Vitreous Glutamate Concentration and Axon Loss in Monkeys With Experimental Glaucoma

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Objective: To evaluate vitreous glutamate concentration and axon loss in monkeys with experimental glaucoma.

Methods: We induced unilateral chronic glaucoma by means of laser trabecular destruction in 14 rhesus and 6 cynomolgus monkeys. Intraocular pressure (IOP) was monitored weekly. We assessed optic nerve damage clinically and photographically. Vitreous, sampled immediately before enucleation, was analyzed for glutamate concentration by means of high-performance liquid chromatography. We quantified percentage of axon loss after histopathologic sectioning of the optic nerve, compared median glutamate concentration ratios, and assessed correlation of glutamate concentration, axon count, IOP, cup-disc ratio, duration of IOP elevation, and age.

Results: Median vitreous glutamate concentration in glaucomatous eyes was 7.0 µmol/L (range, 3.0-88.6 µmol/L) vs 6.7 µmol/L (range, 2.8-87.4 µmol/L) in control eyes. The ratio (glaucomatous to control eyes) was 1.08. We found no significant correlation between vitreous glutamate concentration ratio and any of the other variables. The IOP, disc cupping, and axon loss were correlated.

Conclusions: We found no difference between vitreous glutamate concentration in glaucomatous and contralateral control monkey eyes when the entire data set was examined and no evidence of correlation between vitreous glutamate concentration and axon loss.

Clinical Relevance: Vitreous concentration of the excitotoxic amino acid glutamate, thought to be associated with retinal ganglion cell death in glaucoma, was not altered in this study.

Arch Ophthalmol. 2005;123:64-70

Glucoma is characterized by progressive loss of retinal ganglion cell axons and their cell bodies in the retina, usually in response to abnormally elevated intraocular pressure (IOP). The role of excitatory amino acids has been explored in various central nervous system disorders, and an association between central nervous system injury and glutamate has been demonstrated.1,2 Researchers have speculated about the role of glutamate in retinal ganglion cell injury in glaucoma.3,7

In 1996, Dreyer et al8 found 2-fold higher concentrations of glutamate in the vitreous of patients with glaucoma compared with controls and 6- to 8-fold higher levels of glutamate in 3 glaucomatous monkey eyes compared with fellow control eyes. However, in a group of patients undergoing vitrectomy, Honkanen et al9 reported no difference in vitreous glutamate concentrations between patients with glaucoma and control subjects. Carter-Dawson et al10 found no differences in vitreous glutamate concentration between experimental glaucoma and control monkey eyes. Conflicting results have also been obtained with the rat models of ischemia,11 optic nerve crush,12 and experimental glaucoma.12

Our main objective was to compare vitreous glutamate concentrations and optic nerve axon counts in glaucomatous and contralateral control eyes of monkeys. Our study included monkeys that were being prepared for other investigators whose protocols specified a variety of levels of IOP elevation for varying lengths of time.
4-week intervals, when ocular inflammation had subsided, each time leaving a different quadrant untreated, until an elevation in IOP was achieved. In no case did IOP decrease after elevation, requiring additional ALTS. Anesthesia for ALTS was induced with intramuscular ketamine hydrochloride (10 mg/kg) and acepromazine maleate (0.2-1 mg/kg). Some animals also received intramuscular methohexital sodium anesthesia for stereoscopic fundus photography, which was performed in fewer than half the animals before ALTS and death. Anesthesia for these procedures was induced with intramuscular ketamine hydrochloride (10 mg/kg), acepromazine maleate (0.2-1.0 mg/kg), and, if needed to eliminate eye movements, methohexital sodium (15 mg/kg).

All 6 of the cynomolgus monkeys that underwent ALTS (monkeys 15-20) had been used previously in multiple outflow facility experiments involving perfusion of the anterior chamber with various drug solutions. One rhesus monkey (monkey 14) had also undergone an anterior chamber perfusion.

Intraocular pressure was monitored under ketamine anesthesia weekly using a minifying Goldmann applanation tonometer (Haag-Streit, Koniz, Switzerland), occasionally backed up by measurements with a handheld applanation tonometer (Tonometer X; Mentor O & O, Norwell, Mass) if head or eye movements prevented accurate Goldmann readings. Intraocular pressure was measured before ALTS and weekly thereafter, with the monkey lying prone in a head holder.

Owing to the multiple protocols for which the animals were being prepared, IOP targets in treated eyes varied and ranged from approximately 20 to 30, 30 to 40, 40 to 50, and 50 to 60 mm Hg.

