Vitreal Glutamate Concentration in Monkeys with Experimental Glaucoma

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Purpose. To investigate the hypothesis that the pathophysiology for the death of retinal ganglion cells in glaucoma involves excitotoxic effects from elevated concentrations of vitreal glutamate.

Methods. Experimental glaucoma was induced in the right eyes of 18 rhesus monkeys by argon laser treatments to the trabecular meshwork. After significant visual field defects and/or typical clinical glaucomatous changes had developed (1.5–13 months), the eyes were removed, and a sample (0.1–0.2 mL) of posterior vitreous was collected. Similar vitreous samples also were collected from eight untreated monkeys. The vitreous samples were analyzed in a masked fashion by high-pressure liquid chromatography in two independent laboratories. Mean levels of vitreal glutamate were determined for the treated and control eyes and differences between groups of eyes were evaluated by Student's *t*-test.

Results. The mean level (± SD) of vitreal glutamate in the eight untreated monkeys was 5.0 ± 2.0 μM. A similar level of 5.7 ± 1.8 μM was measured in the untreated eyes of monkeys with experimental glaucoma. In the glaucomatous eyes, the mean concentration of vitreal glutamate was 5.7 ± 2.6 μM, which was not significantly different from the concentrations in the control eyes.

Conclusions. Vitreal glutamate concentrations were not elevated in eyes with anatomic and functional damage from experimental glaucoma. This finding is in contradiction to previous reports that vitreal glutamate increases to toxic levels and probably contributes to glaucomatous damage of retinal ganglion cells. (*Invest Ophthalmol Vis Sci.* 2002;43:2633–2637)

Primary open-angle glaucoma (POAG) is an ocular disorder typically characterized by elevated intraocular pressure and deficits in visual function as a result of ganglion cell injury and death. Several hypotheses have been proposed and investigated to explain the mechanisms that trigger injury and death of ganglion cells, including damage to the optic nerve at the lamina cribrosa,[1–3] blockage of retrograde transport of trophic factors,[4] increased production of nitric oxide,[5] autoimmune mechanisms,[6–10] and elevated vitreal glutamate.[11] In their initial studies, Dreyer et al.[11] reported that vitreal glutamate concentrations were elevated in all forms of glaucoma to concentrations twice that in control eyes in patients, by a factor of six to eight times in monkeys with experimental glaucoma,[11] and by four times in dogs with naturally occurring glaucoma.[12] Based on the reports of elevated vitreal glutamate, glutamate excitotoxicity has been proposed to contribute to ganglion cell death, which has led to clinical trials to test the efficacy of compounds that block the action of glutamate at the *N*-methyl-D-aspartate (NMDA) receptor as potential therapy in glaucoma.

Because of the potential importance of excitotoxicity in the progression and treatment of glaucoma, the present study was designed to replicate the previous results of elevated vitreal glutamate in experimental glaucoma and further elaborate the hypothesis of excitotoxic effects that contribute to the death of retinal ganglion cells. Our studies included a relatively large number of monkeys with experimental glaucoma, a group that was six times larger than that of the previous study by Dreyer et al.[11] The study design included masked analyses of vitreous samples in two independent laboratories by reversed-phase high-pressure liquid chromatography to determine the concentrations of glutamate and 18 other amino acids.

Methods

Subjects

Intraocular pressure was elevated in the right eyes of 18 adult monkeys (*Macaca mulatta*) by Argon laser treatment of the trabecular meshwork.[13–15] In these animals, vitreous samples from the untreated left eyes served as the control but, in addition, vitreous from both eyes of eight untreated monkeys was analyzed. Details of the trabecular ablation and intraocular pressure measurements have been published.[16] The intraocular pressure in both eyes was measured weekly by handheld applanation tonometry, with the mean of three measurements taken as the intraocular pressure. Typically, elevated intraocular pressure was maintained between 35 and 50 mm Hg for 1.5 to 13 months (see Table 3). All procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Visual field defects for the 13 monkeys with experimental glaucoma at the University of Houston were assessed by behavioral perimetry measurements by using methods that have been described in detail.[16–19] For these measurements, a standard clinical field analyzer (Humphrey Field Analyzer; Humphrey Instruments, San Leandro, CA), was attached to a primate-testing cubicle, and the alert monkeys were trained to fixate and perform a manual detection task that is similar to patients' responses for clinical perimetry. After the training was completed, standard automated perimetry, with the 24-2 test pattern and the full-threshold test strategy with the size III white stimulus was used to assess the visual fields. The monkeys were highly competent subjects with visual field data that were essentially identical with data in humans.[16,19] Trabecular ablation was performed on the right eye of monkeys with normal visual fields. The onset and progression of visual field defects caused by experimental glaucoma were followed. Several examples of the gray-scale plots of visual fields are presented in Figure 1 to illustrate the normal (pretreatment data) and visual field defects near the time that the vitreous samples were collected. Only one

