LABORATORY INVESTIGATIONS

Autoantibody Against Neuron-Specific Enolase Found in Glaucoma Patients Causes Retinal Dysfunction In Vivo

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Purpose: In our recent paper, we have reported the presence of serum autoantibody against neuron-specific enolase (NSE) in patients with glaucoma. The purpose of the present study was to investigate further the pathological effects of anti-NSE antibody on retina by comparing them with the effects induced by N-methyl-D-aspartate (NMDA).

Methods: Either a glaucoma patient’s serum or purified anti-NSE antibody, or 10–40 mM NMDA was intravitreously administered into Lewis rat eyes, and electrophysiological, histopathological, and biochemical evaluations were performed. In addition, the neuroprotective effects of anti-glaucoma drugs, such as timolol, betaxolol, nipradilol, and isopropyl unoprostone, and a calcium antagonist were also studied using these animal models.

Results: Electron microscopy revealed that intravitreal administration of a glaucoma patient’s serum, which immunoreacted with retinal 50 kDa in Western blot analysis, and purified anti-NSE antibody induced retinal ganglion cell apoptosis in rat eyes. Functionally, these eyes showed a significant decrease in electroretinogram (ERG) responses and a remarkable decrease in rhodopsin phosphorylation reaction. These changes were comparable to the effects observed after the intravitreal administration of 20 mM NMDA. Co-administration of nipradilol, an β1- and β2-blocker, with anti-NSE antibody or 20 mM NMDA caused marked recovery of the affected ERG responses within 2 weeks. In contrast, administration of timolol or betaxolol showed no recovery effect on the ERG responses. Among these drugs, only betaxolol showed a recovery effect on NMDA-induced decrease of rhodopsin phosphorylation. Nilvadipine functioned beneficially on both impaired ERG and rhodopsin phosphorylation reactions observed in rat eyes injected intravitreously with anti-NSE antibody or NMDA. These effects of nilvadipine were not changed by the addition of endothelin-1. In contrast, isopropyl unoprostone had no effect on these functions.

Conclusion: These observations suggest that serum autoantibody against NSE found in some patients with glaucoma induces retinal dysfunction in vivo, similarly to NMDA.

Key Words: Apoptosis, autoantibody, glaucoma, retinal ganglion cell.

Introduction

Glaucoma optic neuropathy is characterized by loss of retinal ganglion cells and their axons, an excavated appearance of the optic nerve head, and progressive loss of visual field sensitivity. With regard to the etiology of glaucoma, retinal ganglion cell death by apoptosis has been suggested as a cause based upon histopathological studies of human eyes with primary open angle glaucoma (POAG), neovascular glaucoma, and experimental glaucoma models with elevated intraocular pressure (IOP). In terms of the molecular mechanism triggering the apoptosis, several mechanisms, such as deprivation...
of neurotrophic factors, ischemia, chronic elevation of glutamate, and disorganized nitric oxide (NO) metabolism have been proposed. Recently Wax et al and his associates reported that autoantibodies against rhodopsin, 60-kDa heat shock protein (hsp 60), 27-kDa heat shock protein (hsp 27), and α-crystallin were identified in serum from glaucoma patients. Therefore, they suggested that these autoimmune reactions toward retinal components may be related to the apoptotic cell death process in some glaucoma patients, particularly in patients with normal tension glaucoma (NTG). We independently have found the presence of the serum autoantibody against neuron-specific enolase (NSE), detected by Western blot analysis in 20 of 79 patients with glaucoma (NTG, 23 cases; POAG, 56 cases). Clinically, maximum IOP in the serum antibody-positive patients was significantly lower than that in the serum antibody-negative patients. However, no statistical differences were observed in visual field loss, disc cupping, or other clinical factors. Injection of a patient’s serum or purified anti-NSE antibody into the vitreous cavity of Lewis rats caused slight reduction of the b-wave in electroretinography (ERG) and increase of TUNEL (TdT-dUTP terminal nick-end labeling)-positive staining within the retinal ganglion cells. Therefore, based upon these results, we suggested that the serum autoantibody against NSE might be one of the risk factors for retinal ganglion cell death in glaucoma.