If the IOP was higher than desired or if there was any sign of discomfort due to elevated IOP, the monkeys were treated topically once or twice daily with a single drop of 1 or more of the following until the desired IOP was achieved: 0.5% timolol maleate in gel-forming vehicle (Timoptic-XE; Merck & Co, Whitehouse Station, NJ), 0.02% brimonidine tartrate (Alpha-12 µg in 5 µL of isotonic sodium chloride solution (donated by (Trusopt; Merck & Co), or prostaglandin F2α (Parsol; Allergan, Irvine, Calif), 2% dorzolamide hydrochloride (Avalon; Alcon, Fort Worth, Tex), or the fixative of Ito and Karnovsky.17

One rhesus monkey (monkey 14) had also undergone an anterior chamber perfusion.

CUP-DISC RATIO ASSESSMENT
Optic nerve damage was assessed clinically in the 17 monkeys with clear media by means of stereoscopic slitlamp biomicroscopy through dilated pupils. A single unmasked examiner (P.L.K.) estimated the cup-disc (C/D) ratios in experimental glaucoma (ExpG) and control eyes of all animals shortly before ALTS and again shortly before enucleation. The difference between C/D ratio before treatment and before enucleation was calculated for each animal.

TISSUE PROCESSING
Vitreous sampling was performed with the animals under deep pentobarbital sodium anesthesia (35 mg/kg administered intramuscularly or 15 mg/kg administered intravenously) immediately before enucleation in all eyes. Pupils were dilated with 2.5% phenylephrine hydrochloride and 1% tropicamide. A 23-gauge needle attached to a tuberculin syringe was inserted through the pars plana 12 to 14 mm toward the papillomacular nerve fiber bundle under direct visualization with an operating microscope, and 0.1 to 0.2 mL of vitreous was aspirated from each eye. On the basis of ocular geometry, we estimated the needle tip to have been approximately 4 mm from the retinal surface. Vitreous samples were quickly frozen in liquid nitrogen and then stored at −80°C for future assay, and the vitreous cavity volume was restored with hyaluronate sodium viscoelastic material (Healon; Pharmacia Corp). In most cases, the monkeys then underwent perfusion through the heart with 4% paraformaldehyde in 0.1M phosphate buffered saline (pH, 7.4). The eyes were then enucleated, immersed in 4% paraformaldehyde, and shipped to the University of Erlangen-Nürnberg, Erlangen, Germany (to Elke Lütjen-Drecoll, MD) or to one of us (R.H.) at Washington University, St Louis, Mo, where the optic nerve heads were dissected free from the sclera and surrounding tissues. In some cases, only the optic nerve or pieces of the optic nerve were sent in 4% paraformaldehyde, 1% paraformaldehyde, or the fixative of Ito and Karnovsky.17

GLUTAMATE ANALYSIS
Vitreous glutamate concentration was measured by means of high-performance liquid chromatography18 in the following 2 independent laboratories: the University of Wisconsin–Madison Biotechnology Center Peptide Synthesis Facility (laboratory 1) and Cornell University Biotechnology Resource Center, Amino Acid Analysis Facility, Ithaca, NY (laboratory 2). All high-performance liquid chromatography analysis was masked.

At laboratory 1, vitreous samples were thawed, and 40 µL were transferred into a microfuge tube. Each sample was deproteinized at 0°C by adding 10 µL of 10% sulfosalicylic acid containing 1000 pmol of norleucine internal standard. The samples were centrifuged, and the supernatants were transferred to tubes for frozen storage at −80°C. A mixed reference sample was prepared by combining equal portions of 5 samples selected arbitrarily to ensure that there was nothing endogenous to the samples that would alter the size or position of the peak. Analysis was performed in triplicate for each sample by loading 10 µL of sample extract onto an amino acid analyzer (Model 421; Applied Biosystems, Foster City, Calif).

At laboratory 2, vitreous samples were analyzed by means of phenylisothiocyanate derivatization. Thawed samples were deproteinized, vortexed, and centrifuged, and aliquots were evacuated to dryness in a mixture of ethanol, water, and triethylamine (2:2:1 vol/vol/vol). Derivatization of dried samples was performed with a freshly prepared mixture of ethanol and triethylamine phenylisothiocyanate (7:1:1:1 vol/vol/vol/vol). The derivatized samples were evacuated to dryness, resuspended in 0.05M ammonium acetate, and subjected to high-performance liquid chromatography on a C-18 column (in a buffer system consisting of water, sodium acetate, triethylammonium acetate, and acetoniitrile) at fixed-wavelength detection and absorbance of 294 nm (a modified Pico-Tag System; Waters Corporation, Milford, Mass). Acquisition and processing of data was performed with a computer-based system (EZChrom Laboratory, State College, Pa) and external standards (Sigma-Aldrich Corp, St Louis, Mo). Unstable amino acids (asparagine, glutamine, and tryptophan) were freshly prepared. The samples and standard were batch processed, and the injection volume was verified by monitoring the derivatization artifact peaks that were present in each sample.

