Vitreous Amino Acid Concentrations in Patients With Glaucoma Undergoing Vitrectomy

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Objective: To measure vitreous concentrations of glutamate and other amino acids in patients with glaucoma undergoing vitrectomy.

Methods: Undiluted vitreous samples were collected from patients undergoing vitrectomy at the University of Iowa (Iowa City) between 1997 and 1998 (n=69). Vitreous concentrations of 16 amino acids, including glutamate, were determined using high-pressure liquid chromatography. Patients with a history of diabetes mellitus were excluded from the analysis. The study group consisted of those with a history of glaucoma (n=8), and the control group included those with an epiretinal membrane and/or macular hole with no history of glaucoma (n=17). Comparison of amino acid concentrations between the 2 groups was performed using a multifactor main effects model that adjusted for the effect of 10 selected covariates. Power analysis was done to determine the level of significant difference in amino acid concentrations.

Results: The glaucoma group comprised vitreous specimens from patients with primary open-angle (n=3) and angle-closure glaucomas that included aqueous misdirection (n=2), uveitis with secondary angle-closure (n=2), and Axenfeld Rieger syndrome (n=1). Indications for vitrectomy in this group included epiretinal membrane, retinal detachment, aqueous misdirection, and uveitis. The control group included specimens from patients with a macular hole (n=11) and epiretinal membrane (n=7), with 1 eye having both. Surgical indications in controls were macular hole, retinal detachment, and epiretinal membrane. The mean±SD levels of vitreous glutamate, glycine, γ-aminobutyric acid, and alanine were 6.1±2.4, 16.3±7.5, 0.8±0.3, and 260.5±101.9 µM, respectively, in glaucoma and 5.2±2.3, 8.5±2.5, 0.6±0.2, and 159.5±54.9 µM in controls (P<.05 for all). None of the 16 amino acid concentrations measured showed a statistically significant difference between glaucoma and controls (P values between .06 and >.99). A power analysis indicated that a 1.8-fold elevation in the glutamate level was needed to reach significance.

Main Outcome Measures: Vitreous amino acid concentrations.

Conclusions: None of the 16 amino acids measured, including glutamate, were significantly elevated in the vitreous of glaucomatous eyes compared with controls. Our results are not consistent with the simple hypothesis of glutamate excitotoxicity in glaucoma. Instead, our findings indicate the dynamic nature of extracellular glutamate, whose concentration is dependent on complex mechanisms not yet fully understood. Further studies are needed to fully elucidate the role of glutamate in the pathogenesis of glaucoma.

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Glaucoma is an important cause of blindness worldwide. It is the second leading cause of blindness in the United States and is the leading cause among older African Americans. There is evidence that lowering intraocular pressure slows the progression of glaucoma. Recently, much research has been directed at the elucidation of biochemical and molecular mechanisms of optic nerve damage in glaucoma, with a hope of finding additional treatment modalities.

Glutamate, an excitatory amino acid, has been identified as a possible key element in the pathogenesis of glaucoma. There is evidence that experimentally elevated glutamate levels can induce retinal ganglion cell death both in vitro and in vivo. The excitotoxic effect of glutamate on retinal ganglion cells can be mitigated by compounds that block glutamate receptors. One study reported significantly elevated levels of glutamate in the vitreous of monkeys with experimentally induced glaucoma and in human patients with glaucoma who were undergoing cataract sur-
We conducted the present study to provide additional evidence that the level of glutamate in the vitreous is elevated in patients with glaucoma. One important methodological difference between our study and the previous study is the way the vitreous specimen was collected in human patients. Rather than collecting the vitreous sample during unplanned complication of cataract surgery, we collected it at the beginning of planned vitrectomies to avoid inadvertent contamination. Our study did not provide the expected result, and raises questions about the dynamic nature of the levels of extracellular glutamate in glaucoma.

METHODS

PATIENTS

The study protocol was reviewed and approved by the institutional review board at the University of Iowa (Iowa City) prior to initiation of the study. All study patients provided informed consent prior to enrollment. Any patient undergoing vitrectomy surgery over a 15-month period (January 1997 to April 1998) at the University of Iowa was eligible to participate. The following clinical characteristics of the study patients were compiled for statistical analysis: age, sex, race, eye affected, systemic hypertension, number of systemic medications, ocular diagnoses, number of eye medications, number of prior eye surgeries, prior cataract surgery, preoperative and postoperative visual acuity, preoperative intraocular pressure (IOP), and cup-disc ratio. The cup-disc ratio was obtained by averaging the horizontal and vertical cup-disc ratios. All cup-disc ratios were subjectively assessed by one of us (R.A.H.).