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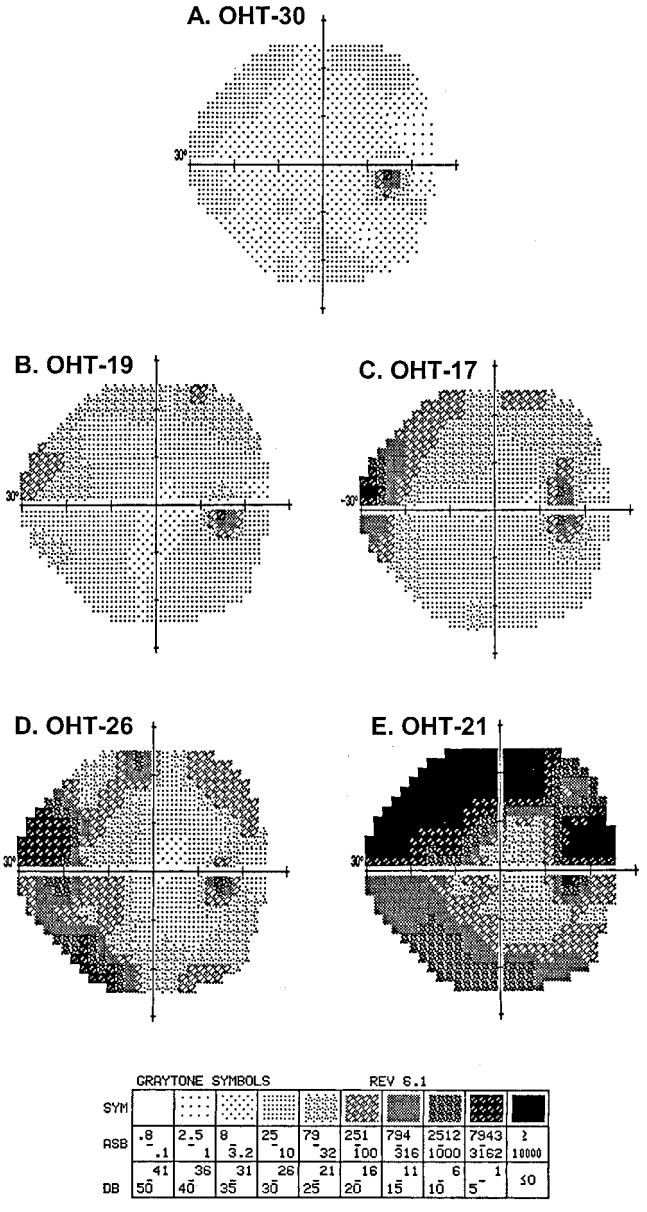

FIGURE 1. Visual field plots of eyes in five of the monkeys included in the study. These examples show (**A**) a normal visual field and gradations of mild to advanced (**B–E**, respectively) glaucomatous visual field damage. SYM, symbol; ASB, apostilbes; DB, decibel.

monkey, OHT-28, did not show development of a clinically significant mean deviation (MD) of the treated eye, even though the intraocular pressure was elevated for 4.5 months. However, the visual field of this monkey showed reliable changes, a superior nasal step and enlargement of the blind spot that are indicative of early glaucoma.

The intraocular pressure was elevated by trabecular ablation in another group of five monkeys at the University of Texas Houston Medical School by the method described earlier. Behavioral perimetric analyses of visual fields were not conducted. However, trabecular ablations and changes in the appearance of the optic nerve head were evaluated by one of the authors (RF), who is a glaucoma specialist. Documentation of the optic nerve head was made by stereophotographs at baseline and during subsequent evaluations. All the animals' optic nerve heads were considered normal at baseline. Additional evaluations were performed in some eyes with a nerve fiber analyzer (GDX; Laser Diagnostic Technologies, Inc., San Diego, CA), which confirmed the findings of glaucomatous optic neuropathy by changes in the nerve fiber layer (flattening of the normal curve).