At laboratory 1, where 17 of the 20 samples were run, triplicate testing was performed on each sample; therefore an error term was provided. The relative percentage error (mean±SEM) in glutamate values was found to be 5.3%±1.0% in glaucomatous eyes and 7.0%±1.5% in control eyes. We analyzed 20 amino acids during each run, and the percentage error (mean±SEM) for all amino acids was found to be 4.3%±0.4% in glaucomatous eyes and 3.1%±0.5% in control eyes. We also analyzed 20 amino acids at laboratory 2, but only single runs were performed.

(REPRINTED) ARCH OPHTHALMOL/VOL 123, JAN 2005 WWW.ARCHOPHTHALMOL.COM

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formed; therefore, no error term was provided. Only 2 vitreous samples (from the glauamous and control eyes of monkey 5) were analyzed in both laboratories. This showed that the average glutamate and glutamine levels were 23% lower in the laboratory 2 analysis than in laboratory 1. The remaining amino acid levels were at least within 25% of each other. Other controls in-cluded a mixed reference sample and amino acid standards as a reference. Glutamate concentrations were calculated on the basis of chromatograph peak height (17/20) or peak area (3/20) analysis, de-pending on the laboratory, or in some cases both (10/20). The area analyses on the basis of the 10 monkeys that underwent both, glutamate concentration by peak height and peak area analyses were used in the data analysis, as there were more peak height–based data points. A conversion factor was cal-culated between glutamate concentration by peak height and peak area analyses on the basis of the 10 monkeys that underwent both.

EVALUATION OF NERVE DAMAGE

We evaluated optic nerve damage microscopically through quantification of retinal ganglion cell axons after histopathologic sectioning of the optic nerve by 2 laboratories using 2 techniques. In 9 of 20 animals, axon counts were measured in the laboratory of Dr Lütjen-Drecoll according to the method described by Quigley et al. In 4 of 20 animals, axon loss ratios were calculated in the laboratory of one of us (R.H.) as previously described. Results were expressed as a ratio of the area of axon degeneration of the experimental eye to the total area of the control eye. In 7 of 20 monkeys, no axon data were available because insufficient tissue remained after other uses or the samples were unusable after freezing.

Actual counts and axon loss ratio were combined by calcu-lating the percentage of axon loss.