Herein, to study further the effects of anti-NSE antibody on the retina, we injected either anti-NSE antibody-positive patient’s serum or purified anti-NSE antibody into the vitreous cavity of Lewis rats caused slight reduction of the b-wave in electroretinography (ERG) and increase of TUNEL (TdT-dUTP terminal nick-end labeling)-positive staining within the retinal ganglion cells. Therefore, based upon these results, we suggested that the serum autoantibody against NSE might be one of the risk factors for retinal ganglion cell death in glaucoma.

Materials and Methods

The studies were performed in accordance with the guidelines set by Hiroasaki University and the Declaration of Helsinki on Biomedical Research Involving Human Subjects. The protocols were approved by the Clinical Research Ethics Board of the University of Hiroasaki General Hospital and this institution’s Committee for the Protection of Human Subjects. All experimental procedures were designed to conform to both the ARVO statements governing the Use of Animals in Ophthalmic and Vision Research and our own institution’s guidelines.

Vitreous Injection of Antibodies

Six-week-old Lewis rats (approximately 180 g) reared in cyclic light conditions (12 hours on/12 hours off) were used. Anesthesia of rats was performed by intramuscular injection with a mixture of ketamine (80–125 mg/kg) and xylazine (9–12 mg/kg) as described by Ohguro et al. 14 To anesthetized rats, a total of 10 μL phosphate-buffered saline (PBS) solution containing anti-NSE antibody (5 μL), 10–40 mM NMDA (5 μL), and/or anti-glaucoma drug (5 μL) (betaxolol, 1 pM–1 nM; nipradilol, 2.5 pM–1 nM; desnitro-nipradilol, 2.5 pM; timolol, 0.1–10 nM) were injected into the vitreous cavity of the eyes as described by Ohguro et al. 14 Two weeks after the treatment, ERG analysis and rhodopsin phosphorylation were carried out as described below. In each experimental condition, ERG was separately measured in 8 eyes and rhodopsin phosphorylation was performed in triplicate. Animals showing apparent traumatic changes following vitreous injection, such as cataract, vitreous hemorrhage, and/or retinal detachment were excluded from the present study. After the treatment, a drop of ofloxacin was administered to avoid infection.

Purified anti-NSE antibody was purchased from UltraClone (Wellow, UK). Betaxolol, nipradilol, and its inactive compound (desnitro-nipradilol) were obtained from Alcon (Ft. Worth, TX, USA) and Kowa (Nagoya), respectively. Timolol was purchased from Sigma (St Louis, MO, USA). Nilvadipine [5-isopropyl 3-methyl 2-cyano-6-methyl-4-(3-nitrophenol)-1, 4-dihydro-3,5-pyridinedicarboxylate] (Fujisawa, Osaka) was dissolved in a mixture of ethanol:polyethylene glycol 400:distilled water (2:1:7) at a concentration of 0.1 mg/mL, diluted twice with physiological saline before use, and injected intraperitoneally (0.5 mL/kg) into anesthetized rats twice a week for 2 weeks. Isopropyl unoprostone or its vehicle solution (Fujisawa) was administered topically into rat eyes twice a day for 2 weeks.

Light and Electron Microscopy

Two weeks after administration of 5 μL of anti-NSE antibody-positive glaucoma patient’s serum
into Lewis rat eyes, animals were transcardially perfused under anesthesia with a total of 100 mL of 82 mM sodium phosphate buffer (pH 7.2) containing 4% paraformaldehyde. Enucleated eyes were fixed in 10% glutaraldehyde and 2.5% formalin in 0.15 M phosphate buffer solution (pH 7.2) for several days. The retinochoroidal tissues adjacent to the pupil-optic nerve block at the posterior region were cut in five to seven small pieces, postfixed in 1.0% osmic acid and embedded in epoxy resin. These sections were stained with Azur for light microscopy. Ultrathin sections double-stained with uranium-lead were examined by electron microscopy. After obtaining informed consent, a blood sample was collected from a 74-year-old woman with POAG, and immediately subjected to serum separation. Her detailed clinical data were described in our previous report.15

Electroretinography

The amplitudes of a- and b-waves on ERG measurement of rats were determined as described by Ohguro et al.14 Briefly, the anesthetized animals were kept in dark adaptation for at least 1 hour in an electrically shielded room. The pupils were dilated with drops of 0.5% tropicamide. The scotopic ERG response was recorded with a contact electrode equipped with a suction apparatus to fit on the rat cornea (Kyoto Contact Lens, Kyoto). A grounding electrode was placed on an ear. Responses evoked by white flashes (3.5 × 10^2 lux, 200 ms duration) were recorded by an ERG recording instrument (Neuropack, MES-3102, Nihon Kohden, Tokyo). The a-wave amplitude was determined from the baseline to the bottom of the a-wave. The b-wave amplitude was determined from the bottom of the a-wave to the top of the b-wave.

