Retinal Neurotoxicity of Nitric Oxide Donors With Different Half-Life of Nitric Oxide Release: Involvement of N-Methyl-d-aspartate Receptor

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Abstract. We compared the degree of neurotoxic outcome in the retina exposed to three nitric oxide (NO) donors with different half-life of NO release. Intravitreal injection of NO donors resulted in a significant decrease in cell density in the ganglion cell layer and thinning of the inner plexiform layer in a half-life time-dependent manner. Concurrent injection of an NO-trapping reagent with an NO donor abolished NO donor-induced retinal damage. (+)-MK-801 also prevented NO-induced retinal damage, indicating that N-methyl-d-aspartate receptors are partly involved in NO-induced neurodegeneration. These results may be relevant to a pathogenic role of NO – glutamate receptor in several ophthalmic disorders.

Keywords: nitric oxide, neurodegeneration, retina

Elevation of intraocular glutamate levels followed by glutamate receptor-mediated excitotoxicity is regarded as one of the important mechanisms in the pathogenesis of neurodegenerative diseases such as diabetic retinopathy and optic neuropathy (1, 2). Abnormal levels of glutamate are likely to cause extensive neurodegeneration via stimulation of N-methyl-d-aspartate (NMDA) receptors, which recruits a series of events including activation of nitric oxide (NO) synthase (NOS). In fact, several neurons of the retina contain NOS, and NOS inhibitors block NMDA-induced retinal damage and ischemic injury (3–5). It has been also reported that NOS expression is enhanced in the ischemic retina (6). Therefore, NO appears to play an important role in the retinal neurotoxicity mediated by NMDA receptors. Recently, we have reported that intravitreal injection of 1-hydroxy-2-oxo-3-(3-aminopropyl)-3-isopropyl-1-triazene (NOC 12), an NO donor which requires no co-factors and has no biological activity in itself (7), induces retinal damage in rats (8). In the present study, we investigated the correlation between the degree of retinal damage and time course of NO release by NO donors, using three donors with different half-life of NO release.

All experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Adult male Sprague-Dawley rats (7-week-old; Nihon SLC, Hamamatsu) were anesthetized by an intraperitoneal injection of pentobarbital sodium (50–80 mg/kg) and then given 1% atropine sulfate drops to the right eye to produce full dilation of the pupil (8). A single intravitreal injection of NO donors (400 nmol in 5 μl of sodium phosphate buffer (PB) containing 0.03 M NaOH) was given to the right eye, through a 33-gauge needle connected to a 25-μl Hamilton syringe. The following NO donors and an NO-trapping reagent (all from Dojindo Laboratories, Kumamoto) were used: 1-hydroxy-2-oxo-3-(3-aminoethyl)-3-ethyl-1-triazene (NOC 12), an NO donor which requires no co-factors and has no biological activity in itself (7), induces retinal damage in rats (8). In the present study,
phosphate-buffered saline, pH 7.4, 22°C and 1260 min, 37°C), and 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazole-1-oxyl-3-oxide (carboxy-PTIO). Drugs were injected slowly over a period of 1 min. The control group of rats received 5 µl of PB containing 0.03 M NaOH to the right eyes. Seven days after intravitreal injection, the animals were killed and both eyes were enucleated. Eyes were immediately fixed in phosphate-buffered 4% formalin and 1% glutaraldehyde aqueous solution (pH 7.4), followed by phosphate-buffered 10% formalin solution (pH 7.4). After fixation, the eyes were embedded in paraffin and sliced into six horizontal meridional sections (5-µm-thick) through the optic disk of each eye. The sections were then stained with hematoxylin and eosin. A microscopic image of each section within 1 mm of the optic disk was scanned digitally with the aid of an image analysis system including a 3CCD camera module (XC-009; Nexus, Tokyo) and an image analysis processor (nexusQube, Nexus). For assessment of the degree of injury for each eye, the number of cells in the ganglion cell layer (GCL) was counted for a 1-mm range from the optic disk to obtain a linear cell density (cells/mm). The thickness of the inner plexiform layer (IPL) and inner nuclear layer (INL) was measured at three randomly-selected points with image analysis software (NIH image, W. Rasband). Finally, the thickness of IPL and INL and the linear cell density in the GCL were expressed as the mean of nine measurements. For each animal, values of the parameters for the right eye were expressed as a ratio (%) to those for the intact left eye. No attempt was made to distinguish cell types in the GCL for counting the cell number. Values were expressed as the means and S.E.M. for 3–8 animals. Statistical analyses were performed with Dunnett’s test, and differences were considered significant at \( P<0.05 \).