Table 1. Clinical Data

<table>
<thead>
<tr>
<th>Monkey No./ Sex/Age, y</th>
<th>Duration of IOP Elevation, d</th>
<th>IOP, Mean ± SEM, mm Hg</th>
<th>Glutamate Ratio, ExpG vs Ctl Eyes</th>
<th>C/D Ratio</th>
<th>Glutamate Concentration, µmol/L</th>
<th>% Axon Loss</th>
<th>Storage Time, d</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/3</td>
<td>168</td>
<td>25</td>
<td>19.7 ± 0.4</td>
<td>13.9 ± 0.4</td>
<td>0.2</td>
<td>0.1</td>
<td>57.00</td>
<td>87.40</td>
</tr>
<tr>
<td>2/F/NA</td>
<td>79</td>
<td>20</td>
<td>31.5 ± 2.7</td>
<td>18.8 ± 0.8</td>
<td>0.2</td>
<td>0.1</td>
<td>7.00</td>
<td>4.38</td>
</tr>
<tr>
<td>3/M/4</td>
<td>71</td>
<td>10</td>
<td>29.3 ± 1.7</td>
<td>14.0 ± 0.5</td>
<td>0.3</td>
<td>0.3</td>
<td>3.23</td>
<td>3.44</td>
</tr>
<tr>
<td>4/M/3</td>
<td>35</td>
<td>15</td>
<td>28.1 ± 3.5</td>
<td>15.1 ± 0.6</td>
<td>1.0</td>
<td>0.3</td>
<td>87.30</td>
<td>76.80</td>
</tr>
<tr>
<td>5/F/3</td>
<td>37</td>
<td>10</td>
<td>45.7 ± 3.7</td>
<td>17.1 ± 0.7</td>
<td>0.5</td>
<td>0.2</td>
<td>3.90</td>
<td>15.93</td>
</tr>
<tr>
<td>6/F/NA</td>
<td>237</td>
<td>68</td>
<td>46.0 ± 1.5</td>
<td>20.4 ± 0.3</td>
<td>NA</td>
<td>NA</td>
<td>88.60</td>
<td>82.80</td>
</tr>
<tr>
<td>7/N/NA/NA</td>
<td>174</td>
<td>48</td>
<td>32.9 ± 2.2</td>
<td>17.4 ± 0.4</td>
<td>NA</td>
<td>NA</td>
<td>17.80</td>
<td>10.00</td>
</tr>
<tr>
<td>8/M/6</td>
<td>77</td>
<td>27</td>
<td>34.9 ± 2.6</td>
<td>22.2 ± 0.5</td>
<td>0.6</td>
<td>0.2</td>
<td>3.40</td>
<td>4.80</td>
</tr>
<tr>
<td>9/F/7</td>
<td>42</td>
<td>11</td>
<td>43.1 ± 2.3</td>
<td>18.1 ± 0.8</td>
<td>0.9</td>
<td>0.3</td>
<td>5.30</td>
<td>4.13</td>
</tr>
<tr>
<td>10/M/16</td>
<td>66</td>
<td>23</td>
<td>28.3 ± 1.2</td>
<td>22.3 ± 0.6</td>
<td>0.7</td>
<td>0.3</td>
<td>53.68</td>
<td>54.58</td>
</tr>
<tr>
<td>11/M/12</td>
<td>82</td>
<td>19</td>
<td>41.0 ± 3.0</td>
<td>19.4 ± 0.5</td>
<td>1.0</td>
<td>0.2</td>
<td>49.59</td>
<td>50.73</td>
</tr>
<tr>
<td>12/M/7</td>
<td>33</td>
<td>12</td>
<td>56.2 ± 2.9</td>
<td>19.0 ± 0.5</td>
<td>1.0</td>
<td>0.3</td>
<td>53.30</td>
<td>52.48</td>
</tr>
<tr>
<td>13/M/10</td>
<td>114</td>
<td>43</td>
<td>62.1 ± 1.8</td>
<td>22.9 ± 0.4</td>
<td>NA</td>
<td>NA</td>
<td>10.90</td>
<td>8.90</td>
</tr>
<tr>
<td>14/M/5</td>
<td>203</td>
<td>49</td>
<td>53.8 ± 2.6</td>
<td>17.7 ± 0.4</td>
<td>1.0</td>
<td>0.2</td>
<td>12.74</td>
<td>4.27</td>
</tr>
<tr>
<td>15/F/5</td>
<td>20</td>
<td>6</td>
<td>41.0 ± 2.7</td>
<td>15.3 ± 0.9</td>
<td>0.7</td>
<td>0.2</td>
<td>6.90</td>
<td>4.30</td>
</tr>
<tr>
<td>16/M/5</td>
<td>108</td>
<td>27</td>
<td>44.2 ± 2.5</td>
<td>18.6 ± 0.6</td>
<td>0.3</td>
<td>0.2</td>
<td>7.08</td>
<td>6.53</td>
</tr>
<tr>
<td>17/R/7</td>
<td>27</td>
<td>5</td>
<td>29.8 ± 2.5</td>
<td>12.4 ± 0.8</td>
<td>0.4</td>
<td>0.3</td>
<td>4.50</td>
<td>6.90</td>
</tr>
<tr>
<td>18/M/NA</td>
<td>134</td>
<td>34</td>
<td>32.6 ± 2.3</td>
<td>14.8 ± 0.3</td>
<td>0.5</td>
<td>0.3</td>
<td>5.98</td>
<td>4.25</td>
</tr>
<tr>
<td>19/M/8</td>
<td>141</td>
<td>37</td>
<td>41.7 ± 2.9</td>
<td>15.6 ± 0.4</td>
<td>0.8</td>
<td>0.2</td>
<td>6.73</td>
<td>5.43</td>
</tr>
<tr>
<td>20/M/7</td>
<td>362</td>
<td>35</td>
<td>55.9 ± 1.0</td>
<td>16.4 ± 0.2</td>
<td>0.9</td>
<td>0.3</td>
<td>2.95</td>
<td>2.77</td>
</tr>
</tbody>
</table>

STATISTICAL ANALYSIS

We used nonparametric statistical procedures, because the data did not satisfy the assumption of normality. The relationship between glutamate concentration in glaucomatous vs fellow control eyes was assessed using a sign test to determine whether the median ratio of glutamate concentration of ExpG to control eyes was different from 1.0. We used the Spearman rank correlation to assess the correlation of glutamate ratios, percentage of axon loss, IOP, C/D ratio, duration of IOP elevation, age, and vitreous storage time. Differences in mean ± SEM values are compared using the 2-tailed paired t test for differences not equal to 0.0.

RESULTS

Table 1 represents the entire data set.

IOP DATA

The mean ± SEM IOP from the point of first IOP elevation to the time of death was 39.9 ± 2.5 mm Hg in ExpG eyes and 17.5 ± 0.7 mm Hg in control eyes (P < .001) (Figure 1). The range of duration of IOP elevation was 20 to 362 days (mean, 110 days). Because of varying levels of pressure elevation during variable periods of time, a composite term was calculated using the area under the curve of the IOP difference between ExpG and contra-lateral control eyes and the duration of pressure elevation in days. This composite term reflected the total excess pressure to which each ExpG eye was subjected.