SAMPLE COLLECTION AND AMINO ACID ANALYSIS

At the start of each vitrectomy, 100 to 500 µL of fluid from the mid-vitreous was obtained with an automated vitrector through a standard pars plana incision, prior to infusion of any irrigating solution into the eye. The samples were placed on ice, taken to the laboratory, and immediately centrifuged for 3 minutes at room temperature to separate cellular components from the liquid vitreous. Only the liquid supernatant was saved and stored at −70°C until the time of amino acid analysis.

Samples were consecutively numbered for identification. Vitreous amino acid analysis was performed in small batches using only the sample number, without the knowledge of patients' clinical information. Sample analysis was performed in the department of psychiatry. Nineteen amino acids, including alanine, arginine, asparagine, aspartate, γ-aminobutyrate (GABA), glutamine, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, taurine, threonine, tyrosine, and valine, were analyzed using high-pressure liquid chromatography (HPLC).10 Concentrations of amino acids were determined by first adding 200 nmol of allothreonine as internal standard and then deproteinizing with 0.20 mL of 50mM picric acid. After centrifugation at 16000g for 3 minutes, the supernatant was filtered through a 0.22-µm filter. Amino acid analysis was performed following precolumn derivatization with ophthaldehyde. Twenty-milliliter samples were mixed with 60 µL of 2.0 mg/mL solution of ophthaldehyde containing 0.01% solution of 2-mercaptobenzothiazole. Samples were derivatized for 2.0 minutes prior to being injected into a reverse-phase C-18 Altima column (3 mm, 15 cm × 4.6 cm) (Alltech, Waukegan, Ill). A Gilson model 231 autosampler (Middleton, Wis) was routinely used for derivatization and injection. The column was maintained at 40°C. The variable mobile phases were 50 mM of sodium acetate (pH 5.65) with 4% acetonitrile and methanol. The HPLC consisted of an SCL-6A system controller, 2 LC-6A pumps, and an RF-535 fluorescence detector (Shimadzu, Columbia, Md). The detector was set at an excitation wavelength of 345 nm and an emission wavelength of 445 nm. Data were collected and analyzed using a Chromatopac C-R4A integrator (Shimadzu).

RELIABILITY OF AMINO ACID ANALYSIS

We tested the reliability of the amino acid analysis performed at the University of Iowa by sending vitreous samples to 2 other independent laboratories. Five randomly selected vitreous samples were divided into 3 equal aliquots and sent to the laboratories of the Mayo Clinic (Rochester, Minn) and St Louis University (St Louis, Mo) as well as University of Iowa. The amino acid analysis was performed using HPLC at the Mayo Clinic and University of Iowa, while gas chromatography was used at St Louis University. The levels of amino acids from the 3 laboratories were compared, and any amino acid whose relative values were not comparable between laboratories was excluded from the study. The acceptable range for comparable values was defined as within 3.3 SDs of the concentration ratio between any 2 laboratories.11 Two amino acids (taurine and valine) exceeded this range and thus, were excluded from further analysis. In addition, arginine was excluded because it was not analyzed at St Louis University. With the remaining amino acids, we calculated the coefficient of variation (CV) for the 3 laboratory values as a measure of interlaboratory variation. The CV for phenylalanine was the lowest at 11.2%. Asparagine had the largest variation (124.2%). Glutamate had a CV of 65.3%. The rest of the amino acids had CV between 52.4% (leucine) and 97.4% (methionine).

Vitreous fluids from the patients were analyzed by HPLC in batches of 15 to 20 samples over a period of 12 months. Multiple calibration standards were included in each batch to be analyzed. As a measure of variation within our own laboratory, the CV was calculated based on 50µM calibration standards repeated 12 different times. The intralaboratory CV was lowest for phenylalanine (2.6%) and highest for lysine (27.0%) and glutamine (28.7%). The CV for glutamate was 6.6%. The CV for the other amino acids ranged from 5.7% (histidine) to 10.7% (glycine and serine).

