occluded with 3-0 silk sutures. These animals were designated as the bilateral common carotid artery occlusion (BCCAO) group. Rats receiving a sham operation served as controls. In the sham operation, occlusion of common carotid arteries was not performed, but all other surgical procedures were the same as in the BCCAO group.

A total number of 91 rats were used in the experiment (77 rats in the BCCAO group and 14 rats in the control group). Rats of the BCCAO group were sacrificed at one, 3, 6, 12, and 24 hours and 3 days (5 animals at each time interval), 1 week (n = 9), and 1, 2, 4, and 6 months (6 animals at each time interval) after surgery. Control rats were sacrificed at 1 week (n = 6) and 4 months (n = 8) after the sham operation.

Immediately after surgery, the ocular blood flow at the posterior pole of the fundus was evaluated using a laser Doppler flowmeter (TBF-LN 1 Unique, Tokyo) with a 0.5-mm-diameter laser Doppler probe.[27] Regional cerebral blood flow was measured 1 hour before the sacrifice of animals using a standard inhalation hydrogen clearance method as described previously.[28]

Tissue Processing

At each time interval after the surgery described earlier, animals were deeply anesthetized with intramuscular injections of ketamine (250 mg/kg) and were perfused transcardially with 0.2 mol/L phosphate buffer (PB) followed by a fixative consisting of 4% (w/v) paraformaldehyde in 0.1 mol/L PB (pH 7.4). After confirming that the rats were dead after the perfusion, both eyes were enucleated and immersed in a fixative solution for 6 hours. One eye of each animal was used for histological studies and the other eye was used for immunohistochemical studies.

Eyes for histological studies were embedded in paraffin and cut at 5-μm thickness using a microtome. Eyes for immunohistochemical studies were incubated at 4°C in 7% (w/v) sucrose in PB for 6 hours, 10% sucrose in PB overnight, 15% sucrose in PB for 6 hours, and 20% sucrose in PB overnight. Eyes were then embedded in OCT compound (Tissue-Tek; Miles, Naperville, IL, USA) and quickly frozen in acetone and dry ice. The frozen eyes were cut at 5-μm thickness parallel to the horizontal meridian using a cryostat (Leica 8400E; Leica, Tokyo). Sections for histology and immunohistochemistry were mounted on glass slides coated with 2% silane.

Histology

Paraffin-embedded sections of the rat eyes were stained with hematoxylin-eosin and examined by light microscopy. The thicknesses of the inner plexiform layer, inner nuclear layer, and outer plexiform layer were measured with the aid of a micrometer in a standard area located at the temporal retina, 200 μm distant from the optic disc. The measurement was performed in a masked fashion. The thickness of each layer of the retina was compared between the BCCAO group and the control.

The statistical analysis was carried out using Student's *t*-test.

Primary Antibodies

The following primary antibodies were used: rabbit polyclonal antibodies against rat brain NOS (bNOS, same as nNOS) (1:800; Wako, Osaka), and rabbit polyclonal antibodies against mouse iNOS (1:500; Wako); mouse monoclonal antibodies against rat 200-K neurofilament (NF) (1:1000; Transformation Research, Framingham, MA, USA) were used to identify the retinal ganglion cells[29] and amacrine cells;[30] mouse monoclonal antibodies against rat vimentin (1:100; Dako Japan, Kyoto) were used to identify Müller cells.[31]

Immunohistochemistry

The frozen sections were immersed in 0.01 mol/L phosphate buffered saline (PBS) (3 times for 5 min-

utes each), incubated with normal goat serum (Vector Lab, Birmingham, CA, USA) in a humid chamber at room temperature for 30 minutes and then incubated with the primary antibodies in a humid chamber at 4°C overnight. The sections were washed in PBS (3 times for 5 minutes each) and incubated with a secondary antibody, fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (1:100; Jackson, West Baltimore, MD, USA) for 1 hour at room temperature. Some sections were immunostained using avidin-biotinylated enzyme complex methods. Namely, after the frozen sections were incubated with normal goat serum and the primary antibodies, the sections were incubated with biotinylated rabbit anti-goat IgG antibodies for 1 hour at room temperature, then with avidin-biotin peroxidase complex (Vector Lab) for 1 hour at room temperature. After each incubation, the sections were washed with PBS. The peroxidase in the tissue was visualized with a mixed solution of 0.05% 3.3′-diamino-benzidine tetrahydrochloride and 0.01% H_2O_2 in 0.05 mol/L Tris HCl buffer (pH 7.5).

To identify the cells positive for nNOS or iNOS, a double-immunostaining was performed as follows. First, the frozen sections were incubated with the primary antibodies against nNOS or iNOS, then incubated with the secondary antibodies of a fluorescein (FITC)-conjugated goat anti-rabbit IgG (1:100). Second, the sections were incubated with another primary antibody against 200-K NF or vimentin, and incubated with another secondary antibody, that is, Texas Red-conjugated rabbit anti-mouse IgG antibody (1:50; Cappel, Aurora, OH, USA).

These sections were mounted using Slow Fade Light Antifade kit components (Molecular Probes, Eugene, OR, USA). Immunostained sections were examined with a light and fluorescence microscope (Nikon, Tokyo) and a confocal laser scanning microscope (TCS NF, Leica, Tokyo).

Results

Fourteen of the 77 (18.2%) rats in the BCCAO group died during the experiments, while none of the control rats died. Animal behavior was carefully observed during the experiments. There were no differences in motion behavior and feeding behavior between the control group and the BCCAO group. In addition, there was no difference in the body weight between the two groups. To confirm if the ocular blood flow was maintained even after BCCAO, the blood flow in the ocular fundus was measured by a laser Doppler flow meter. The percent laser Doppler flowmetry results were 15.4 ± 2.9% (mean ± SD) and 26.7 ± 1.1% at 1 minute and 3 hours after BCCAO, respectively. The cerebral blood flow measured by hydrogen clearance methods was 83.3 ± 11.8 mL/100 g brain per minute in the control group. At 1 hour, 3 hours, 3 days and 4 months after BCCAO, respectively, they were 29.7 ± 7.16 (36% of control), 25.2 ± 9.57 (30%), 38.8 ± 8.48 (47%) and 43.9 ± 12.8 (53%) mL/100 g brain per minute.