Rhodopsin Phosphorylation

Rhodopsin phosphorylation was studied as a retinal photoreceptor function using isolated rod outer segments from rat eyes as described by Ohguro et al16 with some modifications. Briefly, after dark adaptation of enucleated eyeballs (4–6 eyes for each condition) for 1 hour on ice, retinas were dissected and homogenized in 0.5 mL of 45% sucrose in buffer A (100 mM Na-phosphate buffer, pH 7.2, containing 5 mM MgCl_2). After centrifugation at 10,000 g for 5 minutes, the supernatant was diluted twice with buffer A and centrifuged again at 10,000 g for 5 minutes. The pellet was dissolved in 200 μL of buffer B containing 0.5 mM [γ-32P]ATP (100 cpm/nmol) and incubated at 30°C for 5 minutes under a 100-W lamp from a distance of 10 cm. The reaction was terminated by the addition of buffer B (200 mM Na-phosphate buffer, pH 7.2, containing 5 mM adenosine, 100 mM KF, and 200 mM EDTA) and the mixture

Figure 1. Light micrograph (Azur staining) of rat retina after intravitreal administration of glaucoma patient’s serum. Two weeks after administration of 5 μL of anti-neuron-specific enolase (NSE) antibody-positive glaucoma patient’s serum into Lewis rat eyes (4 rats, 8 eyes). Pyknosis of retinal ganglion cell (arrows) is seen in ganglion cell layer.
was centrifuged at 10,000 g for 5 minutes. The pellet was dissolved in 50 μL of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer and analyzed by SDS-PAGE using a 12.5% gel. The gel was stained and de-stained with Coomassie Blue and dried, followed by autoradiography. The band corresponding to rhodopsin was cut out, dissolved with 0.5 mL of H₂O₂, and the radioactivity was counted in a scintillation cocktail.

**Statistical Analysis**

The data are shown as mean ± SD. *P* values < .05 were considered significantly different from controls as assessed by a two-paired Student *t* test.

**Results**

Specimens of retina and choroid after intravitreal administration of glaucoma patient’s serum or purified anti-NSE antibody showed almost the same findings in light micrographs and electron micrographs. Pyknosis of retinal ganglion cells was found by Azur staining at the posterior region in all eyes (Figures 1 and 3). Electron microscopic examination of the pyknotic retinal ganglion cells showed two types of apoptotic findings. In the first type, the nucleus was malformed and segmented; chromatin was still concentrated in the nucleus. There was less cytoplasm and the organelles were significantly reduced (Figure 2). In the second type, a fragmented nucleus, the apoptotic body, was surrounded by Müller cells and axons (Figure 4).

Next, to study the pathologic effect of anti-NSE antibody on retinal function, its effect on retina was compared with NMDA-treated rat retina. Five microliters of PBS containing purified anti-NSE antibody or 10–40 mM NMDA was injected intravitreally in Lewis rat eyes, and the retinal functions were then evaluated. In ERG responses, eyes injected with NMDA showed a significant decrease in the amplitude of b-waves in a concentration-dependent manner when compared with eyes injected with PBS (Figure 5, upper graph). Amplitude of a-waves showed a greater decrease than that of b-waves, data not shown. In addition, anti-NSE antibody also caused a significant decrease in amplitude of the ERG b-wave, and this effect was almost identical with that of 20 mM NMDA. To further study the effects of NMDA and anti-NSE antibody on retina, rhodopsin phosphorylation, which is known to be the most critical reaction controlling the phototransduction pathway in retinal photoreceptors, was examined as a func-

![Figure 2](image-url) **Figure 2.** Electron micrograph of inner layer of retina after intravitreal administration of glaucoma patient’s serum. Two weeks after administration of 5 μL of anti-NSE antibody-positive glaucoma patient’s serum into Lewis rat eyes (4 rats, 8 eyes). Ganglion cell (GC) on left is intact; nucleus (*) of ganglion cell on right is malformed and segmented. V: vitreous.
tion of retinal photoreceptors. The levels of rhodopsin phosphorylation were significantly decreased in NMDA-treated eyes, in a dose-dependent manner. Such inhibition of rhodopsin phosphorylation was also observed in anti-NSE antibody-treated eyes and this effect corresponded to the effect with 20 mM NMDA administration (Figure 5, lower graph). Therefore, these data demonstrate that the effect of anti-NSE antibody on rat retina was functionally identical with that of NMDA.

