Elevated Glutamate Levels in the Vitreous Body of Humans and Monkeys With Glaucoma

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Objective: To explore the possibility that the excitatory amino acid glutamate might be associated with the disease process of glaucoma, which is characterized by the death of retinal ganglion cell neurons and subsequent visual dysfunction.

Methods: Amino acid analyses were performed on vitreous specimens that were obtained from patients who were undergoing cataract extraction. Samples were collected prospectively from those patients who sustained inadvertent rupture of the posterior capsule between 1988 and 1993. An additional set of specimens, obtained from both eyes of monkeys, was analyzed; in these monkeys, glaucoma had been experimentally induced in one eye only.

Results: A twofold elevation in the level of glutamate was detected in the vitreous body of the group of patients with glaucoma when compared with that in a control population of patients with cataracts only. An even greater elevation of the glutamate level was found in the vitreous body of glaucomatous eyes of monkeys when compared with that in control eyes. No statistical differences were detected among other amino acid levels from the vitreous body of glaucomatous and nonglaucomatous eyes in humans or monkeys.

Conclusions: The excitatory amino acid glutamate is found in the vitreous body of glaucomatous eyes at concentrations that are potentially toxic to retinal ganglion cells. The increased level of this known neurotoxin is consistent with an "excitotoxic" mechanism for the retinal ganglion cell and optic nerve damage in glaucoma. Therapies to protect neurons against glutamate toxic effects may prove to be useful in the management of this blinding disease.

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GLAUCOMA IS a leading cause of blindness worldwide.[1,2] Although there are multiple causes for this disease, most are thought to involve impaired aqueous outflow from the anterior chamber of the eye and a consequently elevated intraocular pressure.[3] Glaucomatous damage to the optic nerve, and specifically to retinal ganglion cell axons, has been attributed to an intolerably high level of intraocular pressure. Recently, to understand and control glaucomatous damage, consideration of factors beyond normalization of the intraocular pressure has begun.[4,5]

Studies in the central nervous system during the past three decades have found that both traumatic and ischemic neuronal injury can be mediated by excessive levels of excitatory amino acids, especially glutamate.[6-9] Prior investigations have explored the role of excitatory amino acids in stroke, trauma, epilepsy, and neurodegenerative disorders (eg, Huntington's disease, amyotrophic lateral sclerosis, and acquired immunodeficiency syndrome dementia).[6-9] Given that the neuron damaged in glaucoma—the retinal ganglion cell—is part of the central nervous system, these studies lend credence to the possibility that excitatory amino acids play a role in the neuronal loss that is seen in glaucoma.

The toxic potential of glutamate to neurons in the mammalian retinal ganglion cell layer has been well documented. In 1957, Lucas and Newhouse[10] first reported the toxic effects of glutamate on the mammalian eye. Subcutaneous injection of glutamate in young mice led to severe destruction of the inner retinal layers, most notably the retinal ganglion cell layer. By using ultrastructural techniques, Olney[11] demonstrated similar glutamate-induced toxic effects to the retina in neonatal mice and coined the term "excitotoxicity" for this type of neuronal damage. Sisk and Kuwabara[12] injected glutamate intravitreously in adult albino rats and observed degeneration of the inner nuclear and ganglion cell layers. Azuma et al[13] reported that the optic discs of neonatal rats

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MATERIALS AND METHODS

STUDIES OF HUMAN PATIENTS

Vitreous specimens were collected from patients who were undergoing cataract extraction. Specimens were obtained from 26 patients with documented glaucoma and cataracts and from 21 patients with cataracts alone. Clinical data (**Table 1**) were obtained retrospectively from hospital and physician records. Cataract type and severity, axial length of the eye, race, age, sex, surgical procedure (extracapsular cataract extraction or phacoemulsification), length of time in the operating room, preoperative visual acuities, and degree of postoperative inflammation were recorded. For each patient, all intraocular pressure measurements from the time of diagnosis were averaged to provide a value for the mean intraocular pressure.

Patients were classified as having glaucoma if a review of the medical records indicated the following criteria: (1) an elevated intraocular pressure (>21 mm Hg on two separate occasions) *and* (2) an optic nerve in which the appearance was consistent with glaucoma. Demographics of the population of patients with glaucoma are provided in **Table 2**. Each patient with glaucoma either had been receiving therapy for at least 1 year immediately before cataract surgery or had undergone a trabeculectomy or posterior lip sclerectomy. All patients had intraocular pressures of less than 22 mm Hg at the last preoperative visit. Glaucoma diagnoses included in this study were primary open angle, chronic angle closure, or pseudoexfoliation. Optic nerve changes indicative of glaucoma included the presence of optic nerve asymmetry (ie, a difference of more than 0.2 in cup-to-disc ratios between the two eyes), evidence of progressive enlargement of the optic cup, notching of the nerve rim, vertical elongation of the cup, splinter hemorrhage, or nasal displacement of the vessels.[26] Visual fields were performed on the Goldmann or Humphrey perimeters. Visual fields (for the presence or absence of glaucomatous visual field loss) and cup-to-disc ratios were scored by two reviewers who were masked to the amino acid values. Goldmann visual field defects were to the III4e stimulus or greater, and these defects were larger than 5°. Humphrey visual field defects encompassed three adjacent test stimuli, and these defects were 5 dB or greater. Locations of field defects are provided in Table 2. It was not possible to establish whether patients had evidence of visual field loss at the time of surgery, necessitating the following approximation. Visual fields of patients with glaucoma were obtained between 3 and 6 months after cataract extraction. In all patients, any defects that were detected on these postoperative visual fields were also present on one or more preoperative fields, suggesting that visual field loss had been present at the time of cataract extraction. Although cataract extraction frequently resulted in visual field improvement, these changes in all likelihood reflected visual field compromise owing to lenticular changes, and were not considered when visual fields were scored for the presence of glaucomatous loss. It was not possible to obtain Humphrey perimetry in all patients, and Goldmann perimetry may have underrepresented visual field loss.[27]