Histology

Histological examination disclosed degenerative changes of the entire retina in rats of the BCCAO group. The degenerative changes became more intense with time as demonstrated by a decrease in the number of retinal ganglion cells and in the thickness of the retina (Figures 1 and 2). One week after the surgery, the inner plexiform layer and outer plexiform layer in the BCCAO group were significantly thinner than those of the control group (Figure 2) ($P < .005$). Four months after the surgery, all three layers of the retina were significantly thinner in the BCCAO group than in the control group (Figure 2).

Immunohistochemistry for NOS

Immunoreactivity for nNOS was detected in the cells in the ganglion cell layer both in the control group and in the BCCAO group; the immunoreactivity was more intense in the BCCAO group (Figure 3). The cells positive to nNOS were identified as retinal ganglion cells because the cells were immunoreactive to 200-K NF by double-immunostaining (Figure 4). The immunoreactivity for nNOS was also found in amacrine cells at an early stage (1 hour to 3 days) after BCCAO (Figure 4), but no or minimum immunoreactivity for nNOS was found in Müller cells of the retina at this early stage (Figure 4, left). However, intense immunoreactivity for nNOS was found in Müller cells 1 week after BCCAO (Figure 5) and until 6 months afterward (Figure 6).

The immunoreactivity for iNOS was detected at the ganglion cell layer both in the control group and in the BCCAO group as early as 1 hour after surgery; the immunoreactivity for iNOS was much stronger in the BCCAO group (Figure 7). The cells positive to iNOS were immunoreactive for 200-K NF (Figures 8a,b) and vimentin (Figures 8c,d), indicating that retinal ganglion cells and Müller cells express iNOS. The immunoreactivity for iNOS in

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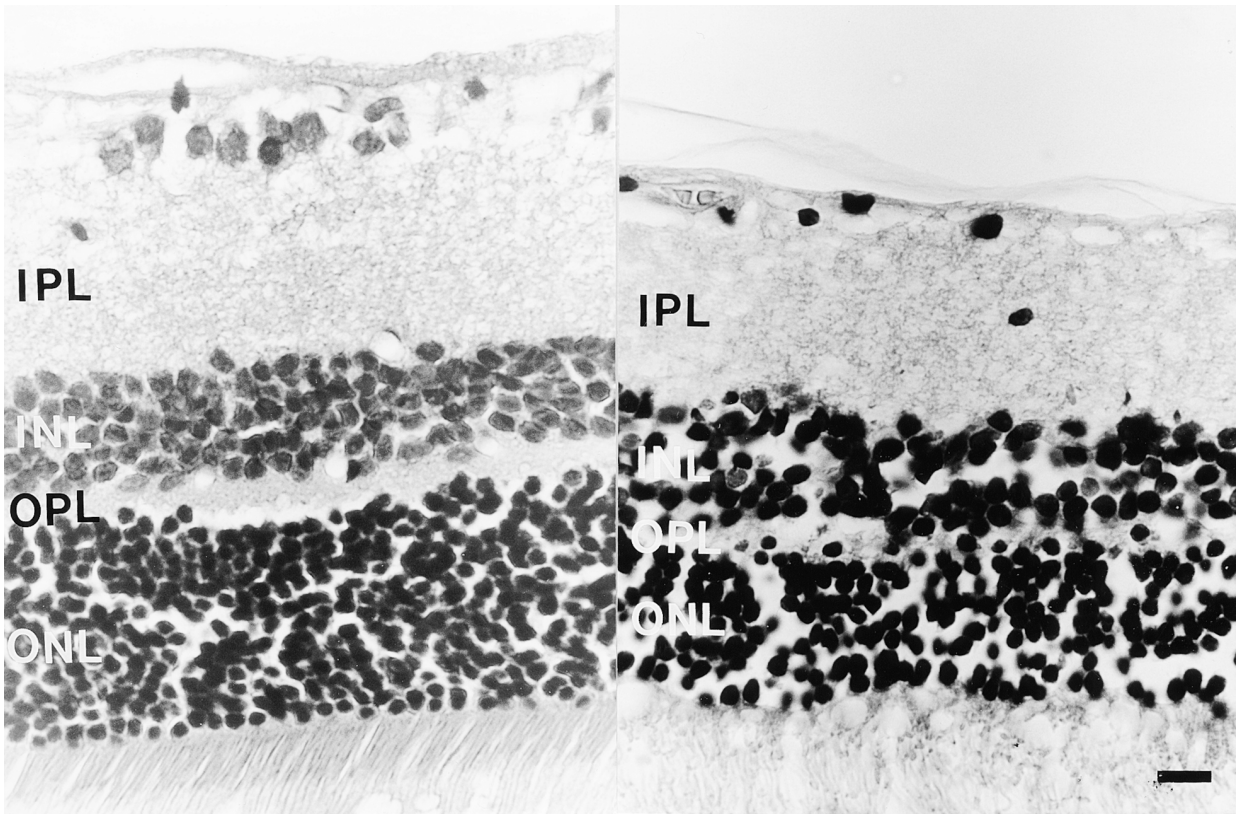

Figure 1. Light micrograph of rat retina, stained with hematoxylin and eosin (left). Control rat (right); 4 months after bilateral common carotid artery occlusion (BCCAO). IPL: inner plexiform layer, INL: inner nuclear layer, OPL; outer plexiform layer, ONL: outer nuclear layer. Thickness of IPL, INL, and ONL was much less in rats after BCCAO than in control rats. Bar = 10 μm.