Collection of Vitreous

Vitreous samples were obtained from deeply anesthetized monkeys, initially by insertion of an 18-gauge needle through the sclera into the posterior vitreous and gently withdrawing 0.1–0.2 mL. Subsequent collections of vitreous from most eyes were made after enucleation to eliminate concerns about blood contamination in the vitreous. However, a comparison of glutamate content in vitreous collected by aspiration to that collected immediately after enucleation and removal of the anterior segment did not show significant differences. In both cases, the undiluted vitreous samples were placed immediately on ice and subsequently frozen and stored at −80°C. Vitreous samples were transported to the two laboratories on dry ice and stored on arrival at −20°C.

Amino Acid Analysis

Two different laboratories analyzed glutamate content in the vitreous with precolumn derivatization and high-pressure liquid chromatography (HPLC) which permits detection of amino acids at picomolar levels. Vitreal glutamate concentration from both eyes of five untreated monkeys and nine treated monkeys was analyzed at Baylor College of Medicine in the Collagen Research Laboratory (laboratory 1) with dabsyl-chloride derivatization.[20] Aliquots of thawed vitreous (0.10 mL) were deproteinized by adding 0.05 mL trichloroacetic acid (5%), vortexed, and centrifuged. The pH of 100 μL of the supernatant was raised to 9.0, with 0.013 mL of 1 N sodium hydroxide; 40 μL of the supernatant was reacted with dabsyl chloride (100 μL) followed by dilution to 1 mL with 25 mM sodium acetate (pH 4.1) containing 4% methanol-acetonitrile. Amino acids were assayed on an amino acid analyzer (Spectra- Physics, Mountain View, CA), with a C-18 column and a linear gradient of 20% to 100% acetonitrile over 23 minutes at a flow rate of 1 mL/min. Absorption was measured at a fixed wavelength of 436 nm. Areas under the peak corresponding to glutamate were calculated by the integrator and the concentration determined from comparison to known concentrations of external standards (Sigma Chemical Co., St. Louis, MO) run at several different concentrations.

The concentrations of glutamate and 17 other amino acids were analyzed in vitreous samples from both eyes of three untreated monkeys, and 10 treated monkeys were also analyzed at Cornell University's BioResource Center, Amino Acid Analysis Facility (laboratory 2), by phenylisothiocyanate (PITC) derivatization.[21] Thawed samples were deproteinized, vortexed, and centrifuged, and aliquots were evacuated to dryness in an ethanol-water-triethylamine mixture (2:2:1 vol/vol/vol). Derivatization of dried samples was performed with freshly prepared ethanol-triethylamine PITC (7:1:1:1, vol/vol/vol/vol). The derivatized samples were evacuated to dryness, resuspended in 0.05 M ammonium acetate, and chromatographed by HPLC on a C-18 column (water-sodium acetate triethylammonium acetate-acetonitrile buffer system) at fixed wavelength detection and absorbance of 254 nm (a modified Pico-Tag System; Waters, Milford, MA). Acquisition and processing of data were performed with a computer-based system (EzChrom; LabAlliance, State College, PA) and external standards (Sigma). Unstable amino acids (asparagine, glutamine, and tryptophan) were freshly prepared. Samples and standard were batch processed and the injection volume verified by monitoring the derivatization artifact peaks that were present in each sample.

Statistical Analyses

The mean ± SD of vitreal amino acid concentrations was computed for each stratification method. Comparisons between laboratories were made using the two-sample *t*-test and multiple paired *t*-tests were used to compare the amino acid concentrations for the left and right eyes. $P < 0.05$, with appropriate adjustment for multiple tests, was considered statistically significant.