STATISTICAL ANALYSIS

Mean amino acid concentrations in patients with glaucoma and controls were first compared using the 2-tailed t test. Ten possible independent variables (age, sex, eye affected, presence of retinal detachment, number of prior eye surgeries, prior cataract surgery, systemic hypertension, number of systemic medications, preoperative and postoperative visual acuity) were examined for any correlation to the observed amino acid levels. Other clinical variables (number of eye medications, number of glaucoma medications, preoperative IOP, and cup-disc ratio) were not separately evaluated because they were already closely correlated with the diagnosis of glaucoma. The 10 variables were each added separately in an analysis of covariance (for continuous variables) or a 2-way analysis of variance (for categorical variables). For each amino acid, variables with a P value less than or equal to .10 were then used as covariates in a multivariate main effects model that tested for the difference in mean amino acid levels between vitreal specimens of patients with glaucoma and controls, adjusting for the effect of the selected covariates. The Bonferroni correction was used to adjust the P values for the 16 amino acids tested. A Bonferroni-adjusted P value less than or equal to .05 was used for
determining statistical significance in the comparison between the glaucoma and control groups. Some of the amino acids did not have a normal distribution. For these amino acids (alanine, aspartate, glycine, lysine, and serine), a natural logarithm transformation was applied to the data values prior to analysis to normalize the data distribution. For glutamine, a reciprocal transformation was used. Results are presented as mean±SD unless otherwise specified.

RESULTS

During the 15-month period beginning January 1997, we obtained 69 vitreous samples from 69 patients. Of these, 8 specimens from the eyes of patients with glaucoma without diabetes comprised the study group (Table 1). Glaucoma diagnoses consisted of primary open-angle glaucoma (n=3) and various angle-closure glaucomas (n=5), including aqueous misdirection (n=2), uveitis with secondary angle-closure (n=2), and Axenfeld-Rieger syndrome (n=1). Indications for vitrectomy in study eyes included epiretinal membrane, retinal detachment, aqueous misdirection, and uveitis (Table 1). For the control group, 17 specimens from eyes with epiretinal membrane and/or macular hole were selected. None of the control patients had a diagnosis of diabetes or glaucoma. Indications for vitrectomy in controls were macular hole, retinal detachment, and epiretinal membrane (Table 1). The remaining 44 specimens obtained from diabetic patients or from those with multiple ocular conditions confounding clear categorization were excluded from the study.

Clinical and ocular characteristics of the 2 groups are presented in Table 2. All patients in both groups were white. The patients with glaucoma were taking more ocular medications, mainly to treat glaucoma (mean, 3.5 vs 0.1 medications). As expected, the preparotheic intraocular pressure (mean, 24.2 mm Hg) and cup-disc ratio (mean, 0.5) were greater in the glaucoma group. The visual acuity decreased following vitrectomy in the glaucoma group, while it increased in the control group, consistent with the greater degree of pathologic ocular findings found in the glaucoma group. Five patients with glaucoma underwent formal visual field examination (Goldmann; Haag-Streit, Bern, Switzerland [n=3]; Humphrey; Humphrey-Zeiss, Dublin, Calif [n=2]) prior to vitrectomy. The 3 Goldmann visual fields showed generalized constriction, while the 2 Humphrey fields showed moderate global depression (mean deviations, –6.3 and –8.4; pattern standard deviations, 5.5 and 4.0). Prior glaucoma surgery for the glaucoma group included laser iridotomy (n=3), laser trabeculoplasty (n=1), trabeculectomy (n=3), and seton tube placement (n=2). Vitreous hemorrhage was not present in either group at the time of vitrectomy.

The vitreous glutamate, glycine, GABA, and alanine levels were 6.1±2.4, 16.3±7.5, 0.8±0.3, and 260.5±101.9 µM, respectively, in glaucomatous eyes and 7.1±2.3, 8.7±2.5, 0.6±0.2, and 158.3±60.5 µM in eyes without retinal detachment; TRD, traction retinal detachment. For the control group, the glutamate, glycine, GABA, and alanine levels were 6.5±2.8, 12.7±2.5, 0.9±0.3, and 256.7±122.6 µM, respectively, in glaucomatous eyes and 4.3±1.6, 12.7±0.9, 0.9±0.3, and 256.7±122.6 µM, respectively, for the open-angle (n=3) and 7.1±2.3, 18.5±9.0, 0.8±0.3, and 262.7±103.1 µM for the closed-angle (n=5) types. Two patients with uveitis and angle-closure glaucoma had relatively higher levels of glutamate (8.7 and 8.2 µM), glycine (21.0 and 26.5 µM), and alanine (421.3 and 313.1 µM). One patient with Axenfeld-Rieger syndrome with aphakic, angle-closure glaucoma also showed relatively higher levels of glutamate (9.1 µM), glycine (26.2 µM), and alanine (191.2 µM). Two patients with retinal detachment showed relatively higher levels of alanine (286.9 µM) but lower levels of glutamate (3.5 µM) and glycine (12.5 µM). Unfortunately, these glaucoma subgroups were too small for any meaningful statistical analysis. Within the control group, the glutamate, glycine, GABA, and alanine levels were 6.5±2.8, 8.8±2.5, 0.6±0.3, and 163.5±37.8 µM, respectively, in eyes with retinal detachment (n=4), and 4.9±2.0, 8.4±2.6, 0.6±0.2, and 158.3±60.5 µM in eyes without retinal detachment (n=13) (P values: .30, .52, 0.73, and .79). Two-tailed Mann-Whitney test).