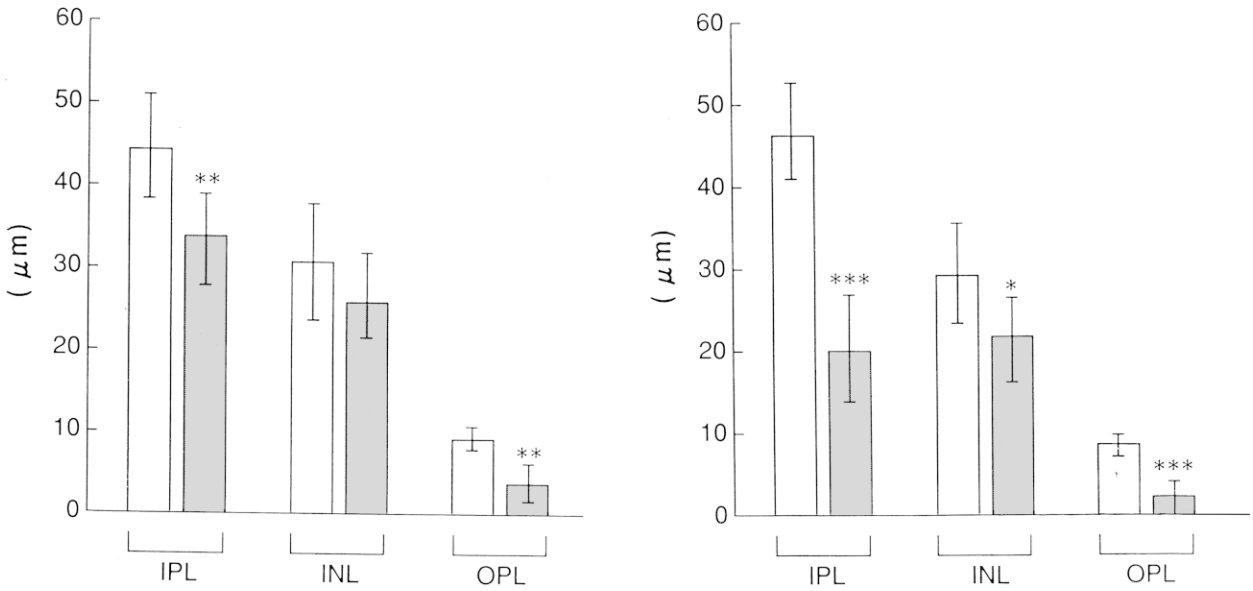

Figure 2. Comparison of thickness of inner plexiform layer (IPL), inner nuclear layer (INL), and outer plexiform layer (OPL) in retinas of control rats and bilateral common carotid artery occlusion (BCCAO) rats at 1 week (left) and 4 months (right) after surgery. □: control group, ■: BCCAO group. *$P < .025$; **$P < .005$; $P < .0005$.

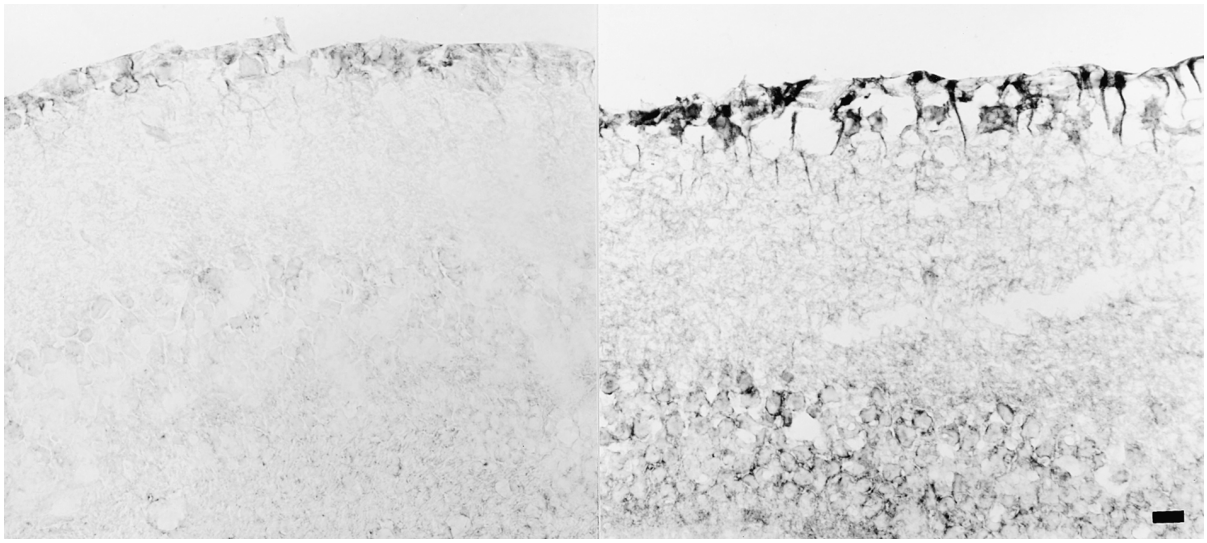

Figure 3. Light micrograph of rat retina from control group (left) and 3 hours after bilateral common carotid artery occlusion (right) immunostained for neuronal nitric oxide synthase (nNOS) using avidin-biotin peroxidase complex method. Immunoreactivity for nNOS was observed in cells in ganglion cell layer. Bar = 10 μm.

Müller cells became stronger at one week after BCCAO (Figure 9) and remained so for 6 months.

