Akita J Med **40** : 135–141, 2013

INVOLVEMENT OF NITRIC OXIDE IN SIMULATED RETINAL ISCHEMIA

Daisuke Jin[1)], Makoto Ishikawa[1)], Takeshi Yoshitomi[1)] and Yukitoshi Izumi[2)]

(received 24 December 2013, accepted 9 January 2014)

[1)]Department of Ophthalmology, Akita University Faculty of Medicine, Akita, Japan
[2)]Department of Psychiatry, Washington University School of Medicine, St. Louis, MO. USA

Abstract

Purpose. To examine the involvement of nitric oxide (NO) in the development of ischemic retinal degeneration using glutamate receptor antagonists, inhibitors of nitric oxide synthetase (NOS), and NO donors.

Methods. Simulated ischemia was induced in the rat *ex vivo* retinal preparations by deprivation of oxygen and glucose from the incubation medium. The NMDA receptor antagonist (MK-801) and non-NMDA receptor antagonist (GYKI) were applied to the ischemic retinal preparations in combination with different types of NOS inhibitors. The neuroprotective effects of these agents were evaluated by morphologically and biochemically. In addition, the deteriorating actions of NO donors to the control and ischemic retina were also examined.

Results. Simulated ischemia produced severe acute neurodegeneration. This neurodegeneration was prevented by the combination of GYKI and MK-801. However, the NO donor induced severe retinal degeneration under the simulated ischemia, in spite of the co-administration of GYKI and MK-801. A neuronal NOS inhibitor offered substantial protection against neuronal damage combined with GYKI. Administration of NO donors invalidated the neuroprotective effects of MK-801and GYKI against the simulated ischemia.

Conclusions. The present study revealed that the NO release after activation of glutamate receptors seems to play an important role in the neuronal cell death induced by ischemia.

Key words : Retina ; simulated ischemia ; glutamate receptor antagonist ; nitric oxide synthetase inhibitors ; nitric oxide donor

Introduction

It has been known that both types of ionotrophic glutamate receptor, N-methyl-D-aspartate (NMDA) and non-NMDA receptor, are associated with the excitotoxity in the central nervous system. In the ischemia of the central nervous system, the NMDA receptors are activated followed by production of nitric oxide (NO). NO is considered to be involved in the development of ischemic degeneration[1-3]. In this context, there has been a series of reports concerning the neuroprotection by nitric oxide synthetase (NOS) inhibitors[4-7].

Similarly, NO is considered to play an important role in the ischemic degeneration of the retina[8]. It has been also reported the possibility that NOS inhibitors may block the ischemic changes in the retina[9,10]. However, it is still unclear whether NO release is mainly due to the activation of NMDA receptors, or in association with both types of receptor in the retina[11,12]. The activation of non-NMDA receptors induced the sodium ion influx,

Correspondence : Makoto Ishikawa, M.D.
Department of Ophthalmology, Akita University Graduate School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan
Tel : 81-18-834-1111
Fax : 81-18-836-2621
E-mail : jin@med.akita-u.ac.jp

which resulted in over-hydration of the cell body, disruption of the cell membrane, and finally death of retinal neurons[13]. Therefore, co-administration of NMDA and non-NMDA receptor antagonists seems necessary to protect the retina from deleterious effects by ischemia[14].

To examine the involvement of NO in the development of ischemic degeneration, we induced the simulated ischemia in the rat retinal *ex vivo* preparation by deprivation of glucose and oxygen. The neuroprotective effects of glutamate receptor antagonists and NOS inhibitors were examined in the simulated ischemia. In addition, the effects of nitric oxide donors to the control and ischemic retina were also examined.

Materials and methods

Animals

All experiments were performed in accordance with the guidelines of the Akita University Animal Study Committee. Male Sprague-Dawley rats obtained from Charies River Laboratories International Inc. (Wilmington, Mass) at postnatal date 30.