Vitreous samples were obtained from patients with glaucoma and control patients who had sustained inadvertent vitreous loss during cataract surgery at the Massachusetts Eye and Ear Infirmary, Boston. Vitreous samples were assayed from 47 such patients (26 with glaucoma plus cataracts and 21 with cataracts alone). All available samples between 1988 and 1993 were evaluated. Although approximately 1200 cases of vitreous loss (Quality Assurance Committee, oral communication, September 1993) occurred at the Massachusetts Eye and Ear Infirmary during the study, many surgeons were reluctant or unable to provide specimens for this study. Seven samples, either insufficient in quantity for amino acid analysis or frankly contaminated with blood, were not included in the study. All other available samples were analyzed, and no amino acid data were excluded from the analyses. Fourteen different surgeons were involved. This study was approved by the Human Studies Board of the Massachusetts Eye and Ear Infirmary. Six vitreous specimens from patients who were undergoing combined cataract extraction and trabeculectomy operations were also obtained and analyzed. However, these data were excluded from the multivariate analyses provided below because of an inability to devise appropriate controls (ie, to exclude the possibility that scleral manipulation during the creation of the trabeculectomy flap could potentially perturb amino acid concentrations).

Vitreous specimens were collected at the time of capsular rupture. The first group of samples was collected by surgical sponge (Weck Cel) vitrectomy (11 patients with documented glaucoma and cataracts and 13 patients with cataracts alone). Small surgical sponges that were used in the vitrectomy were stored at 4°C until the vitreous could be removed. Within 2 hours of collection, clear vitreous was removed from the sponges under magnification, frozen in liquid nitrogen, and stored at −80°C until it could be assayed. The individual who removed the vitreous was masked to the patients' ophthalmic diagnoses (excepting cataract). In the remaining patients, the saline solution that was regularly infused during mechanized vitrectomy was briefly turned off, and a vitreous specimen was collected from the midanterior vitreous.[28] This vitreous biopsy technique reduced possible contamination that could be introduced by manipulation of either the wound or specimen. Vitreous biopsy specimens were collected from 10 patients with cataracts alone and 13 patients with glaucoma plus cataracts. These samples were similarly frozen and stored.

In addition, aqueous specimens were collected by aspiration at the start of cataract surgery from 19 patients (nine with glaucoma of greater than 2 years' duration and 10 with cataracts alone). These patients were similar in age, cataract type, and glaucoma diagnosis (in the group with glaucoma) to those patients who had sustained inadvertent vitreous loss.

that were treated with glutamate were "deeply excavated." Work in our own and other laboratories has established that the predominant form of excitotoxicity of retinal ganglion cells is mediated by overstimulation of the *N*-methyl-D-aspartate (NMDA) subtype of the glutamate receptor, which in turn leads to excessive levels of intracellular calcium.[14-19] Non-NMDA receptors may also play a role in glutamate excitotoxicity.[20]

We have chosen to evaluate the amino acids in the vitreous body for several reasons. Previous investigators found no significant variations in aqueous amino acid levels when glaucomatous samples were compared with

STUDIES OF RATS

To assess the validity of the glutamate-measuring paradigm, Long-Evans rats were anesthetized. Five microliters of a glutamate solution, prepared in a phosphate-buffered saline solution, was injected into the midvitreous body of each eye. The concentration of glutamate was adjusted to achieve a final added intravitreal concentration that ranged from 0- to 40-μmol/L glutamate (assuming a vitreal volume of 40 μL). Three animals were tested at each of five concentrations. The animal was killed immediately after an injection had been made in each eye. Immediately post mortem, an incision was made in one eye, selected at random, along the limbus. The vitreous was gently expressed onto a small surgical sponge by counterpressure, and the vitreous was removed from the sponge under magnification and analyzed for amino acid concentrations. The vitreous of the fellow eye was aspirated under direct visualization into a 16-gauge needle that was placed in the midvitreous. These vitreous specimens were likewise analyzed for amino acid concentrations.

MONKEY GLAUCOMA

All animal experiments were performed in accordance with the standards of the Association for Research in Vision and Ophthalmology, Bethesda, Md. Glaucoma was induced by laser photocoagulation in one eye of each of three monkeys, and the contralateral eye was used as a control. Three cynomolgus monkeys (*Macaca fascicularis*), weighing 3 to 4 kg, of normal development, were selected for use. All animals had undergone baseline eye examinations and had normal anterior and posterior segments, open iridocorneal angles, normal optic nerves, and normal intraocular pressures that were recorded in a 12-hour diurnal variation series by using a calibrated pneumatonometer. Optic nerve photographs were taken that revealed cup-to-disc ratios in all eyes of less than 0.35.