Using these animal models, we evaluated the neuroprotective effects of anti-glaucoma drugs such as timolol, betaxolol, and nipradilol co-administered with either 20 mM NMDA or anti-NSE antibody, and their effects on the retina were examined. In ERG measurement, retinal dysfunction obtained by 20 mM NMDA or anti-NSE serum was not affected by co-injection with 0.1–10 nM of timolol (Figure 6) or 1 pM–1 nM of betaxolol (Figure 7) during the 2 weeks following injection. In contrast, co-injection with 2.5 pM of nipradilol showed significant recovery of the ERG amplitude ($P < .01$), but this effect was diminished by increasing the concentrations of the drug (0.1–1 nM) (Figure 8). Nipradilol was identified as having an NO effect in addition to $\alpha$ and $\beta$ blocker function,\textsuperscript{18} suggesting that the NO effect may be important for the recovery of ERG responses. To test this hypothesis, a biologically NO-inactive derivative, desnitro-nipradilol, was examined. As shown in Figure 8, desnitro-nipradilol (2.5 pM) had no effect on the ERG responses. Rhodopsin phosphorylation was also examined in terms of its retinal outer segment function. Rhodopsin phosphorylation was not altered by the co-administration of timolol (1 nM) with 20 mM NMDA or anti-NSE serum, while the NMDA- or anti-NSE serum-induced suppression of rhodopsin phosphorylation was slightly enhanced or significantly diminished ($P < .01$) by the co-administration of nipradilol (2.5 pM) or betaxolol (1 nM), respectively (Figure 9).

To evaluate the neuroprotective effects of drugs which increase ocular blood flow, such as calcium antagonists and prostaglandin derivatives, the effects of these drugs on retinal functions were evaluated using rat models intravitreally injected with anti-NSE antibody or 20 mM NMDA. Administration of nilvadipine, a calcium antagonist, caused effective recovery from both ERG and rhodopsin phosphorylation impairment after intravitreal injection of 20 mM NMDA (open columns) or anti-NSE antibody (dotted columns). In contrast, isopropyl unoprostone had no effect on these retinal functions (Figures 10 and 11). Based upon these data, we speculated that the recovery-producing effects of nilvadipine

![Figure 3](image-url). Light micrograph (Azur staining) of retina and choroid after intravitreal administration of anti-NSE antibody. Two weeks after administration of 5 μL of purified anti-NSE antibody into Lewis rat eyes (4 rats, 8 eyes). Pyknosis of retinal ganglion cell (arrows) is seen in ganglion cell layer.
may not be related to its vasodilative effects because the other drug increasing intraocular blood flow, isopropyl unoprostone, had no effect. To test our hypothesis, 5 μL of 10 mM endothelin-1, which has been known to cause marked constriction of retinal vessels and pallor of the optic nerve head persisting for 10 days after injection, was co-administered with nilvadipine to anti-NSE antibody-treated rats, and their retinal functions were evaluated. As shown in Figures 10 and 11, endothelin-1 had no influence on the recovery producing effect of nilvadipine.

**Discussion**

In our recent study, we found that 20% of glaucoma patients have the autoantibody toward NSE, and that intravitreal administration of anti-NSE antibody induced a decrease of ERG responses and an increase of TUNEL-positive staining of the retinal ganglion cells in the rat in vivo. Therefore, we suggested that the autoantibody toward NSE may be related to the retinal ganglion cell death found in glaucoma. Here, we confirmed by electron microscopic examination that retinal ganglion cell death was induced by either serum from a glaucoma patient or purified anti-NSE antibody. Functionally, anti-NSE antibody induced a significant reduction in both ERG amplitude and rhodopsin phosphorylation. Interestingly, this anti-NSE antibody-induced retinal dysfunction was comparable to the excitotoxic retinal dysfunction caused by NMDA. In our present data, we found that intravitreal injection of NMDA caused not only impairment of ERG responses but also significant suppression of rhodopsin phosphorylation in a dose-dependent manner. So far, several investigators have claimed that NMDA induced retinal damage, occurring mainly in retinal inner layers, retinal ganglion cell layer and inner nuclear layer, citing the thinning of retinal inner layers and TUNEL positivity in retinal ganglion cells and amacrine cells. However, our present results showing (1) significant suppression of rhodopsin phosphorylation and (2) marked decrease in a- and b-waves of ERG in NMDA-treated retinas, strongly suggested that NMDA may affect both retinal inner and outer layers. This is not surprising since a glutamate-mediated synaptic connection is present between the photoreceptor and bipolar cells. In fact, it is known that both flash and pattern ERG responses were affected in cat eyes with acute IOP elevation, suggesting that damage to retinal outer segments is also involved in such models.