The 16 vitreous amino acid concentrations of the 2 groups are presented in Table 3. None of the 16 amino acid concentrations was significantly different. The alanine concentration difference approached statistical significance (P=.06). We performed a power analysis to determine the minimum amino acid concentration ratio (glaucoma-control) that would reach statistical significance (P=.05 with Bonferroni correction) with our sample size (Table 4). The glaucoma-control concentration ratios of 1.79, 1.92, and 1.89 would have reached statistical significance for glutamate, glycine, and alanine, respectively (with a power of .80).

COMMENT

Animal studies have shown that exogenously applied glutamate can be toxic to the inner retina.7,12 Recently, glutamate has been implicated in the pathogenesis of glaucoma by studies that reported elevated levels of vitreous glutamate in dogs with primary glaucoma13 and in monkeys with experimentally induced glaucoma.9 The latter study also
reported significantly elevated vitreous glutamate levels in human patients with glaucoma. Taken together, these studies suggest that glutamate is an important mediator of the retina ganglion cell damage in glaucoma.

Our study was performed to lend further support to the glutamate hypothesis of glaucoma, with improved methods and patient selection over the previous study as outlined below. (1) Instead of relying on unplanned complications of cataract surgery to obtain specimens, we collected vitreous samples at the beginning of planned posterior vitrectomy procedures, prior to infusion of any irrigating solution into the eye. This minimized the potential for contamination with irrigating solution, blood, or any unwanted ocular tissue (such as lens tissue). This is important because the plasma level of glutamate in humans is 5 to 6 times higher than the vitreous level. (2) We removed cellular components in the sample by centrifuging and taking only the supernatant vitreous fluid, before freezing and storage. The intracellular glutamate concentration is thought to be 10 to 100 times greater than that of the extracellular environment. Removing the cellular component avoided potential contamination from the release of intracellular glutamate arising from disrupted cell membranes. The previous study did not specifically address this issue. (3) We excluded all patients with diabetes from analysis. Ambati et al reported significantly elevated levels of vitreous glutamate in diabetic patients.

### Table 2. Clinical and Ocular Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Glaucoma (n = 8)</th>
<th>Control (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>64 ± 16</td>
<td>68 ± 14</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>9</td>
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<tr>
<td>White race</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Eye (right)</td>
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<td>8</td>
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<tr>
<td>Systemic disease</td>
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<td></td>
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<tr>
<td>Diabetes</td>
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<td>0</td>
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<tr>
<td>Hypertension</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>No. of systemic medications</td>
<td>2.4 ± 1.6</td>
<td>1.8 ± 1.4</td>
</tr>
<tr>
<td>No. of eye medications</td>
<td>3.5 ± 0.9</td>
<td>0.1 ± 0.2</td>
</tr>
<tr>
<td>No. of glaucoma medications</td>
<td>2.6 ± 1.3</td>
<td>0</td>
</tr>
<tr>
<td>No. of prior eye surgeries</td>
<td>1.5 ± 0.9</td>
<td>0.4 ± 0.5</td>
</tr>
<tr>
<td>Prior cataract surgery</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Eye examination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean preoperative visual acuity (logMAR)†</td>
<td>20/126 (0.8 ± 0.6)</td>
<td>20/126 (0.8 ± 0.3)</td>
</tr>
<tr>
<td>Mean postoperative visual acuity (logMAR)‡</td>
<td>20/159 (0.9 ± 0.7)</td>
<td>20/80 (0.6 ± 0.6)</td>
</tr>
<tr>
<td>Preoperative intraocular pressure, mm Hg</td>
<td>24.2 ± 13.0</td>
<td>16.0 ± 2.4</td>
</tr>
<tr>
<td>Cup-disc ratio§</td>
<td>0.5 ± 0.2</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Vitreous hemorrhage</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD unless otherwise indicated. †logMAR is defined as – log (visual fraction). ‡The postoperative visual acuity was taken from their last clinic visit. §The overall cup-disc ratio was obtained by averaging the horizontal and vertical cup-disc ratios. All cup-disc ratios were subjectively assessed by one of us (R.A.H.).