The animals were sedated with ketamine hydrochloride (5 mg/kg) and topically anesthetized with proparacaine hydrochloride (0.5%). To induce glaucoma, the midportion of the trabecular meshwork of one eye, chosen at random, of each animal was treated with argon laser burns by using a specially made lens for monkey eyes.[29] Each animal was treated twice, with approximately 100 burns (diameter of each burn, 50 μm) that were delivered over 360° (power, 1.2 to 1.4 W; exposure time, 0.5 seconds). There were 19 to 41 days between treatments. To control inflammation, animals were treated with prednisolone acetate (1%), one drop to the treated eye every 6 hours for the first 4 days after laser surgery. Frequent intraocular pressure measurements were conducted following the procedures; one eye that did not show sustained pressure elevation (to at least 30 mm Hg) was re-treated a third time. Complete eye examinations and optic nerve photographs were performed periodically. In all animals, typical glaucomatous optic nerve excavation developed in the treated eye; the contralateral, untreated eye was unchanged.

Specimens were obtained from monkeys at 126 to 226 days after the initial treatment. Monkeys were deeply sedated with ketamine hydrochloride (5 mg/kg) intramuscularly and topically anesthetized with proparacaine hydrochloride (0.5%). An 18-gauge needle that was attached to a 1-mL syringe was introduced through the sclera, and the needle tip was positioned either just behind the lens near the equator of the globe (anterior vitreous placement) or just in front of the optic nerve head (posterior vitreous placement). Under visual guidance by indirect ophthalmoscopy, 0.1 to 0.2 mL of vitreous gel was removed from the eye by using gentle aspiration. Specimens were taken from glaucomatous and fellow control eyes. Separate needles and syringes were used for each vitreous biopsy. The syringes were rapidly immersed in liquid nitrogen and stored at −80°C until assayed in a masked fashion for amino acid concentrations. After vitreous sampling, monkeys were given lethal injections of pentobarbital sodium (35 mg/kg intravenously as a bolus).

AMINO ACID ANALYSES

Amino acid analyses were performed by high-pressure liquid chromatography in a masked fashion by the Neurochemistry Laboratory of the Massachusetts General Hospital, Boston. Immediately before analysis, salicylic acid was added to each sample. Analyses were carried out by cation exchange.[30] Duplicate analyses of five samples were carried out at Children's Hospital, Boston, Mass; all values agreed within 5% (minimum level of detection for all amino acid analyses, 5 μmol/L).

STATISTICAL ANALYSES

For the 47 human patients, amino acid levels were compared statistically by standard general linear models (analysis of variance [ANOVA] or regression). Multivariate analysis was performed by using the presence or absence of glaucoma as the explanatory variable and amino acid concentrations as dependent variables. "Demographic variables," including age, race, sex, degree and type of cataract, surgical procedure or surgeon, sampling technique, time in the operating room, and axial length, were also analyzed by regression methods. In the control population alone, multiple regression was performed to evaluate the relationships of the demographic variables with amino acid concentration. Additional multiple regression analyses were performed to assess the relationships between the presence of glaucoma and glutamate elevation using categoric covariates, including glaucoma diagnosis and type of therapy. Years of treatment of glaucoma was included as a continuous predictor that represented the severity of the disease. All *P* values were two-tailed with a Bonferroni correction to adjust the error rate inasmuch as 12 distinct dependent variables were analyzed.[31] Commercially available computer software (SAS Institute, Cary, NC) was used for all analyses.[32] The monkey samples were compared by using the paired Student's *t* test, based on 2 *df*. In all analyses, the nominal significance level was .05, which was used as the criterion to reject the null hypothesis.

control values.[21,22] However, the retinal ganglion cell layer is contiguous with the vitreous body. Any agent that is toxic to retinal ganglion cells would therefore be closer to the target site if it was present in the vitreous body. Aqueous analysis may not adequately represent the local concentration of amino acids at the retina because of diffusion or active transport. Durham and coworkers[23,24] and Welge-Lüssen and Oppermann[25] found significant differences in amino acid levels between the aqueous humor and vitreous body in human and animal eyes. We therefore evaluated amino acid concentrations in the vitreous body and aqueous humor of glaucomatous eyes.

RESULTS

GLUTAMATE LEVEL ELEVATION IN VITREOUS FROM GLAUCOMATOUS EYES

Amino acid analyses of human vitreous revealed an approximately twofold elevation in glutamate levels in patients with glaucoma and cataracts when compared with those in cataractous control patients (**Table 3** and **Figure 1**). Patients with glaucoma had a mean (±SD) vitreous glutamate concentration of 23.1±4.6 μmol/L compared with 10.0±5.1 μmol/L in the population of patients with cataracts alone ($P<.001$). Vitreous specimens that were obtained from six patients who were undergoing combined cataract extraction and trabeculectomy (six patients) had a mean (±SD) vitreous glutamate concentration of 21.2±3.2 μmol/L. As noted above, incision of the sclera during the creation of the trabeculectomy flap introduces a variable that cannot be controlled for in patients who are undergoing cataract extraction alone. Accordingly, these patients were not included in the statistical analyses, and they will not be discussed further. An ANOVA revealed a significant increase in the glutamate level for patients with glaucoma ($F [1, 45]=78.97, P<.001$). No other statistically significant differences in the vitreal amino acid concentrations were detected between the two groups of patients. Although concentrations of amino acids other than glycine and glutamate were slightly higher in the control group, these differences were not significant. No significant variation in the aqueous amino acid concentrations were detected between those patients with cataracts alone or those with glaucoma plus cataracts (data not shown).

