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.

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LAUCOMA 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.

METHODS

SUBJECTS

All animal studies were performed in accordance with institutional guidelines approved by the University of Wisconsin–Madison Research Animal Resources Center.

Argon laser scarification of the trabecular meshwork (ALTS)13,14 was performed in 14 rhesus (Macaca mulatta) and 6 cynomolgus (Macaca fascicularis) male and female monkeys, aged 3 to 16 years. A standard clinical argon laser and slitlamp delivery system were used to produce 50 to 250 spots 50 µm in diameter (1.0-1.3 W of energy; 0.5-second duration) over 270° of the angle circumference. This procedure was repeated at approximately 3- to
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 minified Goldmann applanation tonometer (Haag-Streit, Konz, Switzerland), occasionally backed up by measurements with a handheld applanation tonometer (Tonometer; 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 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 with topically once or twice daily with a single drop of 1 or more of 0.2% dorzolamide hydrochloride (Trusopt; Merck & Co), or prostaglandin F2

GLUTAMATE ANALYSIS

Vitreal glutamate concentration was measured by means of high-performance liquid chromatography16 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, 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, triethylamine, and acetonitrile) at fixed-wavelength detection and absorbance of 254 nm (a modified Pico-Tag System; Waters Corporation, Milford, Mass). Acquisition and processing of data was performed with a computer-based system (EZChrom; LabAlliance, 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.
formed; therefore, no error term was provided. Only 2 vitreous samples (from the glaumatous 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 compared with that of laboratory 1. The remaining amino acid levels were at least within 25% of each other. Other controls in both laboratories. This showed that the average glutamate and glutamine levels were 23% lower in the laboratory 2 analysis compared with that of laboratory 1. The remaining amino acid levels were at least within 25% of each other. Other controls included a mixed reference sample and amino acid standards as already described in the previous paragraphs.

Glutamate concentrations were calculated on the basis of chromatogram peak height (17/20) or peak area (3/20) analysis, depending on the laboratory, or in some cases both (10/20). The glutamate concentrations obtained by means of chromatograph peak height analysis were used in the data analysis, as there were more peak height–based data points. A conversion factor was calculated between glutamate concentration by peak height and peak area analyses on the basis of the 10 monkeys that underwent both. However, no error term was provided. Only two vitreous samples (from the glaucomatous 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 compared with that of laboratory 1. The remaining amino acid levels were at least within 25% of each other. Other controls included a mixed reference sample and amino acid standards as already described in the previous paragraphs.

EVALUATION OF NERVE DAMAGE

We evaluated optic nerve damage microscopically through quantification of retinal ganglion cell axons by 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.²⁰ In 4 of 20 animals, axon loss ratios were calculated in the laboratory of one of us (R.H.) as previously described.²⁰ 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 calculating 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>% Axon Loss</th>
<th>Storage Time, d</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/3</td>
<td>168</td>
<td>35.9 ± 1.0</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>188 ± 0.8</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>3/M/4</td>
<td>71</td>
<td>10</td>
<td>29.3 ± 1.7</td>
<td>140 ± 0.5</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>4/3/3</td>
<td>35</td>
<td>15</td>
<td>28.1 ± 3.5</td>
<td>151 ± 0.6</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>5/3</td>
<td>37</td>
<td>10</td>
<td>45.7 ± 3.7</td>
<td>171 ± 0.7</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>6/3/3</td>
<td>237</td>
<td>68</td>
<td>0.06 ± 10.5</td>
<td>20.4 ± 0.3</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>7/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>
</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>
</tr>
<tr>
<td>9/M</td>
<td>40</td>
<td>11</td>
<td>32.1 ± 2.3</td>
<td>161 ± 0.9</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td>10/M</td>
<td>66</td>
<td>23</td>
<td>28.3 ± 1.2</td>
<td>223 ± 0.6</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>11/M</td>
<td>32</td>
<td>19</td>
<td>41.0 ± 3.0</td>
<td>19.4 ± 0.5</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>12/M</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>
</tr>
<tr>
<td>13/M</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>
</tr>
<tr>
<td>14/M</td>
<td>209</td>
<td>49</td>
<td>53.8 ± 2.6</td>
<td>17.7 ± 0.4</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>15/F</td>
<td>20</td>
<td>6</td>
<td>41.0 ± 2.7</td>
<td>153 ± 0.9</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>16/M</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>
</tr>
<tr>
<td>17/F/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>
</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>
</tr>
<tr>
<td>19/M</td>
<td>141</td>
<td>37</td>
<td>41.7 ± 2.9</td>
<td>156 ± 0.4</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>20/M/7</td>
<td>362</td>
<td>35</td>
<td>55.9 ± 1.0</td>
<td>164 ± 0.2</td>
<td>0.9</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Abbreviations: AUC, area under the curve of the difference in intracocular pressure (IOP) between experimental glaucoma (ExpG) and contralateral control (Ctl) eyes once the IOP became elevated times the number of days until death; NA, not available.

*Indicates the number of measurements averaged to give the IOP for each monkey.

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 contralateral 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 75,487 to 1,715,808 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 = 0.049).