Chemicals

An NMDA type glutamate receptor antagonist dizocilpine (MK-801), and a non-NMDA type glutamate receptor antagonist GYKI52446 (GYKI), four different types of NOS inhibitors, NG-monomethyl-L-arginine acetate (NMMA), N(6)-(1-iminoethyl)-L-lysine (NIL), L-N5-(1-iminoethyl)-ornithine (NIO), and 7-Nitroindazole (7-NIA) were obtained from Sigma Chemical Co (St. Louis, MO). NMMA, NIL, NIO, and 7NIA were inhibitors against pan NOS, inducible NOS, endothelial NOS, and neuronal NOS, respectively. NO donor, 3-morpholinosydnonimine hydrochloride (SIN) and sodium nitroprusside (SNP), were purchased from Sigma Chemical Co.

Retinal preparation

Retinal segments were prepared from 30 (±2)-day-old Sprague-Dawley rats, using previously described methods[14]. Briefly, albino rats were anesthetized with halothane and decapitated. The eyes were carefully enucleated and placed in chilled artificial cerebrospinal fluid (aCSF) (medium). The aCSF contained (in mM) : 124 NaCl, 5 KCl, 2 MgSO4, 2 CaCl2, 1.25 NaH2PO4, 22 NaHCO3, 10 glucose, bubbled with 95% O_2-5% CO_2. After removal of the lens, iris, and vitreous, the empty eyecup was sliced into retinal segments.

Simulated ischemia was induced by deprivation of glucose from aCSF, and O_2 was replaced by N_2[15]. The *ex vivo* retinal preparations were incubated in the condition of simulated ischemia for 60 min.

MK-801 and GYKI were used at the concentration of 10 μM, and applied separately or simultaneously to the incubation medium for 60 min of simulated ischemia. L-NMNA (100 μM), NIL (1 mM), NIO (1 mM), and 7-NIA (100 μM) were also applied separately or in combination with glutamate receptor antagonists to the incubation medium for 60 min.

NO donor, SIN and SNAP, were used at the concentration of 500 μM, and also applied separately or in combination with glutamate receptor antagonists to the incubation medium for 60 min.

The severity of neuronal damage was assessed in each slice using a neuronal damage score (NDS). The NDS rates changes in retinal slices on a 0-4 scale with 0 signifying no neuronal damage and 4 indicating very severe damage. Criteria used in establishing the degree of neuronal damage included the extent of swelling of dendritic processes in the inner plexiform layer (IPL) and the number of neurons in the inner nuclear layer (INL) showing signs of severe cytoplasmic swelling and coarse clumping of nuclear chromatin. Based on prior experiments, retinal neurons do not recover from the latter changes[14].

LDH assays

LDH levels were determined from whole retina following gentle sonication using methods described previously[16]. Whole retinas were placed in an incubation chamber containing gassed aCSF for a 1-h recovery period following isolation. During an experiment, each preparation was incubated at 30°C for 60 min in individual 4 ml vials with caps (Fisher Scientific Hampton, NH, catalogue #03-339-26B). Each vial was gassed every 15 min. For ultrasonic manipulation, a Model 250 Sniffier with micro-tip (Branson Ultrasonics Corp., Danbury, CT) was used at minimal strength. Sonication pulses (100-ms in

duration) were delivered beneath the surface of the aCSF (5 mm) with the tissue located at the bottom of the vial. The 100-ms sonication pulses were repeated every 2 s. After sampling the aCSF, the tissue was washed and homogenized with a buffer containing 250 mM sucrose, 10 mM imidazole, and 10 mM KCl for determination of tissue protein concentrations (BioRad, Hercules, CA). LDH activity was determined from 50 μl samples with a Sigma LDH assay kit (Sigma Chemical Company, St. Louis, MO). Based on our prior studies, there is a good correlation between LDH levels and histological measures of neuronal damage[16].

Results

Compared with the control retina (Fig. 1a), the degeneration induced by simulated ischemia for 60 min was characterized by spongy appearance of the inner plexiform layer and the bull's eye formation of the inner nuclear layer (NDS 3.2±0.5, N=7, Fig. 1b).