CLINICAL EVALUATION OF NERVE DAMAGE

The mean C/D ratio was 0.62 in ExpG eyes and 0.24 in control eyes (P < .001).
HISTOPATHOLOGIC EVALUATION OF NERVE DAMAGE

Axon counts (available in 10 of 20 monkeys) ranged from 350,498 to 1,006,700 in ExpG eyes and from 754,874 to 1,715,868 in control eyes. Axon loss ratios (available in 4 of 20 monkeys) ranged from 0.15 to 0.89. Based on the axon counts and axon loss ratios, the calculated percentage of axon loss among pairs of eyes ranged from 9% to 89%, reflecting the entire spectrum of glaucomatous disease. The mean axon loss in a pairwise comparison of ExpG vs contralateral control eyes was 46%. Axon loss was consistent with the difference in C/D ratio found between ExpG and control eyes. There was a statistically significant relationship between the percentage of axon loss and the C/D ratio difference between ExpG and control eyes (Spearman rank correlation $r = 0.64; P = .049$).

VITREOUS GLUTAMATE CONCENTRATION

The median glutamate concentrations were 7.0 µmol/L (range, 2.9-88.6 µmol/L) in ExpG eyes and 6.7 µmol/L (range, 2.8-87.4 µmol/L) in control eyes (Figure 2). We did not find a significant difference between glutamate levels in ExpG and control eyes. The range of glutamate concentrations was wide in both groups, and the higher levels in ExpG and control eyes. The population sample median ratio (T) of glutamate concentration was 0.98 to 1.28, suggesting that glutamate levels in ExpG eyes could reasonably be about 25% higher than those of the control eyes.8,10

STATISTICAL ANALYSIS

The population sample median ratio (T) of glutamate concentration in ExpG compared with fellow control eyes was not significantly different from 1.0 when analyzed by means of the 2-tailed sign test ($T = 1.08; P = .26$). The median was used instead of mean values to provide a better estimate of central location, because glutamate concentrations in both groups were heavily skewed.

Spearman rank correlation estimates indicated that the only variables of interest with strong correlation were percentage of axon loss and IOP difference between ExpG and control eyes ($r = 0.70; P = .008$), axon loss and C/D ratio difference ($r = 0.64; P = .049$), and IOP difference and C/D ratio difference ($r = 0.57; P = .02$), all suggesting a good model. There were no statistically significant correlations between glutamate concentration ratio and percentage of axon loss, IOP, C/D ratio, duration of IOP elevation, or age. Axon loss was significantly correlated with age ($r = 0.76; P = .01$). Because the population of monkeys was skewed toward younger age, there was insufficient information to comment on the effect of age on axon counts in control eyes. None of these correlations was significant at the level of $P = .05$ when corrected for multiple comparisons.

No correlation was found between the glutamate concentration ratio and the area under the curve (Spearman rank correlation $r = 0.35; P = .13$). The 95% confidence interval for the median glutamate ratio in the population was 0.98 to 1.28, suggesting that glutamate levels in ExpG eyes could reasonably be about 25% higher than those of the control eyes.

SUBSET ANALYSIS

Because of the large spread of glutamate concentrations in both groups, we searched for potential sources of confounding. The glutamate levels appeared higher with longer times for storage of the vitreous samples at −80°C before analysis. Amino acids such as glutamine may be unstable even in low-temperature storage owing to deamination from glutamine to glutamate.21 The recovery of glutamine and glutamate in a reference mixture stored at −80°C showed a gradual increase in glutamate level of up to 8-fold after 180 days of storage, with a corresponding decrease in glutamine level (G.L.C., unpublished data, January 1999). Figure 3A shows a graph of increasing glutamate concentration with increasing duration of storage time of vitreous from control eyes (Spearman rank correlation $r = 0.49; P = .03$). However, there was no effect of sample storage time on the glutamate ratio (Figure 3B) (Spearman rank correlation $r = -0.09; P = .70$). Elevated glutamate levels in both eyes did not appear to be associated with previous protocols for which the
animals were used before they became glaucomatous. Some monkeys with elevated glutamate levels in the vitreous samples of the control eyes had been used only for laser treatments. Drugs used in anterior chamber perfusion experiments were common to monkeys with and without elevated glutamate levels in control eyes. These drugs had been administered at least 3 months before the vitreous taps. Also, the presence (0 to 3+ cells) or absence of inflammation was unrelated to elevated glutamate levels.