![Figure 4. Electron micrograph of ganglion cell layer after intravitreal administration of anti-NSE antibody. Two weeks after administration of 5 μL of purified anti-NSE antibody into Lewis rat eyes (4 rats, 8 eyes). Apoptotic body (*) is surrounded by Müller cells (M) and axons (a).](image-url)
In contrast with the knowledge we have about the excitotoxic neuronal damage by NMDA, we do not know, at present, the precise molecular mechanisms causing retinal ganglion cell apoptosis by anti-NSE antibody. However, it was suggested that antibody against α-enolase which is a homoenzyme with NSE is found in patients with cancer-associated retinopathy (CAR). CAR is characterized as progressive retinal degeneration similar to retinitis pigmentosa. In CAR, it was suggested that photoreceptor apoptosis is primarily caused by autoimmune responses against several kinds of retinal antigens, including α-enolase, recoverin, and hsc 70. Because enolases are known to be important enzymes regulating glycolysis, we speculated that autoimmune responses toward enolases may block their enzyme activities and cause severe damage to target cells. In fact, in our recent paper, we found that anti-NSE antibody was found not only in glaucoma patients (approximately 20%), but also in normal control subjects (approximately 10%). However, serum from glaucoma patients specifically reacted with the retinal ganglion cell layer, while serum from control subjects did not react with the retinal ganglion cell layer in immunocytochemical studies. Therefore, we suggested that antibodies in glaucoma patients may differ from anti-enolase antibodies found in normal individuals, in their affinity, avidity, specificity, or class.

To explain the molecular mechanism of retinal ganglion cell death in glaucoma, an increase in intra-
cellular Ca\(^{2+}\) concentrations resulting from activation of NMDA and kainate/AMPA receptor channels following ischemic changes in retina and optic nerve are thought to be responsible.\(^{23,24}\) The NMDA receptor activation causes an influx of calcium, \([\text{Ca}^{2+}]_i\), through the NMDA receptor channels as well as through voltage-gated calcium channels in retinal ischemia/reperfusion injury models.\(^{23–25}\) It is known that the kainate/AMPA receptors are generally regarded as low \(\text{Ca}^{2+}\)-permeable receptors. Therefore, the activation of these receptors is thought to increase \([\text{Ca}^{2+}]_i\), mainly via a depolarization-induced opening of voltage-gated permeable calcium channels.\(^{23–25}\) In addition, activation of other receptors linked to phospholipase C causes a release of calcium from internal stores,\(^{26}\) and this can theoretically lead to an additional influx of calcium by activation of an unidentified channel in plasma membranes.\(^{27}\) Based upon the above observations, so-called “neuroprotection” is required for effective therapy in glaucoma patients in addition to decreasing IOP levels. Because it is known that betaxolol reduces voltage-gated calcium entry in ciliary and retinal blood vessels, this compound may act neuroprotectively by reducing calcium influx into neurons.\(^{28,29}\) Recently, Osborne et al\(^{30}\) reported that betaxolol is a retinal neuroprotective compound based on the following experimental observations: (1) administration of betaxolol caused significant recovery of ERG b-wave in rat eyes after ischemia/reperfusion; (2) betaxolol remarkably reduced kainite-induced \([\text{Ca}^{2+}]_i\) in retinal cultured cells; (3) betaxolol antagonized hypoxia/reoxygenation effects on the lactose dehydrogenase released from rat cortical cultures and the ischemia-induced effect on the \(\gamma\)-aminobutyric acid immunoreactivity in rabbit retina. In addition, Gross et al\(^{31}\) reported that betaxolol functioned as a neuroprotective agent for retinal ganglion cells by single-unit extracellular and whole-cell voltage clamp re-
Therefore, they suggested that in glaucoma patients betaxolol could act to lower IOP by the action of -receptors in anterior uvea as well as by giving neuroprotection to retinal ganglion cells. However, our present data revealed betaxolol to be ineffective in remedying the NMDA-induced ERG impairment, whereas it produced significant recovery of rhodopsin phosphorylation, suggesting that it may be more effective in retinal outer layers than in retinal inner layers. In fact, it is known that Ca regulation by Ca-binding proteins, including recoverin, GCAP, and calmodulin, is pivotal for signal transduction mechanisms in photoreceptors. Thus, it seems a likely possibility that NMDA may cause photoreceptor dysfunction by misregulation of these Ca-binding proteins induced by an influx of Ca into the photoreceptor cells. If this is indeed the case, our present observations suggest that betaxolol may also function neuroprotectively in photoreceptor cells.