To determine whether ischemic damage results from activation of glutamate receptor, MK-801, a noncompetitive NMDA receptor antagonist, and GYKI, a competitive non-NMDA receptor antagonist, were administered alone or in combination to retina exposed to 60-min simulated ischemia. As previously described[12], MK-801 did not inhibit the ischemic degeneration (NDS 3.0±1.5, N=3, Fig. 1c). GYKI alone was also not neuroprotective (NDS 2.8±0.9, N=3, Fig. 1d). However, when MK-801 and GYKI were combined, neuroprotection was observed against neuronal damage in the INL (NDS 0.9±0.8, N=3, Fig. 1e).

Attenuation of ischemic neuronal damage by glutamate receptor antagonists indicated that this damage was mediated by glutamate. Because NO release occurred downstream of activation of NOS and NMDA receptor, we attempted to examine the neuroprotective effects of NOS inhibitors combined with glutamate receptor antagonists in simulated ischemia. Each four types of NOS inhibitor (LMNA, NIL, NIO, and 7-NIA) alone did not blocked the retinal degeneration induced by simulated ischemia (data not shown).

Combination of each type of NOS inhibitor (LMNA [Fig. 2a], NIL [Fig. 2b], NIO [Fig. 2c], and 7-NIA [Fig. 2d]) and MK-801 did not prevent excitotoxic damage from simulated ischemia. NDS was calculated as 2.8±0.9 (N=8), 3.3±1.5 (N=7), 3.0±0.8 (N=6), and 2.9±1.1 (N=5), respectively.

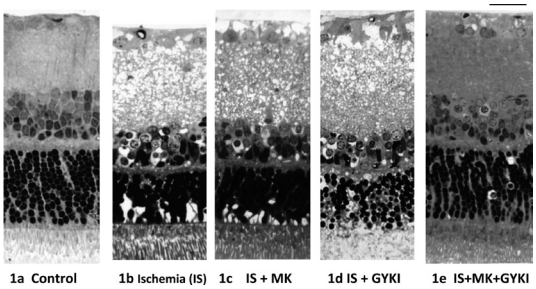

Fig. 1. a. Control retina incubated for 3 h in drug-free solution. b. Sixty min of simulated ischemia induces extreme swelling of the cell bodies with pyknosis in the inner nuclear layer accompanied by severe dendritic swelling. In addition, irregular spaces are observed in the outer nuclear layer. c. Retinas exposed to 60-min simulated ischemia treated with 10 μM MK-801 alone shows a similar irregular appearance in the ONL, along with neuronal and dendritic swelling in the INL and IPL. d. Similarly, 10 μM GYKI alone does not inhibit excitotoxic damage in a retina exposed to 60-min simulated ischemia. e. Combination of MK-801 and GYKI diminishes the neuronal changes in the INL and IPL, although the irregularity of photoreceptor cells in the ONL remains. Scale bar=30 μm

On the other hand, NOS inhibitor showed better neuroprotection against the simulated ischemia combined with GYKI. Combination of LMNA and GYKI offered substantial protection against neuronal damage (NDS 0.7±1.0, N=4, Fig. 3a). NIL (Fig. 3b) and NIO (Fig. 3c) failed to protect retinal neurons from simulated ischemia when co-administered with GYKI (NDS 2.9±0.8, N=7, and NDS 3.3±1.3, N=5, respectively). The neuronal damage was markedly diminished by co-administration of 7-NIA and GYKI offered substantial protection against neuronal damage (NDS 1.2±0.9, N=8, Fig. 3d).

To determine whether ischemic damage results from direct effect of NO, NO donors, SIN and SNP, were administered alone to the control retina (NDS 0.3±0.4, N=3 and NDS 0.5±0.3, N=3, respectively). While SIN (Fig. 4a) and SNP (Fig. 4b) did not induce ischemic degeneration in the control retina, the administration of