Discussion

The present study demonstrated histopathological changes and significant expression of NOS isoforms in the ischemic retina. The ischemic condition of the retina in the present study was induced by surgical occlusion of bilateral carotid arteries in the rat. There are several experimental methods to induce ischemic retina, such as by (1) increasing the intraocular pressure by continuous perfusion of the anterior chamber in the rabbit,[32] (2) surgical occlusion and reperfusion of the common carotid artery in the Mongolian gerbil,[27,33] and (3) surgical permanent occlusion of the bilateral common carotid arteries in the rat,[22–25] as in the present study. Because the present study was aimed at investigating the expression of NOS isoforms in an experimental model of ocular ischemic syndrome, it is essential to cause chronic cerebral hypoperfusion. To achieve this con-

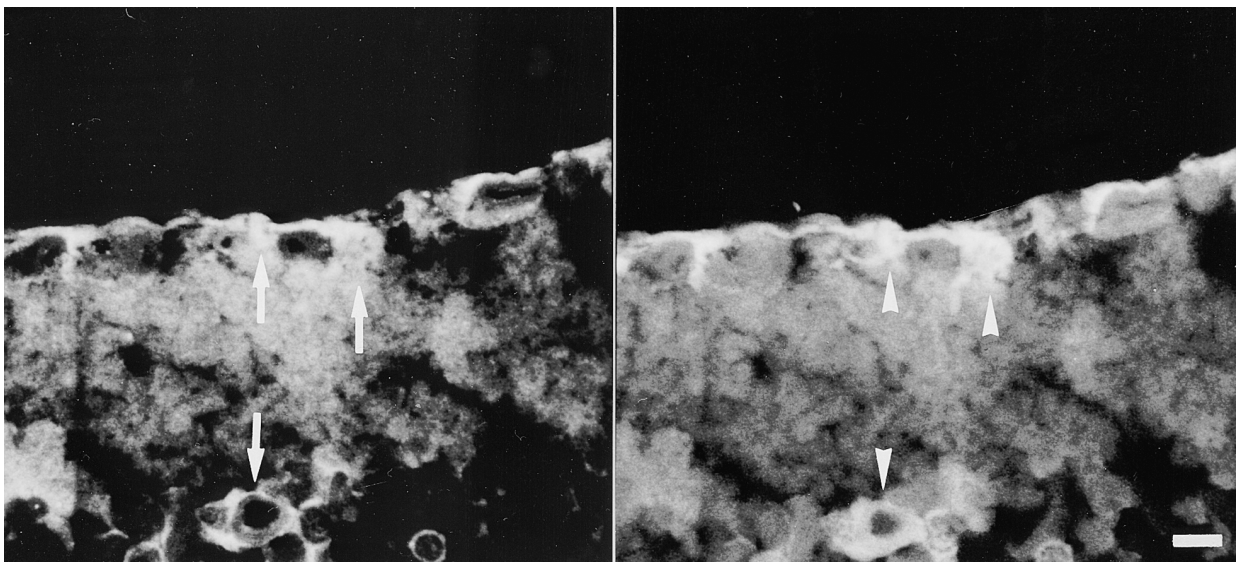

Figure 4. Confocal laser scanning micrograph of rat retinas 1 hour after bilateral common carotid artery occlusion, double-immunostained for neuronal nitric oxide synthase (nNOS) (left) and for 200-K neurofilament (NF) (right). Immunoreactivity for nNOS was observed in cells (arrows) in ganglion cell layer and inner nuclear layer in retina. These cells were identified with retinal ganglion cells and amacrine cells, which were also immunoreactive for 200-K NF (arrowheads). Bar = 10 μm.

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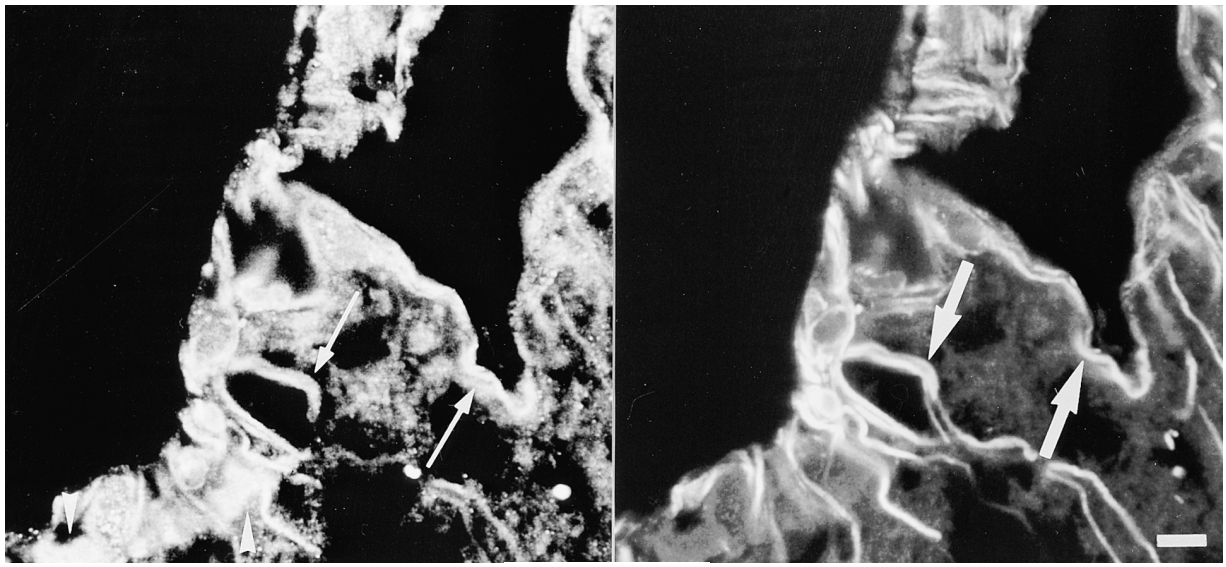

Figure 5. Confocal laser scanning micrograph of rat retinas at 1 week after bilateral common carotid artery occlusion, double-immunostained for neuronal nitric oxide synthase (nNOS) (left) and vimentin (right). Immunoreactivity for nNOS was clearly observed in Müller cells (arrows) which were positive for vimentin (large arrows). Surviving retinal ganglion cells were also immunoreactive for nNOS (arrowhead). Bar = 10 μm.