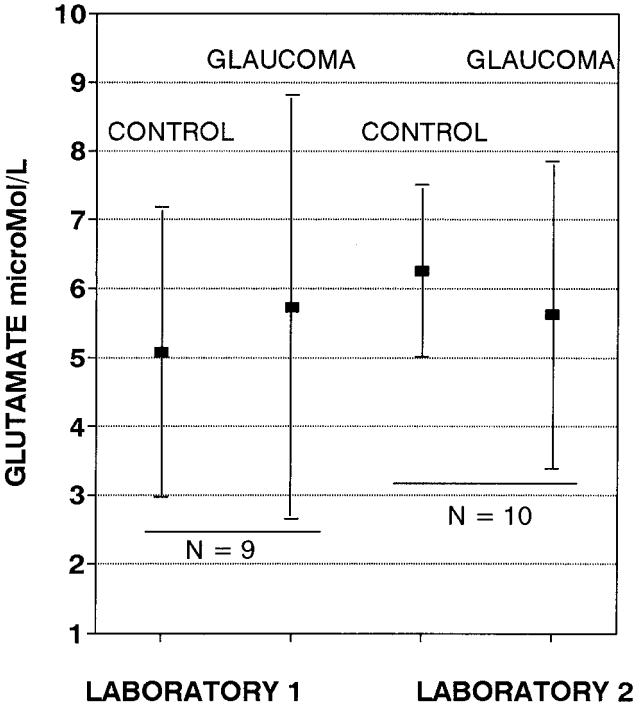

FIGURE 2. The mean ± SD vitreal glutamate concentration (μM) in normal and glaucomatous eyes of 18 monkeys, as determined in two independent laboratories from examination of masked samples.

RESULTS

The average vitreal glutamate concentration in both eyes of five of the untreated control monkeys was 5.1 ± 1.3 μM, as measured by laboratory 1. A similar concentration level of 4.9 ± 3.2 μM was measured in the samples from the remaining three untreated monkeys by laboratory 2 ($P = 0.85$). In nine monkeys with monocular experimental glaucoma, the average concentration of glutamate was 5.1 ± 2.1 μM in the fellow untreated left eyes and 5.7 ± 3.1 μM in the glaucomatous right eyes (laboratory 1; Fig. 2; $P = 0.54$). The average concentration of glutamate measured by laboratory 2 was 6.2 ± 1.3 μM in the fellow untreated eyes of 10 monkeys and 5.6 ± 2.3 μM in the glaucomatous eyes (Fig. 2; $P = 0.53$). None of the differences between any of the groups of vitreous samples analyzed in either of the independent laboratories reached statistical significance. In addition, the average concentrations of 13 of the other amino acids that were assayed were not significantly different in concentration between control and glaucomatous eyes (Table 1).

However, five amino acids (histidine, arginine, alanine, tryptophan, and lysine) were found to be significantly higher in the glaucomatous eyes by paired *t*-test, although not with adjustment for multiple statistical tests. Neither was it apparent that these amino acids share similar properties that promote cell damage. For example, histidine, which is often found in the active sites of enzymes and serves as a precursor for the neurotransmitter histamine, may increase as a result of ganglion cell loss and/or damage to centrifugal histamine containing axons present in macaque retina.[22] As is histidine, tryptophan and lysine are essential amino acids; thus, reduced utilization may lead to increased concentrations. In nervous tissue, arginine and alanine are known to be sequestered in glia,[23–25] and efflux from Müller cells[26] and astrocytes[27] is enhanced by extracellular glutamate. The relationship of these amino acids to glaucoma is not presently understood but warrants further investigation.

TABLE 1. Amino Acids in Vitreous

Amino Acid	Control	Glaucoma	*P*
Asparagine	20.46 ± 3.22	20.82 ± 3.7	0.7019
Serine	57.32 ± 10.05	67.08 ± 19.46	0.0902
Glutamate	6.20 ± 1.26	5.63 ± 2.27	0.525
Glutamine	655.45 ± 93.87	692.31 ± 84.16	0.1531
Glycine	6.93 ± 1.05	6.91 ± 2.33	0.9869
Histidine	17.04 ± 2.18	22.39 ± 5.68	0.0116*
Arginine	54.96 ± 9.97	78.82 ± 25.83	0.0056*
Threonine	30.37 ± 6.46	30.03 ± 5.55	0.8395
Alanine	50.72 ± 9.98	69.7 ± 17.97	0.0082*
Proline	13.33 ± 3.09	31.57 ± 29.68	0.069
Tyrosine	25.93 ± 7.68	28.41 ± 8.22	0.2525
Valine	67.09 ± 11.2	64.61 ± 5.73	0.489
Methionine	17.52 ± 4.17	17.59 ± 5.03	0.9558
Cysteine	4.73 ± 0.67	10.65 ± 8.86	0.0609
Isoleucine	27.49 ± 3.74	25.84 ± 2.35	0.1976
Leucine	71.71 ± 12.34	67.05 ± 7.08	0.2002
Phenylalanine	29.66 ± 6.48	30.03 ± 5.13	0.8329
Tryptophan	18.59 ± 2.7	27.14 ± 9.41	0.0163*
Lysine	59.7 ± 14.03	90.24 ± 35.63	0.0066*

Data from 10 monkeys are expressed as mean micromoles per liter ± SD.