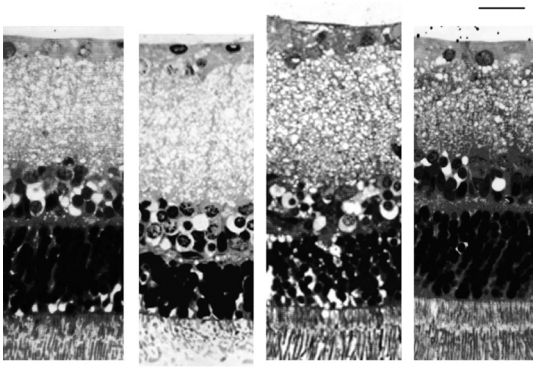

Fig. 2. a. Combination of pan-NOS inhibitor, LMNA, and MK-801 does not inhibit excitotoxic damage in a retina exposed to 60-min simulated ischemia. b. Retinas exposed to 60-min simulated ischemia treated with combination of inducible NOS inhibitor, NIL, and MK-801 show similar irregular appearance in the ONL, along with neuronal and dendritic swelling in the INL and IPL. c. Combination of endothelial NOS inhibitor, NIO, and MK-801 fails to offer neuroprotection under the simulated ischemia. d. Retinas exposed to 60-min simulated ischemia treated with combination of neuronal NOS inhibitor, 7-NIA, and MK-801 show similar retinal damage induced by 60-min simulated ischemia. Scale bar=25 μm.

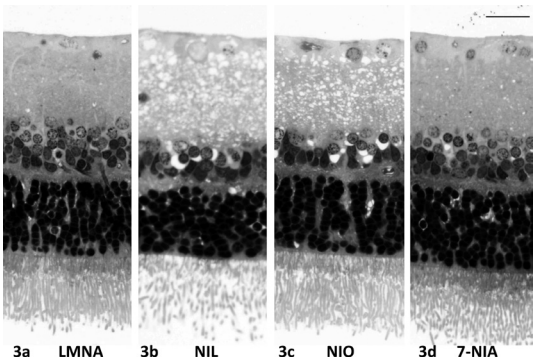

Fig. 3. a. Combination of LMNA and GYKI shows nearly complete neuroprotection against the simulated ischemia, although the irregularity of photoreceptor cells in the ONL remains. b. Combination of NIL and GYKI only partially blocks the ischemic degeneration. c. Combination of NIO and GYKI also shows partial attenuation of the excitotoxic damage in a retina exposed to 60-min simulated ischemia. d. Combination of 7-NIA and GYKI deceases the retinal damage in the IPL and the INL. However, this combination does not prevent the irregular appearance in the ONL. Scale bar=25 μm.

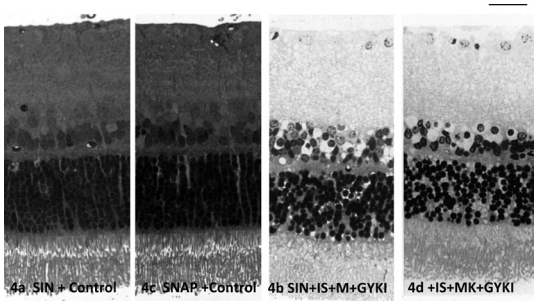

Fig. 4. a. SIN, NO donor, does not induce excitotoxic damage in the control retina. b. Administration of SNP does not alter morphology in the normal condition. c. While SNP does not induce the excitotoxic damage in the control retina, neural damage is clear when administration of SIN is combined with simulated ischemia in the presence of MK-801 and GYKI. d. Administration of SNP shows deleterious effects in the simulated ischemia-induced retina, when combined with simulated ischemia in the presence of MK-801 and GYKI. Scale bar=30 μm.

Table 1. LDH release from the retinal preparation. In four sets of experiments, LDH levels were determined after 60 min incubation of whole retina using gentle sonication as described in the Methods. Retinas were exposed to 60 min silulated ischemia (IS) in the presence of GYKI or GYKI plus 7-NIA. Data are mean±S.E.

Condition	*n*	LDH U/mg protein
Control	5	5±4
Simulated ischemia (IS)	6	206±59
IS + MK801	7	204±69
IS + GYKI	5	189±45
IS + MK801 + GYKI	7	*82±32
IS + GYKI + 7-NIA	5	*75±45
IS + MK801 + GYKI + SNAP	6	199±75

Asterisks mean the significant reduction of LDH ($p<0.001$) compared with simulated ischemia.

both NO donors (Fig. 4c and d) overcame the neuroprotection of MK-801 and GYKI to the retina treated with simulated ischemia (NDS 3.2±0.6, $N=3$ and NDS 3.5±1.3, $N=3$, respectively).