We did not wish to miss hidden data that may have corroborated previous reports. Therefore, despite the potential biases, we performed subset analyses that might control for potential problems in our data set. One subset analysis included monkeys whose vitreous samples were stored for no longer than 30 days (to address the concern of possible glutamate degradation with increased storage time), monkeys with moderately advanced glaucomatous optic neuropathy as defined by axon loss of 30% to 70% (mild axon loss was presumed to cause little or no glutamate release, whereas severe axon loss was theorized to decrease release of glutamate, as the cells are essentially dead), and monkeys with single-digit glutamate concentrations (perhaps representing more physiologic levels). Only 3 monkeys met all of these criteria for this subset analysis (monkeys 16, 18, and 19). The glutamate ratios were 1.08, 1.41, and 1.24, respectively.

A second subset analysis was run for monkeys with control eyes that had glutamate concentrations less than 20 µmol/L and ExpG eyes with moderately advanced glaucomatous optic neuropathy (axon loss, 30%-70%). Six monkeys met these criteria (monkeys 7, 13, 15, 16, 18, and 19), and the glutamate ratios were 1.78, 1.22, 1.60, 1.08, 1.41, and 1.24, respectively (mean±SEM, 1.39±0.11). The ratio was significantly different from 1.0 (P=.02) by the 2-tailed t test, indicating that there was a 40% increase in glutamate in ExpG compared with control animals. However, when the subset analysis was run for all monkeys with glutamate concentrations less than 20 µmol/L in the control eye (n=14), the mean±SEM glutamate ratio was 1.27±0.17, which is not significantly different from 1.0 (P=.14) by the 2-tailed t test.

A final subset analysis was performed for the most homogenous group consisting of rhesus monkeys (n=14). Only 1 of these monkeys had been used in a previous outflow facility experiment (monkey 14). The median glutamate concentration ratio was 1.04 with a 95% confidence interval of 0.71 to 1.60. The mean±SEM glutamate concentration ratio was 1.35±0.29 (P=.27).

**OTHER AMINO ACIDS**

Twenty amino acids were analyzed, including glutamate. The results for all monkeys with elevated IOP are shown in Table 2. Levels of asparagine were significantly decreased, whereas levels of serine, threonine, alanine, proline, tyrosine, cysteine, tryptophan, and lysine were significantly increased by paired t test analysis. When corrected for multiple comparisons, serine, alanine, proline, tyrosine, cysteine, tryptophan, and lysine concentrations were significantly altered. When only samples with glutamate levels less than 20 µmol/L were considered, similar results were found. The exceptions were that serine and threonine levels were no longer significantly elevated and the arginine level was significantly elevated. Carter-Dawson et al10 found increased levels of histidine, arginine, alanine, tryptophan, and lysine in their samples, but no difference after adjustment for multiple statistical tests. No amino acid levels were significantly elevated in samples from monkeys in the current study, in which there was no inflammation in either eye (monkeys 1, 3, 5, and 10).

**COMMENT**

To our knowledge, this is one of only 3 reported studies of vitreous glutamate levels in monkeys with experimental glaucoma.8,10 We found no detectable difference in vitreous glutamate levels between glaucomatous eyes and contralateral control eyes in our overall data set. Our results are in contrast to the findings of Dreyer et al8 and support the report by Carter-Dawson et al.10

Our study has weaknesses. Some of the monkeys included were used in several other protocols, and the study was not originally designed to answer the given question. The magnitude and duration of IOP elevation were variable. Caution must be taken in interpreting results of any study in which the subjects are so dissimilar.
The observer for C/D ratio was unmasked, which may have potentially introduced bias into the data. Fewer than half of the animals had fundus photography performed; we considered this proportion insufficient to provide meaningful data by masked grading. Also, in nearly all cases, the C/D ratio was not evaluated under conditions where compliance of the optic disc to elevated IOP was assured.21 However, if this were the case, it would be reasonable to expect that this phenomenon would apply to samples from both ExpG and control eyes. Indeed, there was no effect of sample storage time on the C/D ratio.22

Finally, vitreous sample storage time was not uniform, and effects of storage time may have added noise to the data. It is possible that the amount of glutamate was overestimated because of glutamine instability and glutamine conversion to glutamate over time despite a low temperature storage environment.21 However, if this were the case, it would be reasonable to expect that this phenomenon would apply to samples from both ExpG and control eyes. Indeed, there was no effect of sample storage time on the C/D ratio. If glutamine were being converted to glutamate, we would also expect the levels of glutamine to decrease with increasing storage time, which was not found. However, because the glutamine concentration is several hundred times that of glutamate, a small percentage of change in glutamine may correspond to a bigger percentage of change in glutamate if the conversion occurred. Therefore, glutamine changes might be undetectable given the sensitivity and reproducibility of the system. In summary, we were unable to fully explain the variability of glutamate concentrations.