### Table 3. Vitreous Amino Acid Concentrations in the Control and Glaucomatous Eyes

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Glaucoma, µM</th>
<th>Control, µM</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>260.5 ± 101.9</td>
<td>159.5 ± 54.9</td>
<td>.06</td>
</tr>
<tr>
<td>Aspargine</td>
<td>42.4 ± 12.6</td>
<td>35.8 ± 11.6</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Aspartate</td>
<td>1.9 ± 1.8</td>
<td>1.4 ± 1.0</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>GABA</td>
<td>0.8 ± 0.3</td>
<td>0.6 ± 0.2</td>
<td>.96</td>
</tr>
<tr>
<td>Glutamate</td>
<td>6.1 ± 2.4</td>
<td>5.2 ± 2.3</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Glutamine</td>
<td>1203.9 ± 555.1</td>
<td>1192.9 ± 404.4</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Glycine</td>
<td>163.3 ± 7.5</td>
<td>8.5 ± 2.5</td>
<td>.16</td>
</tr>
<tr>
<td>Histidine</td>
<td>37.3 ± 13.2</td>
<td>38.4 ± 10.0</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>42.6 ± 9.8</td>
<td>37.9 ± 11.6</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Leucine</td>
<td>94.6 ± 20.1</td>
<td>89.7 ± 28.4</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Lysine</td>
<td>157.4 ± 60.5</td>
<td>115.4 ± 33.7</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Methionine</td>
<td>27.8 ± 8.9</td>
<td>22.3 ± 8.1</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>48.8 ± 14.5</td>
<td>44.4 ± 14.2</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Serine</td>
<td>128.4 ± 39.3</td>
<td>103.9 ± 24.4</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Threonine</td>
<td>100.0 ± 26.8</td>
<td>85.5 ± 28.4</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>62.7 ± 19.2</td>
<td>58.2 ± 18.8</td>
<td>&gt;.99</td>
</tr>
</tbody>
</table>

### Table 4. Power Analysis for the 16 Vitreous Amino Acids

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Glaucoma-Control Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>1.89</td>
</tr>
<tr>
<td>Aspargine</td>
<td>1.60</td>
</tr>
<tr>
<td>Aspartate</td>
<td>3.25</td>
</tr>
<tr>
<td>GABA</td>
<td>1.80</td>
</tr>
<tr>
<td>Glutamate</td>
<td>1.79</td>
</tr>
<tr>
<td>Glutamine</td>
<td>1.48</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.92</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.52</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.53</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.53</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.81</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.67</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.58</td>
</tr>
<tr>
<td>Serine</td>
<td>1.57</td>
</tr>
<tr>
<td>Threonine</td>
<td>1.59</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.59</td>
</tr>
</tbody>
</table>

Abbreviation: GABA, γ-aminobutyric acid.

*Data are presented as mean ± SD unless otherwise indicated. Statistical comparisons between the 2 groups were done using a multifactor main effects model that adjusted for the effect of 10 possible clinical covariates and with Bonferroni correction (see the “Methods” section).
ous glutamate in patients with proliferative diabetic retinopathy. Considering that patients with diabetes may have greater vascular permeability compared with those without diabetes, the exclusion of this population of patients is important. Similarly, we excluded all patients who had vitreous hemorrhage regardless of diabetic status because there are higher concentrations of glutamate in plasma. The previous study also did not specifically address this issue.

Our study demonstrates that vitreous glutamate concentrations are slightly higher in eyes with glaucoma (1.2 times the control) but the difference is not significant (Table 3). The power analysis indicated that we had a sufficient sample size to detect significance at 1.8 times the control level of glutamate. In contrast, the study by Dreyer et al showed that the glutamate level was 2.3 times higher in patients with glaucoma compared with controls (P < .001). The difference was even greater (up to 6.5-fold in the posterior vitreous) in the primate model of glaucoma. There may be several reasons for this discrepancy. First, it is difficult to directly compare the results of our study involving human subjects with those from a primate model of experimentally induced glaucoma. The previous study did not control for the laser exposure to the trabecular meshwork itself (using a sham surgical operation, for example). Thus, it is not clear whether the elevated vitreous glutamate concentration was due to glaucoma per se or to the other damaging effects of the laser application. The clinical characteristics of the primate model of glaucoma were not described in detail and thus, no direct comparison of types or severity of glaucoma is possible. Recently, a primate study failed to find elevated vitreous glutamate levels in a similar experimental model of glaucoma, consistent with our results in humans. Second, most subjects in our glaucoma group had angle-closure glaucoma, while other glaucoma parameters indicate the rest of the amino acids evaluated did not show a statistically significant difference in the previous study9 may have been elevated by factors other than glaucoma. Indeed, the control glutamate concentration in the previous study (∼10μM)9 was almost twice our control level (5.2μM).