To qualify retinal degeneration under ischemic conditions, we assayed LDH release into the media using previously described methods[16]. As shown in Table 1, LDH release 60 min after simulated ischemia was not

blocked by GYKI alone. Consistent with our histology experiments, co-administration of MK-801 and GYKI or 7-NIA and GYKI significantly diminished the LDH release ($p<0.001$).

Discussion

The present study demonstrated that the simulated ischemia induced the degeneration characterized by spongy appearance of the inner plexiform layer and the bull's eye formation in the inner nuclear layer. Such histological appearance seems corresponding to that induced by the application of non-NMDA antagonist, kainite, to the normal *ex vivo* retinal preparation[14]. While NMDA itself does not usually induce the excitotoxicity in the adult rat[14,17], the involvement of NMDA receptor in the development of ischemic degeneration is considered, because anti-NMDA receptor antagonist is necessary to block the ischemic degeneration[18].

The present study revealed that the co-administration of NMDA receptor antagonist (MK801) and non-NMDA antagonist (GYKI) blocked the ischemic degeneration of the retina. These results indicate that glutamate may induce excitotoxicity in the ischemic retina via both NMDA and non-NMDA receptors.

It has been known that the activation of NMDA receptor opens calcium ion channels in the post-synaptic membrane, and increases the intracellular concentration of calcium ion, which induces a series of cellular events involving the NO release[19]. In the hippocampus, which is the most vulnerable portion to ischemia in the central nervous system, NO release after activation of the NMDA receptor is involved in the cellular death by ischemia[3]. It has been shown that NO is produced by induction of NOS after activation of NMDA receptors[20], and involved in the ischemic neuronal death in the retina[21,22]. Therefore, NOS is the key enzyme for the development of ischemic degeneration. There are three types of NOSs (iNOS, eNOS, and nNOS), in which iNOS was reported to be concerned to the development of ischemia[22]. Although there are few reports concerning the involvement of NOS to the development of ischemic degeneration in last decades[24], the immediate activation of nNOS under the ischemia has been recently reported[25].

Our present results that nNOS inhibitor, 7NIA, demonstrated the neuroprotective effect against the simulated ischemia under the presence of GYKI, indicate the involvement of NOS to the development of retinal ischemia. These results also indicated that nNOS is induced after activation of NMDA receptor. In addition, the present results that NO donors overcome the neuroprotective effects of glutamate receptor antagonists suggest that NO release may be apparently involved in the development of ischemic neural death.

In the present study, the most effective NOS inhibitor against the simulated ischemia was nNOS inhibitor. The similar protective effect by LMNA, pan NOS inhibitor, seems due to containing nNOS activity. These results were corresponding to the previous reports of the ischemia in the central nervous system[26,27]. On the other hand, iNOS inhibitor and eNOS inhibitor only showed weak protection against the ischemic degeneration.

However, the present results were due to the acute experiments. The other conclusions, in which iNOS or eNOS are associated with ischemia, might be considered, when the chronic experiment is adopted using ischemia-reperfusion model.

NO donor, sodium nitroprusside (SNP), has been used in the treatment of malignant hypertension[28]. Although SNP induce the degeneration in the hippocampus[29], there are no report of the retinal degeneration by SNP[30]. In fact, our present study did not reveal the toxicity of NO donor in the single administration. However, the present results suggest the possibility that NO donor might induce the toxic effect against the retina under the ischemia. It should be careful to use NO donor including SNP in the retinal ischemia such as diabetic retinopathy, retinal vein occlusion, or retinal artery occlusion. Although it was not confirmed the target of NO in the ischemic retina, the risk of abuse of vasodilators with NO release effect should be mentioned in concern with the retinal protection.

The present study revealed that the activation of both types of glutamate receptors, NMDA and non-NMDA receptors, were involved in the development of retinal ischemic degeneration. The activation of neuronal NOS also associated with ischemic neural death.

