Elevated Glutamate Levels in the Vitreous Body of an In Vivo Model of Optic Nerve Ischemia

Tae Woo Kim, MD; Kyung Bok Kang, MD; Ho-Kyung Choung, MD; Ki Ho Park, MD; Dong Myung Kim, MD

Objective: To explore the possibility that an elevation of glutamate levels in the vitreous might be associated with the microvascular compromise of the optic nerve.

Materials and Methods: Endothelin-1, 0.1 µg/d (5 rabbits), or balanced salt solution (4 rabbits) was delivered to the perineural region of the anterior optic nerve by osmotically driven minipumps for 2 weeks. Vitreous specimens were obtained, and their amino acid contents were determined by high-performance liquid chromatography.

Results: There was a statistically significant elevation in the mean ± SEM vitreous concentrations of glutamate (264% ± 41%; \( P = .04 \)), aspartate (269% ± 31%; \( P = .04 \)), and glycine (232% ± 26%; \( P = .04 \)) in the eyes subjected to endothelin-1 when compared with the fellow control eyes.

Conclusion: Administration of endothelin-1 to the microvasculature of the optic nerve leads to elevation of glutamate, aspartate, and glycine concentrations in the vitreous.

Clinical Relevance: The increase of excitatory amino acids in the vitreous might be associated with various ischemic processes of the optic nerve, including glaucomatous optic neuropathy, and may play a role in the neuronal damage that is seen in these diseases.


Glaucoma is a leading cause of blindness, characterized by retinal ganglion cell loss and excavation of the optic nerve head. The pathogenesis of optic neuropathy in glaucoma is still a matter of debate. Although elevated intraocular pressure (IOP) has been thought to be the primary cause of glaucomatous damage, many clinical observations lend credence to the potential role of microcirculatory changes in glaucoma, either as the primary abnormality or as a cofactor that increases the susceptibility to pressure damage.

Recent studies suggest that glutamate, a major mediator of nerve degeneration in the injured central nervous system, may play an important role in the pathogenesis of glaucoma. Subcutaneous injection of glutamate in young mice led to severe destruction of the inner retinal layers, most notably the retinal ganglion cell layer. The predominant form of excitotoxicity of retinal ganglion cells is mediated by overstimulation of the N-methyl-D-aspartate (NMDA) subtype of the glutamate receptor. Larger retinal ganglion cells, which are affected first in glaucoma, showed greater sensitivity to NMDA-mediated cell death. More recently, elevated levels of glutamate in the vitreous body were demonstrated in the eyes of monkeys and humans with high-tension glaucoma.

The purpose of the present study was to investigate whether elevation of glutamate levels is also associated with the microcirculatory compromise of the optic nerve. We used a rabbit in which endothelin-1 (ET-1; Peptides International, Louisville, Ky) was delivered to the perineural region of the anterior optic nerve by an osmotically driven minipump as a model system for optic nerve ischemia. A reduction of approximately 38% of the optic nerve blood flow in the ET-1–administered eyes compared with the fellow control eyes was previously demonstrated in this model.

RESULTS

In the rabbit groups implanted with ET-1–filled and BSS-filled osmotic minipumps,
MATERIALS AND METHODS

ANIMALS

Nine male New Zealand white rabbits, weighing 2.5 to 3.5 kg, were used. All experiments conformed to the Association for Research in Vision and Ophthalmology statement for the Use of Animals in Ophthalmic and Vision Research. Rabbits were anesthetized with intramuscular ketamine hydrochloride, 50 mg/kg, and xylazine hydrochloride, 0.5 mg/kg. To rule out the probability that the change of the glutamate level in the vitreous may be caused by the difference of the IOP between the eyes, we measured IOP with a tonometer (Tono-Pen 2; Oculab, Glendale, Calif) at baseline and immediately before vitreous sampling.

INDUCTION OF ANTERIOR OPTIC NERVE ISCHEMIA

Anterior optic nerve ischemia was induced as previously described by Ciccof et al.16-17 In brief, ET-1 was delivered to the perineural region of the anterior optic nerve by osmotically driven minipumps (Alzet minipumps; Alza Corporation, Palo Alto, Calif) that deliver a test agent at a controlled and constant flow rate (0.5 µL/h). The minipumps were implanted in a surgically created space, superior and nasal to the right eyes. A polyethylene delivery tube was directed from the minipump through the upper eyelid into a surgically created superiortemporal sub-Tenon channel and under the superior rectus muscle and was fixed in place using a scleral fixation suture adjacent to the optic nerve and its vascular supply. A 0.1-µg/d dosage of ET-1 was delivered for 14 days to 5 rabbits. The ET-1 was diluted in balanced salt solution (BSS). In 4 control rabbits, minipumps filled with BSS were implanted.

VITREOUS COLLECTION

Vitreous specimens were collected after 14 days of local drug administration (5 rabbits, ET-1; 4 rabbits, BSS). There was no significant difference between the eyes in IOP at baseline and before vitreous sampling (Table 1).