Table 1. Demographics Summary

Demographic Variable	Patient Group: Cataracts	Patient Group: Glaucoma+ Cataracts
No. of patients	21	26
Mean±SD age, y	68.7±14.9	71.5±10.4
Race, No. (%) of patients		
African American	11 (52.4)	13 (50.0)
White	10 (47.6)	11 (42.3)
Hispanic	0	2 (7.7)
Sex, No. (%) of patients		
F	8 (38.1)	14 (53.8)
M	13 (61.9)	12 (46.2)
Mean±SD axial length of the eye, mm	23.1±0.4	23.2±0.7
Surgical procedure, No. (%) of patients		
Phacoemulsification	10 (47.6)	13 (50.0)
Extracapsular extraction	11 (52.4)	13 (50.0)
Mean±SD time in the operating room, min	73.7±6.3	74.5±9.7
Glaucoma diagnosis, No. (%) of patients		
Open-angle glaucoma	...	18 (69.2)
Chronic angle-closure glaucoma	...	4 (15.4)
Pseudoexfoliative glaucoma	...	4 (15.4)

CONTRIBUTORY VARIABLES

From these analyses, our data indicate that the increased vitreous glutamate concentration correlates with the presence of glaucoma ($r=.80, P<.001$). Several variables were explored to identify possible factors that contributed to the glutamate concentrations. Cataract type, age, sex, race, axial length of the eye, and preoperative acuity were not significant variables alone or in combination in the analysis of vitreous glutamate levels. Factors that related to the surgery itself, including the surgeon, procedure, length of time in the operating room, or vitreous collection technique, were also not significant.

A second analysis then explored whether aspects of a patients' glaucoma status were related to the elevation of the vitreous glutamate concentration. These included the type of glaucoma, treatment with any of several modalities (β-blockers, miotics, adrenergics, carbonic anhydrase inhibitors, laser trabeculoplasty, trabeculectomy, or posterior lip sclerectomy), length of time that the patient had been treated for glaucoma, or intraocular pressure either at presentation or the mean pressure during the course of treatment. These variables did not correlate independently with the presence of glutamate in the vitreous of patients with glaucoma. The statistical power of these calculations was 0.83 by using a two-sided test. Furthermore, intraocular pressure—either at presentation or the mean pressure during the course of treatment—did not correlate with the elevation of the glutamate level.

VALIDATION OF HUMAN GLUTAMATE ANALYSES IN A RAT VITRECTOMY MODEL

High-pressure liquid chromatography reliably quantified the concentration of glutamate that was injected into the vitreous of a rat. These data are illustrated in **Figure 2**. There was good agreement between the values measured by high-pressure liquid chromatography and predicted concentrations (the sum of endogenous plus injected glutamate), whether the vitreous was expressed onto a small surgical sponge or aspirated by syringe. The Pearson correlation coefficient was $r=.97$ ($P<.001$). These data suggest that our technique of high-pressure liquid chromatography analysis of vitrectomy specimens accurately reflected the vitreal glutamate concentrations.

MONKEY MODEL

The mean (±SD) glutamate concentration in the anterior vitreous of glaucomatous eyes of monkeys was 59.7±7.3 μmol/L, and it was 80.3±7.8 μmol/L in the posterior vitreous (**Table 4**). These values were substantially higher than the 12.3±1.5 μmol/L that was found in the control anterior vitreous and the 12.3±2.3 μmol/L in the control posterior vitreous.

COMMENT

The results presented herein indicate that the glutamate concentration is elevated in patients with glaucoma to a