References

1) Dawson, V.L., Dawson, T.M., London, E.D., Bredt, D.S. and Snyder, S.H. (1991) Nitric oxide mediates glutamate neurotoxicity in primary cortical culture. *Proc. Natl. Acad. Sci. USA*, **88**, 6368–6371.
2) Izumi, Y., Benz, A.M., Clifford, D.B. and Zorumski, C.F. (1996) Nitric oxide inhibitors attenuate ischemic degeneration in the CAI region of rat hippocampal slices. *Neurosci. Lett.*, **210**, 157–160.
3) Trindade, V.M., Daniotti, J.L., Raimondi, L., Chazan, R., Netto, C.A. and Maccioni, H.J. (2001) Effects of neonatal hypoxia/ischemia on ganglioside expression in rat hippocampus. *Neurochem. Res.*, **26**, 591–597.
4) Wallis, R.A., Panizzon, K.L. and Gircard, J.M. (1996) Traumatic neuroprotection with inhibitors of nitric oxide and ADP-ribosylation. *Brain Res.,* **710**, 169–177.
5) Centeno, J.M., Oriti, M., Salom, J.B., Sick, T.J. and Perez-Pinzon, M.A. (1999) Nitric oxide is involved in anoxic preconditioning neuroprotection in rat hippocampal slices. *Brain Res.*, **836**, 62–69.
6) Hicks, C.A., Ward, M.A., Swettenham, J.B. and O'Neill, M.J. (1999) Synergistic neuroprotective effects by combining an NMDA or AMPA receptor antagonist with nitric oxide synthase inhibitors in global cerebral ischemia. *Eur. J. Pharmacol.*, **381**, 113–119.
7) Zhao, H.G., Sun, X.C., Xian, X.H., Li, W.B., Zhang, M. and Li, O.J. (2007) The role of nitric oxide in the neuroprotection of limb ischemic preconditioning rats. *Neurochem. Res.*, **32**, 1919–1926.
8) Osborne, N.N., Barnett, N.L. and Herrera, A.J. (1993) NADPH diaphorase localization and nitric oxide synthase activity in the retina and anterior uvea of the rabbit eye. *Brain Res.*, **610**, 194–198.
9) Okuno, T., Oku, H., Sugiyama, T., Yang, Y. and Ikeda, T. (2002) Evidence that nitric oxide is involved in autoregulation in optic nerve head of rabbits. *Invest. Ophthalmol. Vis. Sci.*, **43**, 784–789.
10) Neufeld, A.H., Kawai, S., Das, S., Vora, S., Gachi, E., Connor, J.R. and Manning, P.T. (2002) Loss of retinal ganglion cells following retinal ischemia : the role of inducible nitric oxide synthase. *Exp. Eye Res.*, **75**, 521–528.
11) Sucher, N.J., Lipton, S.A. and Dreyer, E.B. (1997) Molecular basis of glutamate toxicity in retinal ganglion cells. *Vision Res.*, **37**, 3483–3493.
12) Izumi, Y., Hammerman, S.B., Kirby, C.O., Benz, A.M., Olney, J.W. and Zorumski, C.F. (2003) Involvement of glutamate in ischemic neurodegeneration in isolated retina. *Vis. Neurosci.,* **20**, 97–107.
13) Zeevalk, G.D. and Nicklaus, W.J. (1991) Mechanisms underlying initiation of excitotoxicity associated with metabolic inhibition. *J. Pharmacol. Exp. Ther.*, **257**, 870–878.
14) Izumi, Y., Benz, A.M., Kirby, C.O., Labruyere, J., Zorumski, C.F., Price, M.T. and Olney, J.W. (1995) An ex vivo *rat retinal preparation for excitotoxicity studies. J. Neurosci. Methods*., **60**, 219–225.
15) Romano, C., Price, M.T., Almli, T. and Olney, J.W. (1998) Excitotoxic neurodegeneration induced by deprivation of oxygen and glucose in isolated retina. *Invest. Ophthalmol. Vis. Sci.,* **39**, 416–423.
16) Izumi, Y., Izumi, M., Benz, A.M. and Zorumski, C.F. (2001) Lactate dehydrogenase release is facilitated by brief sonication of rat hippocampal slices and isolated retinas following acute neuronal damage. *J. Neurosci. Methods.,* **108**, 49–55.
17) Siliprandi, R., Canella, R., Carmignoto, G., Schavo, N., Zenellato, A., Zanoni, R. and Vantini, G. (1992) N-Methyl-D-aspartate-induced neurotoxicity in the adult rat retina. *Vis. Neurosci*., **8**, 567–573.
18) Adachi, K., Kashii, S., Masai, H., Ueda, M., Morizane, C., Kaneda, K., Kume, T., Akaike, A. and Honda, Y. (1998) Mechanism of the pathogenesis of glutamate neurotoxicity in retinal ischemia. *Graefe's Arch. Clin. Ophthalmol*., **236**, 766–774.
19) Hoyt, K.R., Tang, L.-H., Aizenman, E. and Reynolds I.J. (1992) Nitric oxide modulates NMDA-induced increases in intracellular Ca^{2+} in cultured rat forebrain neurons. *Brain Res.*, **592**, 310–316.
20) Vorwerk, C.K., Hyman, B.T., Miller, J.W., Husain, D., Zurakowski, D., Huang, P.L., Fishman, M.C. and Dreyer, E.B. (1997) The role of neuronal and endothelial nitric oxide synthase in retinal excitotocity. *Invest. Ophthalmol. Vis. Sci.,* **38**, 2038–2044.
21) Morizane, C., Adachi, K., Furutani, I., Fujita, Y., Akaike, A., Kashii, S. and Honda, Y. (1997) N-nitro-L-arginine methyl ester protects retinal neurons against N-methyl-D-aspartate-induced neurotoxicity in vivo. *Eur. J. Pharmacol*., **328**, 45–49.