* Significantly elevated according to paired *t*-test, but not according to multiple tests.

Table 2 shows the content of vitreal glutamate concentration in the right and left eyes in each of the eight untreated control monkeys. Vitreal glutamate concentration in the eyes of monkeys with monocular glaucoma is seen in Table 3, along with mean, peak, and intraocular pressure at or near death; mean deviation; and duration of elevated intraocular pressure. In most of the eye pairs, the concentrations of glutamate were similar between the two eyes, and they were within 2 standard deviations of the mean value.

DISCUSSION

We have determined the vitreal glutamate concentration in 26 monkeys, 8 normal and 18 with unilateral experimental glaucoma, as measured from masked samples by two independent laboratories. There were no significant differences in vitreal glutamate concentration between vitreous from normal control eyes and glaucomatous eyes, nor was there a significant difference in the results between the analyses performed in two independent laboratories. The data from all analyses showed the vitreal glutamate concentration to be approximately 5 μM and to be unrelated to the condition of glaucoma.

The current finding is in marked contrast to the report by Dreyer et al.[11] that glutamate concentration in vitreous of normal monkey eyes was approximately 12 μM, whereas in glaucomatous eyes the anterior vitreous concentration was

TABLE 2. Glutamate Concentration in Vitreous of Untreated Control Monkeys

Laboratory	Monkey	Left Eye	Right Eye
1	1	5.7	6.6
	2	5.3	3.8
	3	5.7	4.1
	4	5.7	6.9
	5	4.0	3.3
2	6	3.5	3.3
	7	3.4	4.4
	8	11.3	3.4

Data are expressed in micromoles per liter.

TABLE 3. Monocular Glaucoma

Laboratory	Monkey	Control Vitreous Glutamate (μmol/L)	Glaucoma Vitreous Glutamate (μmol/L)	Mean Control IOP (mm Hg)	Mean Elevated IOP (mm Hg)	Peak IOP (mm Hg)	IOP at or Near Death	Mean Deviation (dB)	Duration (mo)
1	OHT-01	9.4	3.8	19.8 ± 2.4	26.0 ± 4.1	30	25		1.5
	OHT-17	4.3	4.4	19.7 ± 4.8	37.0 ± 18.2	60	60	−5.93	1.5
	OHT-18	7.9	13.2	13.5 ± 2.5	28.4 ± 10.9	41	20	−18.58	13
	OHT-19*	3.9	4.5	17.0 ± 2.8	37.1 ± 13.8	56	56	−3.85	3.0
	OHT-20	4.3	5.9	18.8 ± 3.4	31.7 ± 16.8	56	37	−17.51	12
	OHT-21	4.8	4.3	15.6 ± 1.7	35.4 ± 12.7	43	25	−19.22	3.5
	OHT-22	3.8	3.9	14.1 ± 3.1	50.3 ± 5.1	56	26	−23.20	3.0
	OHT-23	3.2	7.8	14.2 ± 3.3	44.4 ± 14.8	60	44	−11.70	3.0
	OHT-24	4.2	4.0	17.2 ± 3.6	51.9 ± 19.2	60	51	−5.19	11
2	OHT-19*	7.2	3.6	17.0 ± 2.8	37.1 ± 13.8	53	56	−3.85	10
	OHT-25	5.1	6.7	11.0 ± 0.8	47.0 ± 6.4	56	18	−7.44	12
	OHT-26	5.2	3.3	13.1 ± 1.1	32.3 ± 14.0	52	52	−8.53	2.0
	OHT-28	4.9	3.8	12.3 ± 1.9	34.8 ± 14.5	51	45	−0.06	4.5
	OHT-30	6.7	7.6	8.4 ± 2.2	31.8 ± 11.1	51	51	−12.21	2.0
	OHT-31	4.9	10.4	13.8 ± 3.4	35.7 ± 17.2	60	60	−30.35	2.0
	OHT-749	7.6	6.3	17.4 ± 1.0	45.9 ± 6.6	54	40		10.0
	OHT-923	7.4	6.4	18.8 ± 1.5	39.1 ± 14.4	52	52		6.1
	OHT-937	7.9	4.0	17.2 ± 2.2	39.2 ± 12.2	50	50		7.2
	OHT-967	5.1	4.2	21.6 ± 1.4	33.1 ± 7.2	46	25		6.5

* Vitreous analyzed in both laboratories.