Table 2. Demographics of Patient Population With Glaucoma*

Patient No.	Mean±SD IOP, mm Hg	Mean±SD Cup-Disc Ratio†	Visual Field Type	Visual Field Finding	Medical Treatment‡ β-Blocker	Miotic	Adrenergic	CAI	Surgical Treatment Filtration Procedure	Laser Trabeculoplasty
1	16.8±1.2	0.6±0.04	Goldmann	Full	Y	Y	Y	N	N	Y
2	17.1±1.4	0.6±0.09	Goldmann	Full	Y	Y	Y	N	N	Y
3	16.2±1.0	0.6±0.04	Goldmann	Full	Y	Y	Y	N	N	Y
4	15.1±0.7	0.8±0.08	Goldmann	Full	Y	Y	Y	N	N	Y
5	15.3±1.9	0.6±0.09	Humphrey	Full	Y	N	N	Y	N	N
6	17.0±0.9	0.9±0.07	Goldmann	Full	Y	N	N	Y	N	N
7	17.8±0.9	0.6±0.03	Humphrey	Full	Y	N	Y	N	N	N
8	19.8±0.5	0.6±0.08	Goldmann	Full	Y	Y	Y	N	N	Y
9	20.3±1.7	0.8±0.02	Humphrey	Full	Y	Y	Y	Y	N	Y
10	18.3±1.6	0.8±0.07	Humphrey	Nasal step, arcuate	Y	Y	Y	Y	Y	Y
11	19.7±1.2	0.9±0.09	Humphrey	Arcuate	N	N	N	Y	N	N
12	19.8±1.1	0.9±0.02	Humphrey	Arcuate	Y	N	N	N	Y	Y
13	20.5±1.5	0.8±0.06	Humphrey	Nasal step	N	Y	Y	N	Y	Y
14	15.0±1.3	0.5±0.09	Humphrey	Full	Y	Y	Y	N	N	Y
15	18.5±1.2	0.6±0.05	Goldmann	Full	Y	Y	Y	N	N	Y
16	15.2±1.9	0.6±0.08	Goldmann	Full	Y	Y	N	Y	N	Y
17	15.1±0.9	0.7±0.05	Goldmann	Full	Y	N	Y	Y	N	N
18	18.9±0.4	0.6±0.01	Goldmann	Full	Y	N	N	Y	N	Y
19	18.5±1.9	0.7±0.04	Humphrey	Full	N	Y	Y	Y	N	Y
20	20.6±1.8	0.7±0.06	Humphrey	Full	N	Y	Y	N	N	Y
21	15.0±1.9	0.8±0.10	Goldmann	Paracentral scotoma	N	N	N	N	N	N
22	20.2±1.1	0.9±0.03	Humphrey	Split fixation, nasal step	Y	Y	Y	N	N	Y
23	17.8±1.5	0.9±0	Humphrey	Nasal step	Y	N	N	Y	Y	N
24	16.8±1.1	0.8±0.06	Goldmann	Paracentral scotoma	Y	Y	Y	Y	Y	Y
25	19.7±1.5	0.8±0.03	Humphrey	Arcuate	N	Y	Y	N	Y	Y
26	19.5±0.8	0.8±0.05	Humphrey	Arcuate	Y	Y	Y	N	Y	Y

IOP indicates intraocular pressure; CAI, carbonic anhydrase inhibitor; Y, yes; and N, no.
†Cup-disc ratio is the mean (±SD) of the vertical and horizontal extension, measured twice by each of two observers.
‡Medical treatment indicates whether the patient had been prescribed a member of the indicated pharmacologic class for at least 3 months.

Table 3. Amino Acid Concentrations in Human Vitreous Body

Amino Acid	Mean±SD Concentration, μmol/L: Patients With Cataracts (n=21)	Patients With Glaucoma+ Cataracts (n=26)
Alanine	177.9±18.9	154.0±15.5
Glutamate	10.0±4.6	23.1*±5.1
Glutamine	525.2±33.5	484.5±29.4
Glycine	45.4±9.0	60.7±14.5
Histidine	44.9±5.2	37.2±2.4
Isoleucine	38.0±2.2	31.0±1.7
Leucine	88.0±5.5	79.2±4.1
Lysine	114.1±8.8	89.8±7.1
Methionine	23.6±1.6	20.6±1.5
Phenylalanine	69.2±4.4	57.2±3.2
Serine	118.8±10.2	97.4±5.3
Threonine	82.3±8.5	67.8±5.0
Tyrosine	40.6±8.1	36.0±7.0

**Indicates statistically significant difference in comparison with control (P<.001).*

level that is potentially toxic to neurons.[6,8,9,33,34] Glutamate levels were elevated not only in patients with visual field loss but also in those patients with glaucomatous optic nerve damage but no visual field loss, suggesting that this amino acidopathy may be present in the earlier stages of the disease. To date, we have been unable to analyze samples from patients with ocular hypertension (elevated intraocular pressure but no clinical evidence of optic nerve damage). The elevation of the glutamate concentration in the vitreous of patients with glaucoma is potentially of significance both in the pathophysiologic and therapeutic considerations of the retinal ganglion cell degeneration and visual loss that are seen in this disease.

The elevation of the glutamate level in untreated glaucoma in monkeys indicates that this finding is not a consequence of therapy for glaucoma, but rather, it is associated with the disease process itself. In monkeys with glaucoma, the greatest level of glutamate elevation was observed in the vitreous overlying the retina, compared with that seen in the anterior vitreous. These results therefore suggest that the source of the glutamate is toward the posterior aspect of the eye, and most likely, it is produced within the retina.

The findings suggested by our investigations have been supported in a rabbit model of steroid-induced glaucoma. Two weeks after the intraocular pressure becomes elevated, the vitreal glutamate concentration is more than twice normal in affected eyes (B. Becker, MD, oral communication, June 1995).