dition, the high intraocular pressure attained by anterior chamber perfusion is not adequate. Furthermore, because of the anatomical structure of the blood vessels in the central nervous system of the Mongolian gerbil, permanent surgical occlusion of the bilateral common carotid arteries in the Mongolian gerbil causes complete termination of the blood supply to the forebrain and 40–60% of animals die shortly after the surgery.[34–37] Therefore, the Mongolian gerbil model can be used as a transient ischemia and reperfusion model.[27,38] The present experiment demonstrated that the blood flow after BCCAO was

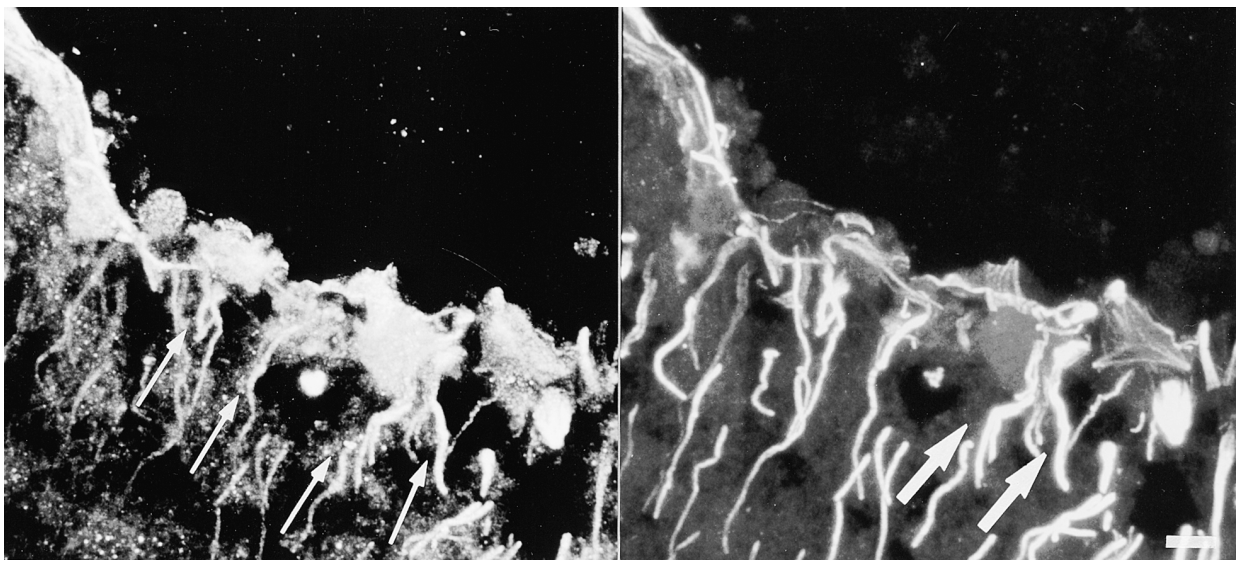

Figure 6. Confocal laser scanning micrograph of rat retinas 6 months after bilateral common carotid artery occlusion, double-immunostained for neuronal nitric oxide synthase (nNOS) (left) and vimentin (right). Immunoreactivity for nNOS was observed in Müller cells (arrows) which were positive for vimentin (large arrows). Bar = 10 μm.

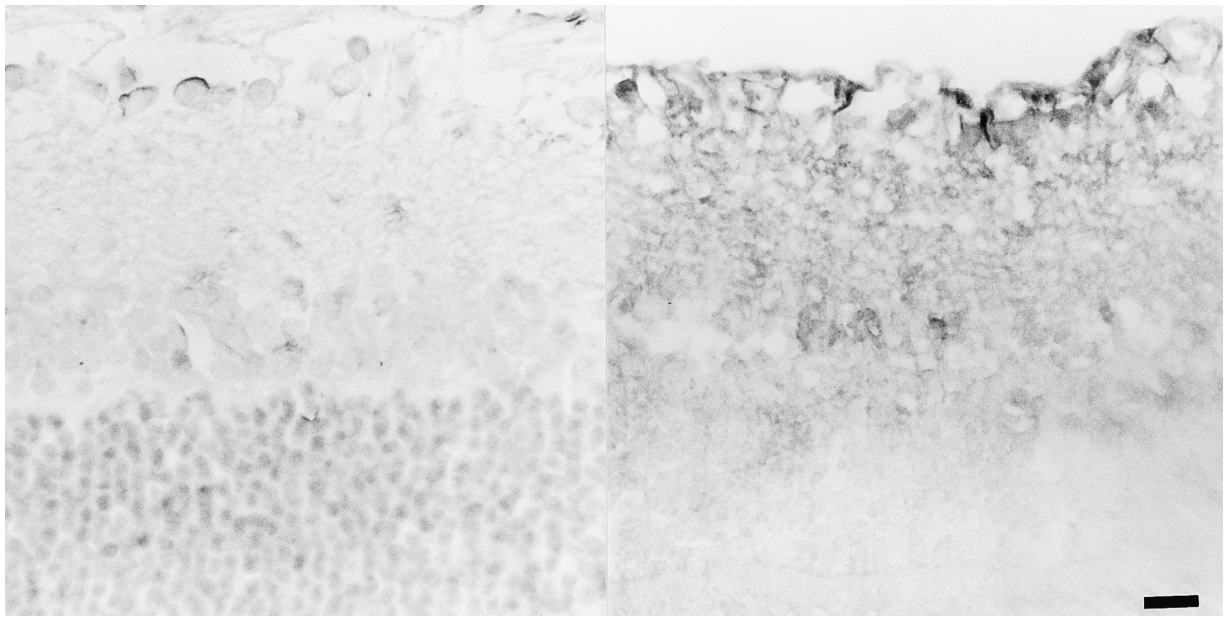

Figure 7. Light micrograph of rat retina from control group (left) and 1 hour after (right) immunostaining for inducible nitric oxide synthase (iNOS) using avidin-biotin peroxidase complex method. Immunoreactivity for iNOS was observed in cells in innermost retina. Bar = 10 μm.