59.7± 7.3 and 80.3± 7.8 μM in posterior vitreous. None of the average concentrations from the current studies approach these levels. However, the present data are in close agreement with those reported recently for vitreal glutamate in patients with glaucoma (6.1 ± 1.6 μM) and in control subjects (5.3± 2.2 μM).[28]

The disparity between the current findings and those reported by Dreyer et al.[11] is difficult to explain, especially the exceptionally high concentrations that they reported. For example, an inappropriate handling of the samples before analysis can cause an increase in glutamate and aspartate through nitrogen loss from asparagine and glutamine, but from the information provided, it is not apparent that such technical problems were involved. Otherwise, the small sample size of three monkeys in the previous study may represent a random selection of extreme values, but that would be unlikely. In short, the current results cast doubt on the validity of the previous results, as discussed previously.[29]

It is very important to note, however, that the current findings on glutamate concentration in the vitreous chamber do not eliminate the role for glutamate excitotoxic damage in glaucoma. Glutamate is normally removed from the extracellular space by glutamate transporters. In the inner plexiform layer, there are three transporters involved in this task: GLT-1, located in the bipolar cell terminals; EAAC1 on ganglion cells; and GLAST in Müller cells. The glutamate that is transported into Müller cells is converted to glutamine in large part, but some is also used to form the glutathione that is found in abundance in Müller cells. Image analysis of both glutamine[30] and glutathione (Carter-Dawson et al., unpublished observation, 1997) immunoreactivity have shown that both are significantly elevated in Müller cells in monkeys' eyes with experimental glaucoma. Immunolabeling for GLAST is also increased in these retinas. Increases in glutamine, glutathione, and GLAST content in glaucomatous monkey eyes indicate an elevation in extracellular glutamate and enhanced glutamate transport and metabolism. Thus, although the results from the present study refute the hypothesis that vitreal glutamate is found at concentrations that are toxic to ganglion cells in monkeys with experimental glaucoma, the possibility of excitotoxic damage to ganglion cells as a consequence of elevated extracellular levels should not be dismissed.

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References

1. Gaasterland D, Tanishima T, Kuwabara T. Axoplasmic flow during chronic experimental glaucoma. 1: light and electron microscopic studies of the monkey optic nerve head during development of glaucomatous cupping. *Invest Ophthalmol Vis Sci.* 1978;17:838–846.
2. Minckler DS, Spaeth GL. Optic nerve damage in glaucoma. *Surv Ophthalmol.* 1981;26:128–148.
3. Quigley HA, Addicks EM. Chronic experimental glaucoma in primates. II: effect of extended intraocular pressure elevation on optic nerve head and axonal transport. *Invest Ophthalmol Vis Sci.* 1987;24:1305–1307.
4. Quigley HA. Ganglion cell death in glaucoma: pathology recapitulates ontogeny. *Aust NZ J Ophthalmol.* 1995;23:85–91.
5. Neufeld AH. Nitric oxide: a potential mediator of retinal ganglion cell damage in glaucoma. *Surv Ophthalmol.* 1999;43(suppl 1): S129–S135.
6. Cartwright MJ, Grajewski AL, Friedberg ML, et al. Immune-related disease and normal-tension glaucoma. *Arch Ophthalmol.* 1992; 110:500–502.
7. Wax MB, Barrett DA, Pestrok A. Increased incidence of paraproteinemia and autoantibodies from patients with normal pressure glaucoma. *Arch Ophthalmol.* 1994;117:561–568.
8. Wax MB, Tezel G, Edward DP. Clinical and pathological findings of a patient with normal pressure glaucoma. *Arch Ophthalmol.* 1998; 116:993–1001.
9. Wax MB. Is there a role for the immune system in glaucomatous optic neuropathy? *Curr Opin Ophthalmol.* 2000;11:145–150.
10. Patil RV, Yu H, Gordon M, Wax MB. T cell subsets and sIL-2R/IL-2 levels in patients with glaucoma. *Am J Ophthalmol.* 2001;31:421–426.