Glutamate, or a congener, is the principal excitatory amino acid neurotransmitter in the central nervous system and specifically in the retina. Glutamate is present at high levels in neurons, but it is usually synaptically released for brief periods in localized areas; hence, it is normally not toxic. Nevertheless, a doubling of glutamate can be critical in determining the retinal ganglion cell sur-

vival. Studies in the central nervous system suggest that damage to hippocampal or cortical neurons can occur at glutamate concentrations of 2 to 5 μmol/L.[8,33] Prior evidence indicates that these concentrations of excitotoxins can be lethal to retinal ganglion cells in vitro as well.[14-16,18] An in-depth discussion of the concentration at which glutamate or its congeners are toxic to neurons in vitro and in vivo is beyond the scope of this work; other factors (eg, extracellular Ca^{2+} and Mg^{2+}, other cell types present in any given culture system) can be significant variables.[6,8,9,33,34] Other investigators have shown that a single injection of as little as 20 nmol of NMDA (which is roughly equipotent to 2 nmol of glutamate[35]) can be strikingly toxic to the retinal ganglion cell layer in the rat eye.[17] We have previously demonstrated that a long-term doubling of the vitreal glutamate concentration is toxic to mammalian retinal ganglion cells in vivo.[36] Therefore, it might well be that long-term exposure of retinal ganglion cells to a twofold elevation of glutamate plays a role in glaucomatous neuronal loss in humans. It is not possible to tell from this study whether the elevated vitreous glutamate levels play a primary or secondary role in glaucomatous damage; however, at the observed levels, glutamate may contribute to this damage.

There are several possible sources for the excess glutamate found in the glaucomatous vitreous. The neuronal injury observed in glaucoma is generally considered to be

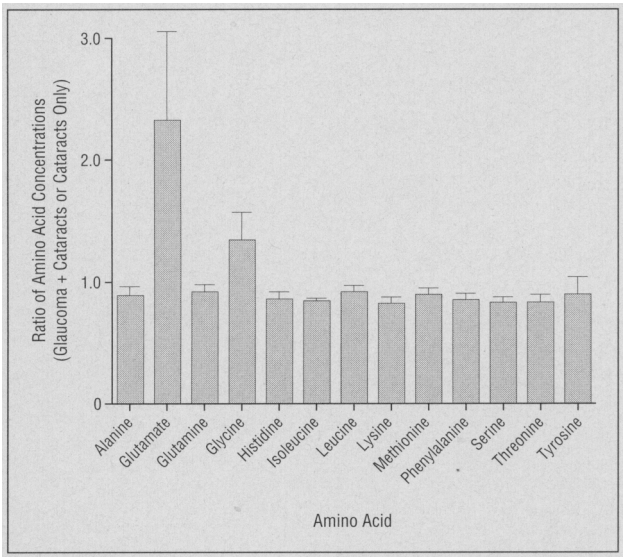

Figure 1. *Comparison of amino acid concentrations between vitreous specimens from patients with glaucoma plus cataracts and those with cataracts alone. The values detected in the vitreous of patients with glaucoma were divided by those found in the population of patients with cataracts only. Error bars to approximate 95% confidence intervals were determined as follows. For the upper limit of the ratio for each amino acid, twice the SEM was added to the amino acid concentration in the glaucomatous vitreous, and this sum was divided by the cataract-only value minus twice the SEM. Similarly, for the lower limit of the ratio, twice the SEM was subtracted from the amino acid concentration in the glaucomatous vitreous, and the sum was divided by the cataract-only value plus twice the SEM. The "whisker" indicates one half of the difference between these two limiting ratios.*

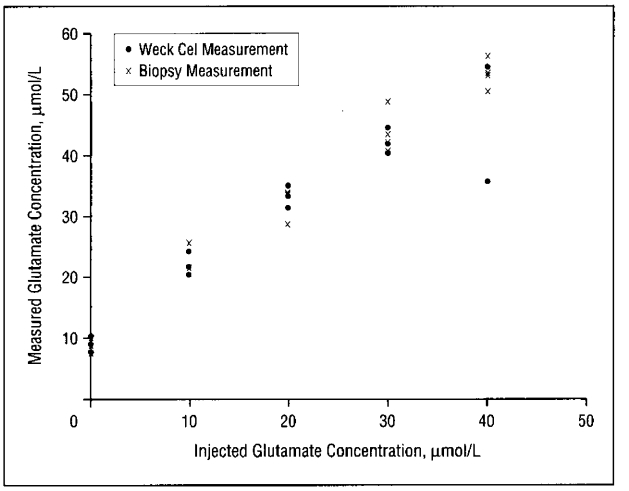

Figure 2. *A rat model of glutamate analyses. A known quantity of glutamate was injected into the vitreous of an adult rat eye, and the vitreous was then removed by either surgical sponge (Weck Cel) vitrectomy (circles) or biopsy needle aspirate (x symbols). There was good agreement between the injected values and the values measured by high-pressure liquid chromatography. Note that the injected values appear to be additive to the baseline glutamate concentration of approximately 8 μmol/L. Data points illustrate the actual measurements. All concentrations were evaluated in three eyes each by either Weck Cel vitrectomy or needle aspirate.*