Vitreous levels of glycine were not significantly different between glaucoma and control eyes (Table 3). This finding is consistent with previous results in human and primate subjects. However, vitreous levels of glycine were found to be significantly lower in dogs with glaucoma, and can also bind to coactivate the N-methyl-d-aspartate receptor. Presently, the role of glycine in glaucoma remains unclear. Vitreous levels of alanine were higher in glaucomatous eyes (1.63 times the control level) and this difference approached statistical significance. Previous studies found that vitreous alanine levels in glaucomatous eyes were significantly different from normal eyes (Table 1). The rest of the amino acids evaluated did not show a significant difference between glaucomatous eyes and controls, consistent with the previous studies.

There are several limitations of our study. (1) The number of study subjects was small and consequently, the study required at least 1.8 times the elevation of glutamate levels for statistical significance. However, it is pos-
sible that the 1.2 times the elevation of levels of vitreous glutamate found in our study, although not statistically sig-
nificant, may still be physiologically sufficient to mediate
cellular damage in glaucoma. (2) Our glaucoma popula-
tion was heterogeneous. If the vitreous glutamate level is not
uniform among different types of glaucoma, lumping them
all into a single group could lead to results that are diffi-
cult to interpret. In our study, the eyes with angle-closure
glaucoma showed higher glutamate levels (mean, 7.1µM,
n=5), especially when associated with uveitis (mean, 8.3µM,
n=2). Unfortunately, we are unable to draw any conclu-
sions from this observation because of the small number
of specimens in each subgroup. (3) The presence of reti-
nal detachment in some eyes may have affected vitreous
amino acid levels, including glutamate. There is evidence
for large shifts in the intracellular glutamate concentra-
tions in the retina following experimentally induced reti-
nal detachment in animals.21 However, we did not ob-
serve any significant difference in the vitreous concentra-
tions of glutamate, glycine, GABA, and alanine between control
eyes with and without retinal detachment (P>0.05, see the
“Results” section). Furthermore, we evaluated and ac-
counted for any possible correlation between the pres-
ence of retinal detachment and each of the 16 amino acid
concentrations before comparing the glaucomatous and con-
trol eyes, using the multifactor main effects model (see the
statistical analysis in the “Methods” section). (4) Our con-
trol group was not entirely normal. It is possible that the
epiretinal membrane or macular hole by itself could have
elevated vitreous glutamate levels, thus lessening the dif-
ference between the study and control groups. However,
the glutamate levels in our controls were lower than those
of the previous study.9 (5) While our intralaboratory varia-
tion of glutamate analysis was fairly reliable (CV, 6.6%),
the interlaboratory variation was much less reliable (CV,
65.5%). It is difficult to precisely identify the reason(s) for
discrepancies in the interlaboratory results. Differences in
sample collection and preparation, derivatization meth-
ods, chromatographic separation methods, and detection
methods may all contribute to observed variations. We have
previously reported large differences between HPLC and
gas chromatography/mass spectrometry levels of plasma
amino acids.22 On the other hand, our methods were able
to detect differences in glutamate concentrations as low as
0.5µM in brain synaptosome preparations (assuming a syn-
aptosomal protein level of about 8 mg/mL).23

In conclusion, we have shown that vitreous levels of
 glutamate were not significantly elevated in our human pa-
tients with glaucoma. As a major excitatory neurotrans-
mittor, glutamate’s extracellular level is tightly controlled
by an efficient glial reuptake mechanism.24 If glutamate is
truly a mediator of ganglion cell damage in glaucoma, then
it seems likely that it is subject to modulation by a com-
plex set of regulatory mechanisms for its reuptake, break-
down, and synthesis.25 Given the dynamic nature of glu-
tamate-induced cellular activity in both the extracellular
and intracellular compartments, it may not be surprising
to find different vitreous levels of glutamate among differ-
ent subjects and in different stages of glaucoma. Further
animal and human studies are needed to fully elucidate
the role of glutamate in glaucoma.

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