30–53% of the control in the brain and 16–27% of the preoperative condition in the eye. This indicates that the blood supply was maintained long after BCCAO in the rat. Due to this level of blood supply to the eye and to the brain, experimental animals maintained their vision judged by the criteria of animal behavior and only 18.2% of the animals died during the experiments. Thus, the present experimental method is considered to be adequate to induce chronic hypoperfusion of the eye and ischemic retina as a model of ocular ischemic syndrome.

Using this experimental model, the data recorded here indicate that hypoperfusion of the eye causes degenerative changes and high expression of nNOS and iNOS in the ischemic retina. Histological examinations revealed a decrease in the number of retinal ganglion cells and in the thickness of the inner plexiform layer, the inner nuclear layer, and outer plexiform layer after BCCAO. The degenerative changes in the retina progressed with time. Immunohistochemical examinations showed high expression of nNOS in the retinal ganglion cells and in the amacrine cells shortly after BCCAO, but not in the Müller cells. High expression of nNOS in the Müller cells was detected 1 week after BCCAO and remained for as long as 6 months. The expression of iNOS was detected in the retinal ganglion cells and in the Müller cells shortly after BCCAO, but the expression of iNOS in the Müller cells became stronger 1 week after BCCAO and the high expression remained for 6 months. These data suggest that the ischemic condition of the eye induced by BCCAO causes the activation of the Müller cells. The Müller cells are glial cells[30,37] and there is experimental evidence indicating that BCCAO causes the activation of glial cells in the central nervous system. It was demonstrated following BCCAO that (1) the expression of major histocompatibility complex (MHC) antigen class I, class II, and leucocyte common antigen was upregulated at the microglia; (2) the expression of glial fibrillary acidic protein (GFAP) was also upregulated at the astroglia; and (3) the activation of these glial cells persisted as long as 90 days after BCCAO.[38] In the eye, Müller cells did not express GFAP under normal conditions,[39] but our unpublished data disclosed that Müller cells expressed GFAP after BCCAO and the expression became stronger with time after BCCAO.

Therefore, it is suggested that the activation of glial cells occurs in the ischemic retina and may be involved in the process of tissue damage in the ischemic retina.

In bacterial infection, a high expression of iNOS was detected in macrophages, which produced large

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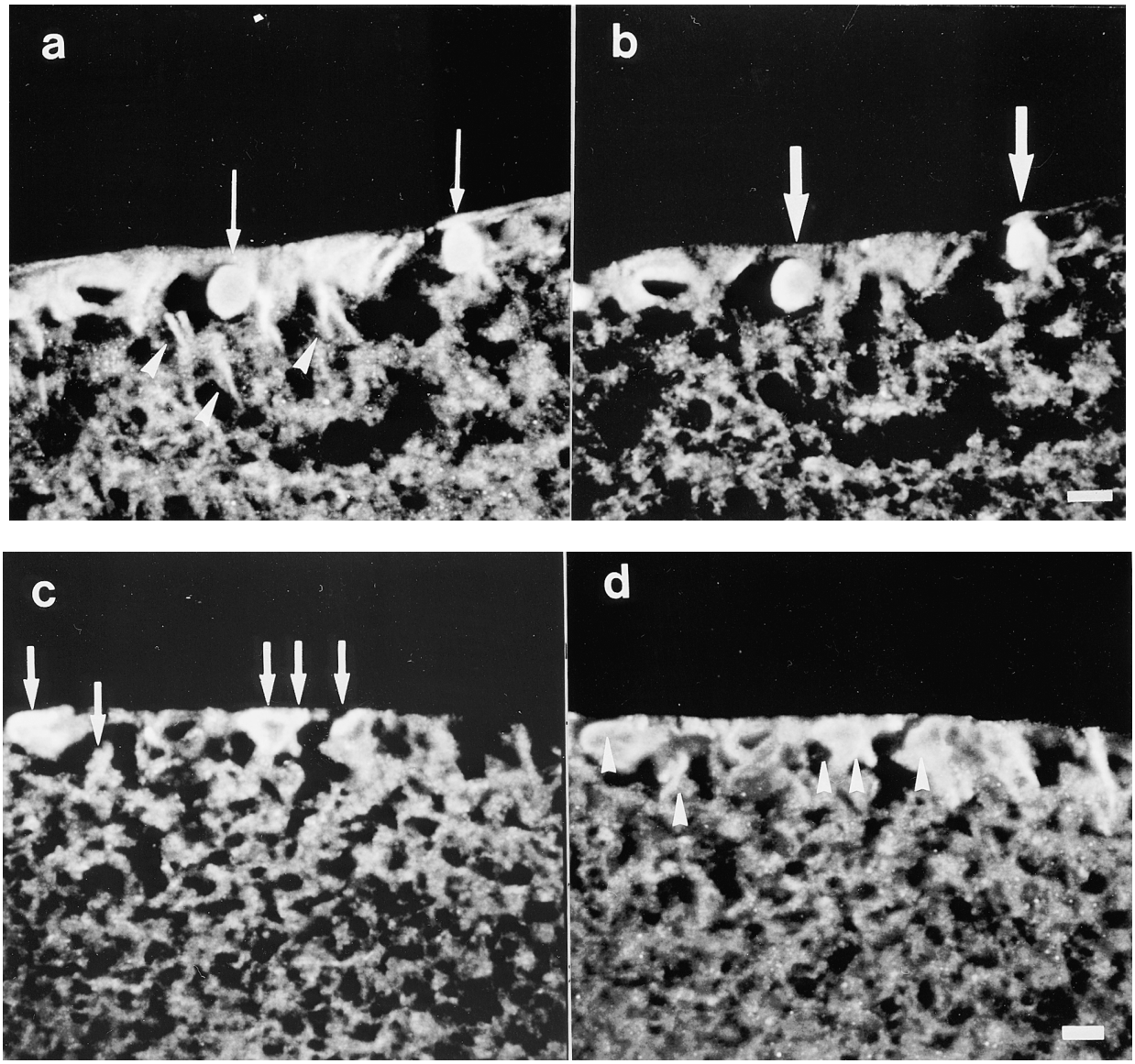