22) Osborne, N.N., Casson, R.J., Wood, J.P., Chidlow, G., Graham, M. and Melena, J. (2004) Retinal ischemia : mechanisms of damage and potential therapeutic strategies. *Prog. Retin. Eye Res*., **23**, 91–147.

23) Kobayashi, M., Kuroiwa, T., Shimokawa, R., Okeda, R. and Tokoro, T. (2000) Nitric oxide synthase expression in ischemic rat retinas. *Jpn. J. Ophthalmol*., **44**, 235–244.

24) Toda, T. and Nakanishi-Toda, M. (2007) Nitric Oxide : ocular blood flow, glaucoma, and diabetic retinopathy. *Prog. Retin. Eye Res.*, **26**, 205–238.

25) Choi, J.S., Kim, D., Hong, Y.M., Mizuno, S. and Joo, C.K. (2006) Inhibition of nNOS and COX-2 expression by lutein in acute retinal ischemia. *Nutrition,* **22**, 668–671.

26) Yoshida, T., Limmroth, V., Irikura, K. and Moskowitz, M.A. (1994) The NOS inhibitor, 7-nitroindazole, decreases focal infarct volume but not the response to topical acetylcholine in pial vessels. *J. Cereb. Blood Flow Metab*., **14**, 924–929.

27) Kelly, P.A.T., Ritche, I.M. and Arbuthnott, G.W. (1995) Inhibition of neuronal nitric oxide synthase by 7-nitroindazole : effects upon local cerebral blood flow and glucose use in the rat. *J. Cereb. Blood Flow Metab*., **15**, 766–773.

28) Curran, M.P., Robinson, D.M. and Keating, G.M. (2006) Intravenous nicardipine : its use in the short-term treatment of hypertension and various other indications. *Drugs*, **66**, 1755–1782.

29) Izumi, Y., Benz, A.M., Clifford, D.B. and Zorumski, C.F. (1993) Neurotoxic effect of sodium nitroprusside in rat hippocampal slices. *Exp. Neurol.*, **121**, 14–23.

30) Levy, H., Twig, G. and Perlman, I. (2004) Nitric oxide modulate the transfer function between cones and horizontal cells during changing conditions of ambient illumination. *Eur. J. Neurosci*., **20**, 2963–2974.