Table 4. Amino Acid Concentrations in Monkey Vitreous Body

	Mean±SD Concentration, μmol/L			
Amino Acid	Anterior Control	Anterior Glaucoma	Posterior Control	Posterior Glaucoma
Alanine	396.7±49.7	407.3±54.9	428.0±40.0	464.7±11.3
Glutamate	12.3±1.5	59.7*±7.3	12.3±3.2	80.3*±7.7
Glutamine	852.0±190.5	833.0±173.0	938.3±93.7	961.7±165.7
Glycine	71.0±14.0	92.0±15.4	83.3±13.7	91.3±2.3
Histidine	58.0±5.5	73.3±11.6	72.7±0.9	64.5±4.5
Isoleucine	62.0±8.5	64.0±6.5	70.0±7.0	79.0±5.5
Leucine	122.3±16.3	131.0±10.0	140.3±10.7	144.7±3.2
Lysine	244.0±26.0	253.0±22.2	271.0±27.0	286.0±3.3
Methionine	40.0±6.0	41.0±6.1	48.3±5.2	46.7±0.3
Phenylalanine	147.7±20.2	146.3±17.0	172.0±9.0	170.0±3.8
Serine	226.3±32.0	219.0±20.6	245.0±23.1	268.0±13.2
Threonine	127.3±14.1	123.0±10.5	144.7±4.9	144.0±6.5
Tyrosine	27.3±3.3	28.3±2.4	31.3±2.3	30.7±2.4
Valine	340.3±38.8	315.7±33.1	340.0±30.0	402.7±7.7

**Indicates statistically significant difference in comparison with corresponding control by the paired Student's t test (P=.003).*

a consequence of either chronic trauma from pressure on the retinal ganglion cell body or axon, or ischemia caused by vascular compromise.[37] Traumatic injury and ischemic insult can lead to energy depletion and extracellular glutamate accumulation.[38,39] Dying cells could release their intracellular store of glutamate as a direct consequence of the glaucomatous process. The glutamate thereby released could, in turn, lead directly to further neuronal injury. An additional possibility depends on the presence of glutaminase in retinal ganglion cells.[40] Glutaminase, normally found only within cells, can convert glutamine (found in high concentrations within the vitreous) to glutamate. Glaucoma (perhaps through elevated pressure on retinal ganglion cells) may lead to increased membrane permeability of damaged retinal cells, exposing the intracellular glutaminase and thereby promoting the conversion of glutamine to glutamate. A third possibility is that the ability of Müller cells or retinal ganglion cells to detoxify glutamate may be impaired in glaucoma. Future investigations are required to explore these and other potential explanations.

Glutamate can be toxic to retinal ganglion cell neurons via overstimulation of various glutamate receptors, but predominantly the NMDA subtype.[15,16,18] Clinically tolerated NMDA antagonists are being developed to control glutamate-induced excitotoxicity.[41] In addition, voltage-dependent calcium channel antagonists (eg, nimodipine) have recently been shown to diminish partially NMDA receptor–mediated retinal ganglion cell excitotoxicity.[14] Therefore, these drugs could be potentially useful in the management of glaucoma.