Figure 8. Confocal laser scanning micrograph of rat retinas 3 hours after bilateral common carotid artery occlusion (BCCAO), double-immunostained for inducible nitric oxide synthase (iNOS) (**a**) and 200-K neurofilament (NF) (**b**). Immunoreactivity for iNOS was observed in retinal ganglion cells (arrows) and Müller cells (arrowheads) in retina. Müller cells were negative for 200-K NF while retinal ganglion cells were positive for 200-K NF (large arrows). Confocal laser scanning micrograph of rat retinas 3 hours after BCCAO, double-immunostained for iNOS (**c**) and vimentin (**d**). Immunoreactivity for iNOS was observed in Müller cells (arrows) that were positive for vimentin (arrowheads) in retina. Bar = 10 μm.

amounts of NO and contributed to the elimination of the infectious agents.[40] It was also reported that Müller cells expressed high levels of iNOS and produced a large amount of NO in vitro.[41] Based on these previous observations, the high expression of nNOS and iNOS in the Müller cells is considered to contribute to the process of retinal degeneration of the ischemic retina after BCCAO. However, the mechanism by which the NOS expressed in the Müller cells and in the retinal ganglion cells causes tissue damage to the ischemic retina remains to be elucidated.

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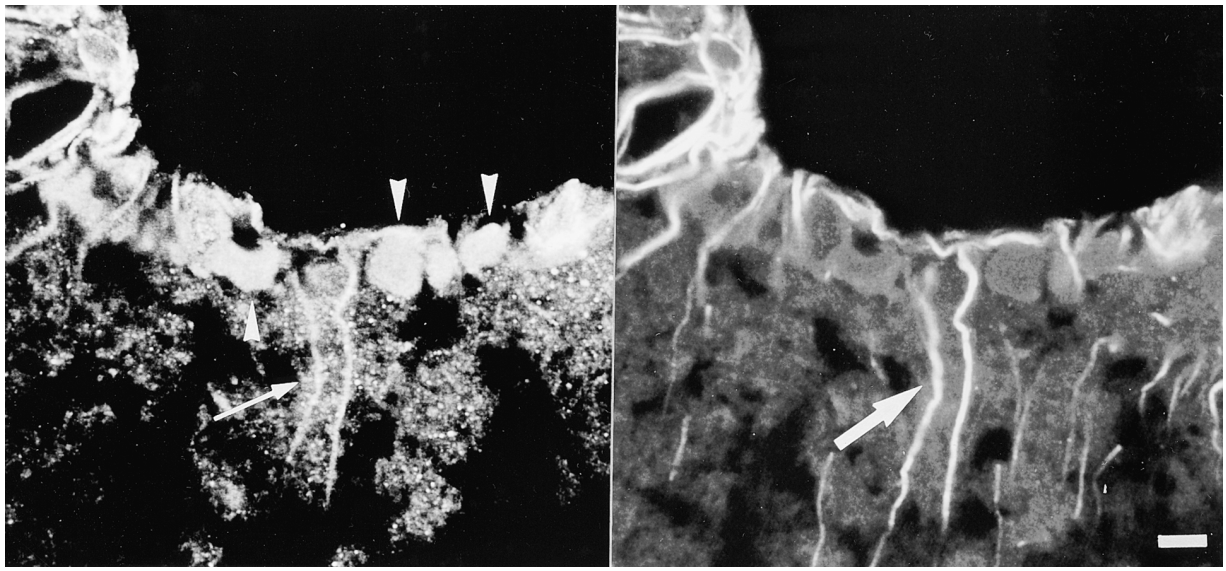

Figure 9. Confocal laser scanning micrograph of rat retinas 1 week after bilateral common carotid artery occlusion, double-immunostained with inducible nitric oxide synthase (iNOS) (left) and vimentin (right). Immunoreactivity for iNOS was clearly observed in Müller cells (arrow) (left), which were positive for vimentin (large arrow) (right). Surviving ganglion cells were also immunoreactive for iNOS (arrowheads) (left). Bar = 10 μm.