We made a great effort to examine the data thoroughly, as we understood that the information might support or refute controversial findings in a previous report by another laboratory. For this reason, we performed subset analyses, and even they failed to detect the severalfold difference in vitreous glutamate levels between glaucomatous and control monkey eyes reported by Dreyer et al.8 Although there were too few animals in the idealized subsets to draw firm conclusions, the subset that included monkeys with glutamate concentrations less than 20 µmol/L in the control eyes and moderately advanced glaucomatous optic neuropathy in the ExpG eyes showed an average of 40% increase in glutamate levels in glaucomatous compared with control eyes. This trend may be biologically important.

Ocular inflammation was present in at least 11 of 20 monkeys, which could have affected vitreous amino acid levels. However, elevated levels of vitreous protein in rats did not appear to correlate with elevated levels of some amino acids in vitreous samples from experimental glaucomatous compared with fellow control eyes.12 In human control eyes, there also were no differences in vitreous concentrations of amino acids between different age groups or between a control group and a group with pathologic eye conditions that presumably would result in inflammation.23

For more than a decade, elevated levels of glutamate have been thought to play a role in glaucoma through excitotoxic effects of overstimulating the N-methyl-D-aspartate receptor24 and subsequent increases in intracellular calcium levels, followed by a cascade of events leading to apoptosis of the retinal ganglion cell. Accruing evidence suggests that N-methyl-D-aspartate antagonists such as memantine may decrease retinal ganglion cell loss, presumably through mechanisms involving glutamate.25-28 Much promising work is currently being performed on the role of immunity on
glutamate toxicity, including vaccination for protection of retinal ganglion cells against death due to glutamate cytotoxicity. 25, 26 Although our findings and those of Carter-Dawson et al 35 do not support the hypothesis of an elevation of vitreous glutamate levels in primate glaucomatous eyes to the level that was reported by Dreyer et al, 35 we cannot conclude that glutamate has no role in the pathophysiology of glaucoma. It is also important to remember that the 95% confidence interval for the glutamate concentration ratio in the entire data set was 0.98 to 1.28, indicating that a small (approximately 25%) increase in glutamate level is possible. Power calculations indicate that Carter-Dawson et al 10 had a 90% chance of detecting a difference of 2 µmol/L between glaucomatous and control eyes, which would be equivalent to a ratio of 1.35 for ExpG eyes and control eyes with glutamate concentrations of 7.7 and 5.7 µmol/L, respectively. Modest changes in glutamate levels such as this could have a significant impact in the mechanism of glaucomatous neuronal damage.

Carter-Dawson et al 35 found elevated levels of glutamine, glutathione, and glutamate transporter in the Müller cells in glaucomatous monkeys eyes, which suggests elevations in extracellular glutamate levels and enhanced glutamate transport and metabolism. 10, 25 Conversely, measurable decreases in the glutamate transporters excitatory amino acid transporter 1 (GLAST) and excitatory amino acid transporter 2 (GLT-1) in rats after experimental glaucoma injury were postulated to increase the potential for glutamate-induced injury. 35 Levels of glutamate in the extracellular space in excess of what could normally be removed by glutamate transporters may not be so high as to diffuse into the vitreous at levels that would be detected as elevated. If such levels were achieved, they would not necessarily remain elevated for prolonged periods. Glutamate excitotoxicity could still play a role in glaucoma damage, because elevated levels may still exist in proximity to the retinal ganglion cells.

Submitted for Publication: May 28, 2003; final revision received February 19, 2004; accepted June 15, 2004.

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Funding/Support: This study was supported by grants EY02698, EY07119, EY10009, EY02687, and EY06416 from the National Institutes of Health, Bethesda, Md; unrestricted grants and a Physician Scientist Award (Dr Kaufman) from Research to Prevent Blindness, New York, NY; the Cornell Biotechnology Program; and grant SFB 539 Glaukome from the German Science Foundation, Bonn.

Acknowledgment: We thank Olga Agapova, PhD, in Dr Hernandez’ laboratory, who determined the axon loss ratios.

REFERENCES


early course of the retinitis. We were compelled to treat this patient’s retinitis in light of the advanced zone 1 involvement on initial examination and the subsequent progression during the first week of oral induction therapy.

This case emphasizes the need to include CMV along with herpes simplex virus, varicella-zoster virus, toxoplasmosis, and syphilis in the differential diagnosis of necrotizing retinitis in healthy patients. Furthermore, physicians should be reminded to administer corticosteroids judiciously and to frequently reappraise their effect on inflammatory ocular disease.

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**Financial Disclosure:** None.

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**Correction**

Error in Table. In the Laboratory Sciences article by Wamsley et al titled “Vitreous Glutamate Concentration and Axon Loss in Monkeys With Experimental Glaucoma,” published in the January issue of the *ARCHIVES* (2005;123:64-70), an error occurred in Table 2 on page 69. In the far right column of that table, eighth row, the ratio for arginine should have been indicated as being significant at \(P<.001\). The corrected Table 2 is reprinted here.