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REFERENCES

1. Hiller R, Kahn HA. Blindness from glaucoma. *Am J Ophthalmol.* 1975;80:62-69.
2. Javitt JC, McBean AM, Nicholson GA, Babish JD, Warren JL, Krakauer H. Undertreatment of glaucoma among black Americans. *N Engl J Med.* 1991;325:1418-1422.
3. Epstein DL. *Glaucoma.* Philadelphia, Pa: Lea & Febiger; 1986.
4. Neufeld AH. Protection of the optic nerve in glaucoma. In: Drance SM, van Buskirk EM, Neufeld AH, eds. *Pharmacology of Glaucoma.* Baltimore, Md: Williams & Wilkins; 1992:292-300.
5. Schumer RA, Podos SM. The nerve of glaucoma! *Arch Ophthalmol.* 1994;112:37-44.
6. Choi DW. Glutamate neurotoxicity and diseases of the nervous system. *Neuron.* 1988;1:623-634.
7. Macaione S, Campisi R, Albanese A. Glutamate decarboxylase and gamma-aminobutyrate transaminase in the retina. *Boll Soc Ital Biol Sper.* 1970;46:785-789.
8. Meldrum B, Garthwaite J. Excitatory amino acid neurotoxicity and neurodegenerative disease. *Trends Pharmacol Sci.* 1990;11:379-387.
9. Lipton SA, Rosenberg PA. Excitatory amino acids as a final common pathway for neurologic disorders. *N Engl J Med.* 1994;330:613-622.
10. Lucas DR, Newhouse JP. The toxic effect of sodium L-glutamate on the inner layers of the retina. *Arch Ophthalmol.* 1957;58:193-201.
11. Olney JW. Glutamate-induced retinal degeneration in neonatal mice: electron microscopy of the acutely evolving lesion. *J Neuropathol Exp Neurol.* 1969;28:455-474.
12. Sisk DR, Kuwabara T. Histologic changes in the inner retina of albino rats following intravitreal injection of monosodium L-glutamate. *Graefes Arch Clin Exp Ophthalmol.* 1985;223:250-258.
13. Azuma N, Kawamura M, Kohsaka S. Morphological and immunohistochemical studies on degenerative changes of the retina and the optic nerve in neonatal rats injected with monosodium-L-glutamate. *Nippon Ganka Gakkai Zasshi.* 1989;93:72-79.
14. Sucher NJ, Lei SSZ, Lipton SA. Calcium channel antagonists attenuate NMDA receptor–mediated neurotoxicity of retinal ganglion cells in culture. *Brain Res.* 1991;551:297-302.
15. Sucher NJ, Wong LA, Lipton SA. Redox modulation of NMDA receptor–mediated Ca^{2-} flux in mammalian central neurons. *Neuroreport.* 1990;1:29-32.
16. Sucher NJ, Aizenmann E, Lipton SA. *N*-Methyl-D-aspartate antagonists prevent kainate neurotoxicity in rat retinal ganglion cells in vitro. *J Neurosci.* 1991;11:966-971.
17. Siliprandi R, Canella R, Carmignoto G, et al. *N*-Methyl-D-aspartate–induced neurotoxicity in the adult rat retina. *Vis Neurosci.* 1992;8:567-573.
18. Hahn JS, Aizenman E, Lipton SA. Central mammalian neurons normally resistant to glutamate toxicity are made sensitive by elevated extracellular Ca^{2+}: toxicity is blocked by the *N*-methyl-D-aspartate antagonist MK-801. *Proc Natl Acad Sci U S A.* 1988;85:6556-6560.
19. Dreyer EB, Pan ZH, Storm S, Lipton SA. Greater sensitivity of larger retinal ganglion cells to NMDA-mediated cell death. *Neuroreport.* 1994;5:5.
20. Mosinger JL, Price MT, Bai HY, Xiao H, Wozniak DF, Olney JW. Blockade of both NMDA and non-NMDA receptors is required for optimal protection against ischemic neuronal degeneration in the in vivo adult mammalian retina. *Exp Neurol.* 1991;113:10-17.
21. Hannappel E, Pankow G, Grassl F, Brand K, Naumann GO. Amino acid pattern in human aqueous humor of patients with senile cataract and primary open-angle glaucoma. *Ophthalmic Res.* 1985;17:341-343.
22. Schonheyder F, Ehlers N, Hust B. Remarks on the aqueous humor/plasma ratios for amino acids and related compounds in patients with various chronic ocular disorders. *Acta Ophthalmol (Copenh).* 1975;53:627-634.
23. Durham DG. Distribution of free amino acids in human intraocular fluids. *Trans Am Ophthalmol Soc.* 1970;68:462-500.
24. Durham DG, Dickinson JC, Hamilton PB. Ion-exchange chromatography of free amino acids in human intraocular fluids. *Clin Chem.* 1971;17:285-289.
25. Welge-Lüssen LL, Oppermann W. Amino acid determination in the aqueous humor and vitreous body of single eyes. *Graefes Arch Clin Exp Ophthalmol.* 1969;177:346-354.
26. Shingleton BJ, Anderson LS, Berson FG, Cantor L, Lee DA. *Glaucoma.* San Francisco, Calif: American Academy of Ophthalmology; 1993:42.
27. Katz J, Tielsch JM, Quigley HA, Sommer A. Automated perimetry detects visual field loss before manual Goldmann perimetry. *Ophthalmology.* 1995;102:21-26.
28. Belfort R, Nussenblatt RB. Surgical approaches to uveitis. *Int Ophthalmol Clin.* 1990;30:314-317.
29. Lee PY, Podos SM, Howard WJ, Severin CH, Rose AD, Siegel MJ. Pharmacological testing in the laser-induced monkey glaucoma model. *Curr Eye Res.* 1985;4:775-781.
30. Lipton SA, Sucher NJ, Kaiser PK, Dreyer EB. Synergistic effects of HIV coat protein and NMDA receptor–mediated neurotoxicity. *Neuron.* 1991;7:111.
31. Hochberg Y, Tamhane AC. *Multiple Comparison Procedures.* New York, NY: John Wiley & Sons Inc; 1987.
32. *SAS/STAT User's Guide v6.* 4th ed. Cary, NC: SAS Institute; 1989.
33. Rosenberg P, Amin S, Leitner M. Glutamate uptake disguises neurotoxic potency of glutamate agonists in cerebral cortex in dissociated cell culture. *J Neurosci.* 1992;12:56-61.
34. Choi DW, Maulucci-Gedde MA, Kriegstein AR. Glutamate neurotoxicity in cortical cell culture. *J Neurosci.* 1987;7:357-368.
35. Patneau DK, Mayer ML. Structure-activity relationships for amino acid transmitter candidates acting at *N*-methyl-D-aspartate and quisqualate receptors. *J Neurosci.* 1990;10:2385-2399.
36. Samy CN, Lui CJ, Kaiser PK, Lipton SA, Dreyer EB. Toxicity of chronic glutamate administration to the retina. *Invest Ophthalmol Vis Sci.* 1994;35(suppl):497.
37. Fechtner RD, Weinreb RN. Mechanisms of optic nerve damage in primary open angle glaucoma. *Surv Ophthalmol.* 1994;39:23-42.
38. Faden AI, Demediuk P, Panter SS, Vink R. The role of excitatory amino acids and NMDA receptors in traumatic brain injury. *Science.* 1989;244:798-800.
39. Benveniste H, Drejer J, Schousboe A, Diemer NH. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J Neurochem.* 1984;43:1369-1374.
40. Svenneby G, Torgner IA. Localization and function of glutamine synthetase and glutaminase. *Biochem Soc Trans.* 1987;15:213-215.
41. Lipton SA. Prospects for clinically tolerated NMDA antagonists. *Trends Neurosci.* 1993:16:527-532.