To date, two reports demonstrated in vivo retinal toxicity of NO donors by intravitreal injection of S-nitro-L-acetyl-DL-penicillamine (SNAP) and NOC 12 (8, 9). We have recently shown that intravitreal injection of NOC 12 (400 nmol) resulted in significant cell loss in the GCL and thinning of IPL, and expended NOC 12 that had undergone incubation for 7 days at 37°C exerted no effects either on cell density in the GCL or on the thickness of IPL (8). Another study reported that intravitreal injection of SNAP, an NO donor, resulted in a decrease in cell density in the GCL and INL of albino rabbit retina (9). However, several other studies performing SNAP treatment in pigmented rabbit eye and performing sodium nitroprusside in the rat eye failed to demonstrate any retinal damage (10, 11). These discrepant results may be due to the differences of NO donors and species of animals. To ascertain whether retinal neurotoxicity of NOC 12 observed in our previous study resulted from NO release, we performed concurrent intravitreal injection of an NO-trapping reagent carboxy-PTIO with NOC 12. Intravitreal injection of NOC 12 (400 nmol) resulted in a significant cell loss in the GCL and thinning of IPL but not of INL (Fig. 1), which was in good agreement with the results in our previous study (8). Carboxy-PTIO (400 nmol) completely prevented NOC 12-induced retinal damage (Fig. 1), indicating that NOC 12-induced retinal damage is attributable to the action of NO released from the compound. Notably, the inner retinal layers selectively injured by the NO donor correspond to the area susceptible to glutamate toxicity as well as the area of NOS localization (3–6).

Next, we compared the effects of three NO donors with different half-life of NO release to assess whether the rates of NO release from NO donors are correlated with the degree of retinal neurotoxic outcome. As was the case with NOC 12, intravitreal injection of NOC 5 (400 nmol) resulted in marked cell loss in the GCL and thinning of IPL (Fig. 2: A, B-1, C-1). On the other hand, NOC 18 (400 nmol) caused a small but significant decrease in the cell density in the GCL, but exerted no effects on the thickness of IPL (Fig. 2: A, B-1, C-1). No histological alterations were observed following intravitreal injection of the vehicle solution. NO donors with a short-life time, such as NOC 12 and NOC 5, appeared to be effective in inducing retinal damage (Fig. 2: B-2, C-2). Hence, these results suggest that the amount of NO released during intraocular retention of
Fig. 2. Morphometric analysis of NO donor-induced retinal damage on day 7 of posttreatment. Light micrographs of representative retinal sections from eyes that received an injection of vehicle, NOC 5 (400 nmol), NOC 12 (400 nmol), and NOC 18 (400 nmol) (A). Scale bar = 20 μm. Intravitreal injection of NO donors (400 nmol) results in cell loss in the GCL (B-1) and thinning of IPL (C-1). There were moderate correlations between the half-life of NO release from NO donors and the degree of retinal damages in the GCL (B-2) and IPL (C-2). Closed triangles, diamonds and circles in B-2 and C-2 represent the results of NOC 5, NOC 12, and NOC 18-treated retina. Each value represents the average for a group of 6 – 8 rats, and vertical bars represent S.E.M. *P<0.01 vs vehicle-treated control group.
NO donors is a critical determinant of the degree of retinal neurotoxic outcome, on the basis of the assumption that the intravitreal retention time of these NO donors is equal.

Additionally, we investigated whether NO-induced retinal neurotoxicity is affected by blockade of the NMDA receptor. Treatment with an NMDA-receptor antagonist (+)-MK-801 (10 mg/kg, s.c.; Sigma, St. Louis, MO, USA) 30 min before intravitreal injection of NMDA (200 nmol; Nacalai Tesque, Kyoto) completely prevented retinal cytotoxicity induced by NMDA (Fig. 3A). Treatment with (+)-MK-801 in a similar manner before NOC 12 injection partially but significantly inhibited NO-induced retinal damage (Fig. 3B), suggesting that NO-induced neuronal damage was mediated in part by activation of the NMDA receptor. Involvement of NMDA receptors in NO-induced cell death has not been hitherto demonstrated in retinal neurons, although protective effects of (+)-MK-801 against NO-induced neurotoxicity have been reported in neuron–glia co-cultures prepared from the cerebral cortex and the cerebellum (12, 13). In addition, NO has been reported to induce glutamate release in neuron–glia co-cultures (12) and in the striatum in vivo (14). Therefore, NO may be able to elicit release of glutamate in the retina, which in turn activates NMDA receptor and stimulates further formation of reactive nitrogen species via NOS, leading to mitochondrial dysfunction and neuronal death. Possible involvement of secondary release of glutamate from dead surrounding cells should also be taken into consideration.

In conclusion, the present results support the notion that NO plays a pathogenic role in degenerative retinal diseases and suggest that NO–glutamate pathways have a role in the neurodegenerative process. Under pathological conditions, NO is produced by several forms of NOS. The constitutive NOS releases NO for short periods of time, whereas the inducible NOS releases NO for extended periods of time. The use of NO donors with different half lives of NO release may provide valuable information concerning the mechanisms that underlie retinal neurotoxicity mediated by different NOS isoforms in several ophthalmic disorders.

References

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