Another neuroprotective mechanism was suggested to be NO serving to protect retinal ganglion cells against glutamate-mediated toxicity. Recently, npradilol, an - and -adrenergic receptor antagonist possessing NO function, which was initially produced as a drug for hypertension, has been used as an anti-glaucoma drug. Therefore, this drug may be expected to be effective not only in decreasing IOP by the action of its - and -adrenergic receptor antagonists but also for neuroprotection because of its intrinsic NO function. As expected, our present data showed that npradilol causes significant recovery of the NMDA-induced ERG impairment at a relatively low dose. However, this effect on ERG was diminished by an increase in the concentration of the drug, and levels of rhodopsin phosphorylation were fur-
Calcium antagonists, which have been widely used in treatment of systemic hypertension, inhibit the entry of calcium ion intracellularly, relax vascular smooth muscle cells, and increase regional blood flow in several organs. Calcium antagonists, increased blood flow in the optic nerve head, but not in retinal vessels when measured by laser Doppler flowmetry in the cat. More recently, it was reported that nilvadipine, another dihydropyridine calcium antagonist, with minimum effects on systemic blood pressure in subjects without hypertension, increased vertebrate blood flow more effectively than nifedipine or nicardipine in the dog, and increased blood velocity and blood flow in the optic nerve head as well as in the choroid and retina in the rabbit. In addition, it also increased blood velocity in the optic nerve head of NTG patients. In the present study, administration of nilvadipine caused significant recovery from impairment of ERG and rhodopsin phosphorylation in rat eyes treated with anti-NSE antibody or NMDA. It was reported that clinical oral administration of calcium antagonists produced beneficial effects on glaucomatous visual field loss in some POAG and NTG patients. The mechanism of action of the calcium antagonist is not yet understood, but it may be related to its vasodilating action on vessels within the central nervous system as well as its effect in decreasing intracellular calcium levels, which are known to trigger apoptotic cell death in neurons.

Isopropyl unoprostone, a prostaglandin derivative, is used mainly in Japan as an anti-glaucoma drug. As the mechanism for decreasing IOP, isopropyl unoprostone promotes aqueous outflow and has no effect on aqueous production. In addition, it was suggested that isopropyl unoprostone increased intraocular blood flow. Therefore, similarly to calcium antagonists, such effects on increase of the intraocular blood flow may function as neuroprotection. However, in the present study, we could not find any effects on ERG or rhodopsin phosphorylation in rat eyes administered anti-NSE antibody or NMDA. In addition, suppression of the nilvadipine-dependent increase in the intraocular blood flow by co-administration of endothelin-1, produced no change in the recovery of rhodopsin phosphorylation and ERG amplitude, suggesting that the decrease of intracellular Ca\(^{2+}\) levels may be important for the recovery effect rather than the increase of the intraocular blood flow. Therefore, taken together, the combination of lowering intracellular Ca\(^{2+}\) and decreasing IOP may be suitable treatment for glaucomatous optic neuropathy.

In conclusion, our present study demonstrated that anti-NSE antibody found in serum autoantibody in a glaucoma patient caused retinal cell dysfunction similar to NMDA-mediated excitotoxic damage, and that rat models intravitreally administered either anti-
NSE antibody or NMDA may be useful for furthering our understanding of the molecular pathology of glaucoma as well as for evaluating anti-glaucoma drugs.

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