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 concentrations were not consistent with what has been previously reported for monkeys.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 = 0.26). 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 = 0.008), axon loss and C/D ratio difference (r = 0.64; P = 0.049), and IOP difference and C/D ratio difference (r = 0.57; P = 0.02), 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 = 0.01). 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 = 0.05 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 = 0.13). 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 = 0.03). However, there was no effect of sample storage time on the glutamate ratio (Figure 3B) (Spearman rank correlation r = −0.09; P = 0.70). Elevated glutamate levels in both eyes did not appear to be associated with previous protocols for which the

**Figure 1.** Box plot summarizing intraocular pressure (IOP) data. We found a significant difference in mean IOP between monkey eyes with experimental glaucoma (ExpG) and control monkey eyes (39.9 ± 2.5 and 17.5 ± 0.7 mm Hg, respectively; P < .001).

**Figure 2.** We found no difference in median vitreous glutamate concentration between monkey eyes with experimental glaucoma (ExpG) and contralateral control eyes (7.0 µmol/L [range, 2.9-88.6 µmol/L] and 6.7 µmol/L [range, 2.8-87.4 µmol/L], respectively; P > .99). Elements of the box plots summarizing the data are described in Figure 1.
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 al 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. 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 al and support the report by Carter-Dawson et al.

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.
port or refute controversial findings in a previous report. Overall, as we understood that the information might sup-
tem. In summary, we were unable to fully explain the vari-
tectable given the sensitivity and reproducibility of the sys-
tercentage of change in glutamate if the conversion
converted to glutamate, we would also expect the levels of glu-
take eyes. Indeed, there was no effect of sample storage time
mine conversion to glutamate over time despite a low tem-
the data. It is possible that the amount of glutamate was
is currently being performed on the role of immunity on
by another laboratory. For this reason, we performed sub-
set analyses, and even they failed to detect the sever-
fold difference in vitreous glutamate levels between glau-
comatous and control monkey eyes reported by Dreyer
et al. Although there were too few animals in the ideal-
ized subsets to draw firm conclusions, the subset that in-
cluded 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 glau-
comatous compared with control eyes. This trend may
be biologically important.
Ocular inflammation was present in at least 11 of 20 mon-
keys, which could have affected vitreous amino acid lev-
el. 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. 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.
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 recep-
tor (NMDAR) and subsequent increases in intracellular calcium lev-
eels, 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. Much promising work
is currently being performed on the role of immunity on

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
ruled out by lowering the pressure before measure-
ments and photographs were taken.

Finally, vitreous sample storage time was not uni-
form, 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 gluta-
mine conversion to glutamate over time despite a low tem-
perature storage environment. However, if this were the
case, it would be reasonable to expect that this phenom-
emon would apply to samples from both ExpG and con-
trol eyes. Indeed, there was no effect of sample storage time
on the glutamate ratio. If glutamine were being con-
verted to glutamate, we would also expect the levels of glu-
tamine to decrease with increasing storage time, which was
not found. However, because the glutamine concentra-
tion is several hundred times that of glutamate, a small per-
centage of change in glutamine may correspond to a big-
ger percentage of change in glutamate if the conversion
occurred. Therefore, glutamine changes might be unde-
tectable given the sensitivity and reproducibility of the sys-
tem. In summary, we were unable to fully explain the vari-
ability of glutamate concentrations.

We made a great effort to examine the data thor-
oughly, as we understood that the information might sup-
port or refute controversial findings in a previous report

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>No. of Eyes*</th>
<th>ExpG Mean ± SEM, µmol/L</th>
<th>Ctl 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>3.09 ± 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>1.09 ± 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>
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<td>1.06 ± 0.04</td>
</tr>
<tr>
<td>Glycine</td>
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<td>1.67 ± 0.12</td>
</tr>
<tr>
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<td>36.79 ± 3.73</td>
<td>35.54 ± 3.81</td>
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<td>73.22 ± 6.72</td>
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</tr>
<tr>
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<td>44.41 ± 6.20</td>
<td>15.23 ± 1.32</td>
<td>3.18 ± 0.48§</td>
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<tr>
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</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>
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<td>15.76 ± 1.67</td>
<td>15.22 ± 1.79</td>
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<tr>
<td>Cysteine</td>
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<td>11.70 ± 0.85</td>
<td>2.67 ± 0.42§</td>
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<td>Phenyldalanine</td>
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<td>29.87 ± 3.39</td>
<td>1.02 ± 0.07</td>
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<td>Tryptophan</td>
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<tr>
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<td>122.40 ± 12.52</td>
<td>78.92 ± 6.89</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<.005.
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glutamate toxicity, including vaccination for protection of retinal ganglion cells against death due to glutamate cytotoxicity. 29,30 Although our findings and those of Carter-Dawson et al 19 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, 8 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 19 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 19 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. 10,29 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 glaucomatous injury were postulated to increase the potential for glutamate-induced injury. 21 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.

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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>3.09 ± 0.62</td>
<td>3.69 ± 0.80</td>
<td>0.98 ± 0.10</td>
</tr>
<tr>
<td>Glutamate</td>
<td>20</td>
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