### Table 2. Amino Acid Analysis Results

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>No. of Eyes*</th>
<th>ExpG Level, Mean ± SEM, µmol/L</th>
<th>Ctl Level, Mean ± SEM, µmol/L</th>
<th>ExpG/Ctl, Mean ± SEM, µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>17</td>
<td>3.99 ± 0.62</td>
<td>3.69 ± 0.80</td>
<td>0.98 ± 0.10</td>
</tr>
<tr>
<td>Glutamate</td>
<td>20</td>
<td>24.40 ± 6.47</td>
<td>24.54 ± 6.76</td>
<td>1.18 ± 0.12</td>
</tr>
<tr>
<td>Asparagine</td>
<td>20</td>
<td>20.30 ± 1.38</td>
<td>22.83 ± 1.59</td>
<td>0.91 ± 0.04†</td>
</tr>
<tr>
<td>Serine</td>
<td>20</td>
<td>84.10 ± 6.11</td>
<td>73.50 ± 4.54</td>
<td>1.17 ± 0.07†</td>
</tr>
<tr>
<td>Glutamine</td>
<td>20</td>
<td>845.09 ± 38.47</td>
<td>806.46 ± 31.80</td>
<td>1.06 ± 0.04</td>
</tr>
<tr>
<td>Glycine</td>
<td>15</td>
<td>12.05 ± 3.87</td>
<td>6.01 ± 2.49</td>
<td>2.02 ± 1.03</td>
</tr>
<tr>
<td>Histidine</td>
<td>20</td>
<td>49.20 ± 7.31</td>
<td>49.43 ± 7.48</td>
<td>1.13 ± 0.10</td>
</tr>
<tr>
<td>Arginine</td>
<td>20</td>
<td>78.01 ± 6.46</td>
<td>47.29 ± 2.12</td>
<td>1.67 ± 0.12‡</td>
</tr>
<tr>
<td>Threonine</td>
<td>20</td>
<td>36.79 ± 3.73</td>
<td>35.54 ± 3.81</td>
<td>1.09 ± 0.09†</td>
</tr>
<tr>
<td>Alanine</td>
<td>20</td>
<td>73.22 ± 6.72</td>
<td>58.24 ± 3.22</td>
<td>1.29 ± 0.10§</td>
</tr>
<tr>
<td>Proline</td>
<td>20</td>
<td>44.41 ± 6.20</td>
<td>15.23 ± 1.32</td>
<td>3.18 ± 0.48‡</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>20</td>
<td>28.25 ± 2.31</td>
<td>22.67 ± 1.59</td>
<td>1.27 ± 0.07§</td>
</tr>
<tr>
<td>Valine</td>
<td>20</td>
<td>66.86 ± 3.12</td>
<td>69.65 ± 3.28</td>
<td>0.98 ± 0.04</td>
</tr>
<tr>
<td>Methionine</td>
<td>20</td>
<td>15.76 ± 1.67</td>
<td>15.22 ± 1.79</td>
<td>1.08 ± 0.08</td>
</tr>
<tr>
<td>Cysteine</td>
<td>20</td>
<td>28.55 ± 3.93</td>
<td>11.70 ± 0.85</td>
<td>2.67 ± 0.42‡</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>20</td>
<td>23.96 ± 1.74</td>
<td>24.46 ± 1.21</td>
<td>0.98 ± 0.04</td>
</tr>
<tr>
<td>Leucine</td>
<td>20</td>
<td>68.27 ± 3.45</td>
<td>68.32 ± 3.03</td>
<td>1.06 ± 0.04</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>20</td>
<td>29.77 ± 3.89</td>
<td>29.87 ± 3.39</td>
<td>1.02 ± 0.07</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>13</td>
<td>29.86 ± 9.02</td>
<td>24.48 ± 9.34</td>
<td>1.64 ± 0.19§</td>
</tr>
<tr>
<td>Lysine</td>
<td>19</td>
<td>122.40 ± 12.52</td>
<td>78.92 ± 4.69</td>
<td>1.61 ± 0.14‡</td>
</tr>
</tbody>
</table>

Abbreviations: Ctl, contralateral control eyes; ExpG, eyes with experimental glaucoma.

*If an amino acid was detectable in the vitreous sample from one eye of a monkey but not the opposite eye, the value of the opposite eye was designated as 0. In no case was the amino acid level of the Ctl eye undetectable and that of the ExpG eye measurable. If an amino acid was not detected in both eyes of an animal, then no value was designated for that amino acid, which is why the numbers of eyes are not the same for all amino acids.

†\(P<.05\).

‡\(P<.001\).

§\(P<.005\).