References

1. Bennett LW. Ocular ischemic syndrome as initial manifestation of bilateral carotid occlusive disease. J Am Optom Assoc 1997;68:250–60.
2. Ros MA, Magargal LE, Hedges TR Jr, et al. Ocular ischemic syndrome: long-term ocular complications. Ann Ophthalmol 1987;19:270–2.
3. Duker JS. Ocular ischemic syndrome secondary to carotid artery dissection. Am J Ophthalmol 1988;106:750–2.
4. Ferrante RJ. Selective sparing of a class of striatal neuronal nitric oxide synthase. Science 1994;265:1883–5.
5. Hyman BT. Relative sparing of nitric oxide synthase-containing neurons in the hippocampal formation in Alzheimer's disease. Ann Neurol 1992;32:812–20.
6. Ueyama Y. Selective sparing of NADPH-diaphorase-somatostatin-neuropeptide Y neurons in ischemic gerbil striatum. Ann Neurol 1990;27:620–5.
7. Zhang ZG. Upregulation of neuronal nitric oxide synthase and mRNA, and selective sparing of nitric oxide synthase-containing neurons after focal cerebral ischemia in rat. Brain Res 1994;654:85–95.
8. Hibbs JB, Taintor RR, Vavrin Z. Macrophage cytotoxicity: role for L-arginine deiminase activity and imino nitrogen oxidation. Science 1987;235:473–6.
9. Iyengar R, Stuehr DJ, Marletta MA. Macrophage synthesis of nitrite, nitrate and N-nitrosamines: precursors and role of respiratory burst. Proc Natl Acad Sci USA 1987;84:6369–73.
10. Forstrmann U, Closs EI, Pollock JS, et al. Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions. Hypertension 1994;23:1121–31.
11. Dawson VL. Nitric oxide: role in neurotoxicity. Clin Exp Pharmacol Physiol 1995;22:305–8.
12. Bredt DS, Hwang PM, Snyde SH. Localization of nitric oxide synthase indicating a neural role for nitric oxide. Nature 1990;347:768–70.
13. Ahmad I, Leinders-Zufall T, Kocsis JD, et al. Retinal ganglion cells express a cGMP-gated cation conductance activatable by nitric oxide donors. Neuron 1994;12:155–65.
14. Uemura Y. Selective sparing of NADPH-diaphorase-somatostatin-neuropeptide Y neurons in ischemic gerbil striatum. Ann Neurol 1990;27:620–5.
15. Huang Z, Huang PL, Panahian N, et al. Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase. Science 1994;65:1883–5.
16. Zhang ZG, Chopp M, Gautam S, et al. Upregulation of neuronal nitric oxide synthase and mRNA, and selective sparing of nitric oxide synthase-containing neurons after focal cerebral ischemia in rat. Brain Res 1994;654:85–95.
17. Huang Z, Huang PL, Panahian N, et al. Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase. Science 1994;265:1883–5.
18. Schwab M, Rauer R, Zweiener U. The distribution of normal brain water content in Wistar rats and its increase due to ischemia. Brain Res 1997;749:82–7.
19. Nagafuji T, Sugiyama M, Muto A, et al. The neuroprotective effect of a potent and selective inhibitor of type I NOS (L-MIN) in a rat model of focal cerebral ischemia. Neuroreport 1995;6:1541–5.
20. Kashii S. The role of nitric oxide in the ischemic retina. Nippon Ganka Gakkai Zasshi (J Jpn Ophthalmol Soc) 1995;99:1361–76.
21. Ostwald P, Goldstein IM, Pachnanda A, et al. Effect of nitric oxide synthase inhibition on blood flow after retinal ischemia in cats. Invest Ophthalmol Vis Sci 1995;36:2396–403.
22. Hangai M, Yoshimura N, Hiroi K, et al. Inducible nitric oxide synthase in retinal ischemia-reperfusion injury. Exp Eye Res 1996;63:501–6.

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23. Hart CT, Haworth S. Bilateral common carotid occlusion with hypoxic ocular sequelae. Br J Ophthalmol 1971;55:383–8.
24. Fujishima M, Ishitsuka T, Nakatomi Y, et al. Change in local cerebral blood flow following bilateral carotid artery occlusion in spontaneously hypertensive and normotensive rats. Stroke 1981;12:874–6.
25. Tsuchiya M, Sako K, Yura S, et al. Cerebral blood flow and histopathological changes following permanent bilateral carotid artery ligation in Wistar rats. Exp Brain Res 1992;89:87–92.
26. Block F, Schwarz M, Sontag KH. Retinal ischemia induced by occlusion of both common carotid arteries in rats as demonstrated by electroretinography. Neurosci Lett 1992;144:124–6.
27. Kuroiwa T, Bonnekoh P, Hossmann KA. Laser doppler flowmetry in CA1 sector of hippocampus and cortex after transient forebrain ischemia in gerbils. Stroke 1992;23:1349–54.
28. Kuroiwa T, Shibutani M, Okeda R. Blood-brain barrier disruption and exacerbation of ischemia brain edema after restoration of blood flow in experimental focal cerebral ischemia. Acta Neuropathol 1988;76:62–70.
29. Drager UC, Hofbauer A. Antibodies to heavy neurofilament subunit detect a subpopulation of damaged ganglion cells in retina. Nature 1984;309:624–6.
30. Blank H, Müller B, Korf H. Comparative investigations of the neuronal apparatus in the pineal organ and retina of the rainbow trout: immunocytochemical demonstration of neurofilament 200-kDa and neuropeptide Y, and tracing with DiI. Cell Tissue Res 1997;288:417–25.
31. Robinson SR, Dreher Z. Müller cells in adult rabbit retinae: morphology and implication for function and development. J Comp Neurol 1990;292:178–92.
32. Veriac S, Tissie G, Bonne C. Oxygen free radicals adversely affect the regulation of vascular tone by nitric oxide in the rabbit retina under high intraocular pressure. Exp Eye Res 1993;56:85–8.
33. Tomimoto H, Wakita H, Akiguchi I, et al. Temporal profiles of accumulation of amyloid β/A4 protein precursor in the gerbil after graded ischemic stress. J Cereb Blood Flow Metab 1994;14:565–73.
34. Berry K, Winiewski HM, Svarzbein L, et al. On the relationship of brain vasculature to production of neurological deficit and morphological changes following acute unilateral common carotid artery ligation in gerbils. J Neurol Sci 1975;25: 75–92.
35. Harrison MJG, Brownbill D, Lewis PD, et al. Cerebral edema following carotid artery ligation in the gerbil. Arch Neurol 1973;28:389–91.
36. Kahn K. The natural course of experimental cerebral infarction in the gerbil. Neurology 1972;22:510–5.
37. Schonfeld AR, Glick SD. Cerebrovascular abnormalities associated with seizure susceptibility in the Mongolian gerbil. Brain Res 1979;173:147–51.
38. Wakita H, Tomimoto H, Akiguchi I, et al. Glial activation and white matter changes in the rat brain induced by chronic cerebral hypoperfusion: an immunohistochemical study. Acta Neuropathol 1994;87:484–92.
39. Schnitzer J. Distribution and immunoreactivity of glia in the retina of the rabbit. J Comp Neurol 1985;240:128–42.
40. Nomura Y, Kitamura Y. Inducible nitric oxide synthase in glial cells. Neurosci Res 1993;18:103–7.
41. Goureau D, Hicks D, Coutois Y, et al. Induction and regulation of nitric oxide synthase in retinal Müller glial cells. J Neurochem 1994;63:310–7.