11. Dreyer EB, Surakowski D, Schumer RA, Podos SM, Lipton SA. Elevated glutamate levels in the vitreous body of humans and monkeys with glaucoma. *Arch Ophthalmol.* 1996;114:299–305.
12. Brooks DE, Garcia GA, Dreyer EB, Zurakowski D, Franco-Bourland RE. Vitreous body glutamate concentration in dogs with glaucoma. *Am J Vet Res.* 1997;58:864–867.
13. Gassterland DE, Kupfer C. Experimental glaucoma in the rhesus monkey. *Invest Ophthalmol.* 1974;13:455–457.
14. Pederson JE, Gaasterland DE. Laser-induced primate glaucoma. 1: progression of cupping. *Arch Ophthalmol.* 1984;102:1689–1692.
15. Quigley HA, Holman RM. Laser energy levels for trabecular meshwork damage in the primate eye. *Invest Ophthalmol Vis Sci.* 1987;24:1305–1307.
16. Harwerth RS, Smith EL, DeSantis L. Behavioral perimetry in monkeys. *Invest Ophthalmol Vis Sci.* 1993;34:31–40.
17. Harwerth RS, Smith EL, DeSantis L. Experimental glaucoma: perimetric field defects and intraocular pressure. *J Glaucoma.* 1997; 6:390–401.
18. Harwerth RS, Carter-Dawson L, Shen F, Smith EL III, Crawford ML. Ganglion cell losses underlying visual field defects from experimental glaucoma. *Invest Ophthalmol Vis Sci.* 1999;40: 2242–2250.
19. Harwerth RS, Crawford MLJ, Frishman LJ, Viswanathan S, Smith EL, Carter-Dawson L. Visual field defects and neural losses from experimental glaucoma. *Prog Retinal Eye Res.* 2002;21:91–125.
20. Drnevich D, Vary TC. Analysis of physiological amino acids using dabsyl derivatization and reversed-phase liquid chromatography. *J Chromatogr.* 1993;613:137–144.
21. Bidlingmeyer BA, Cohen SA, Tarvin TL. Rapid analysis of amino acids using pre-column derivatization. *J Chromatogr.* 1984;336: 93–104.
22. Gastinger MJ, O'Brien JJ, Larsen NB, Marshak DW. Histamine immunoreactive axons in the Macaque retina. *Invest Ophthalmol Vis Sci.* 1999;40:487–495.
23. Yudkoff M, Nissim I, Hummeler K, Medlow M, Pleasure D. Utilization of [^{15}N]glutamate by cultured astrocytes. *Biochem J* 1986; 234:185–192.
24. Poitry-Yamate CL, Poitry S, Tsacopoulos M. Lactate released by Müller glial cells is metabolized by photoreceptors from mammalian retina. *J Neurosci.* 1995;15:5179–5191.
25. Aoki E, Semba R, Mikoshiba K, Kashiwamata S. Predominate localization in glial cells of free L-arginine: immunocytochemical evidence. *Brain Res.* 1991;559:159–162.
26. Poitry S, Poitry-Yamate CL, Ueberfeld J, MacLeish PR, Tsacopoulos M. Mechanisms of glutamate metabolic signaling in retinal glial (Müller) cells. *J Neurosci.* 2000;20:1809–1821.
27. Grima G, Benz B, Do KQ. Glutamate-induced release of the nitric oxide precursor, arginine from glial cells. *Eur J Neurosci.* 1997;9: 2248–2258.
28. Honkanen RA, Weaver YK, Baruah S et al. Vitreous amino acid levels in patients with glaucoma undergoing vitrectomy [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 2001;42(4):**S**314. Abstract 1701.
29. Dalton R. Private investigations. *Nature.* 2001;411:129–130.
30. Carter-Dawson L, Shen F, Harwerth RS, Smith EL, Crawford MLJ, Chuang A. Glutamine immunoreactivity in Müller cells of monkey eyes with experimental glaucoma. *Exp Eye Res.* 1998;66:537–545.