Amino acid concentrations in the vitreous measured by high-performance liquid chromatography are outlined in Table 2 and the Figure. There was a statistically significant elevation in the mean ± SEM vitreous concentration of glutamate (264% ± 41%; P = .04), aspartate (269% ± 31%; P = .04), and glycine (232% ± 26%; P = .04) in the ET-1–administered eyes when compared with the fellow control eyes. In rabbits implanted with BSS-filled minipumps, vitreal amino acid concentrations of both eyes were all comparable. This finding rules out the possibility that the increase in glutamate, aspartate, and glycine levels may have been caused by any procedure during induction of optic nerve ischemia.

COMMENT

We demonstrated that microvascular compromise of the optic nerve is associated with an elevation of glutamate and aspartate concentrations in the vitreous body with the use of an ET-1–administered model.

Glutamate and aspartate are known major sources of cytotoxic effects in brain injuries and disorders that are accompanied by secondary degeneration.10-22 Their excitotoxic potential to neurons in the mammalian retinal ganglion cell layer has been well documented.22 The excitotoxic action of glutamate is primarily mediated by overstimulation of the NMDA subtype of glutamate receptor, triggering an increase in intracellular calcium23 and initiating a cascade of events that finally leads to apoptosis or necrosis, depending on glutamate levels. Non-NMDA receptors may also play a role in glutamate excitotoxicity.24 Recently, a 42% reduction of retinal ganglion cells was demonstrated after long-term elevation of vitreal glutamate levels, which was induced by intravitreal glutamate injection.25 In the ET-1–administered model, in addition to the reduction of optic nerve blood flow, a significant change in topometric parameters was demonstrated by a confocal scanning laser ophthalmoscope, indicating an increase in optic nerve cupping and a decrease of the neural rim (19.4% reduction of volume above surface, which represents optic nerve rim volume) after 4 weeks of ET-1 administration.26 In view of the above information, our
results suggest that an elevation of glutamate and aspartate concentrations in the vitreous may be involved in this neuronal damage induced by microvascular compromise of the optic nerve.

Our finding is in line with the recently reported increase in glutamate levels in the vitreous bodies of human and monkey with high-tension glaucoma and mutant quail with a glaucomalike disorder and in the aqueous humor of rats with partial lesion of the optic nerve. Moreover, elevated glutamate and γ-aminobutyric acid levels were also found in the vitreous of patients with proliferative diabetic retinopathy, suggesting that retinal degeneration, regardless of its cause, is associated with anomalies in intracellular amino acid concentration.

The source of the vitreal glutamate and aspartate remains unknown, but these amino acids are synthesized and released by all categories of retinal cells. As for optic nerve ischemia, direct ischemic insult to the axon of the retinal ganglion cells and interference of axonal flow and subsequent deprivation of neurotrophic factor may induce retinal ganglion cell death. The vitreal accumulation of glutamate and aspartate may be caused by release from the dying retinal ganglion cells. The glutamate and aspartate thereby released, in turn, lead directly to further neuronal injury, and this abnormal process will become self-perpetuating, with resulting neurotoxic effects on the retinal cells. Otherwise, the possibility that retinal cells initially died from retinal ischemia, which might be induced by ET-1 administration, cannot be ruled out.

The degenerative response of the central nervous system to ischemic insult is known to have an acute nature. In a partial ischemia model, which was induced by middle cerebral artery occlusion with collateral blood flow from the posterior circulation remaining open, ischemic infarcts in the cortex and the subcortical basal ganglia in the distribution of the middle cerebral artery were demonstrated 72 hours after the onset of occlusion. Based on this information and considering the rapid vasoconstrictor effect of ET-1, which is known to be initiated within seconds or minutes, it might be reasonable to propose that some of the previously demonstrated neuronal loss after 4 weeks of ET-1 administration occurred during the 2-week study period of the present work.

In addition to the elevation of glutamate and aspartate levels in the vitreous body, more than a 2-fold elevation of glycine was demonstrated in the ET-1 eye, which agrees with previous data. In a rat model of pressure-induced retinal ischemia, glycine levels were elevated by 428% compared with the nonischemic fellow eyes.

Several experiments have shown that NMDA receptor–mediated retinal ganglion cell death can be reduced by neuroprotective agents. Memantine, an NMDA antagonist, was efficacious at protecting ganglion cells from chronic low-dose glutamate toxicity and from high-pressure–induced retinal ischemia in rats. In addition, voltage-dependent calcium channel antagonists (eg, nimodipine) have recently been shown to diminish partially NMDA receptor–mediated retinal ganglion cell death.

### Table 1. Intraocular Pressure (IOP) in Rabbits Implanted With Endothelin-1 (ET-1)– or Balanced Salt Solution (BSS)–Filled Osmotic Minipumps for 2 Weeks

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Control</th>
<th>ET-1–Filled Minipumps (n = 5)</th>
<th>BSS-Filled Minipumps (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>117.29 ± 5.52</td>
<td>101.04 ± 10.41</td>
<td>81.64 ± 5.18</td>
</tr>
<tr>
<td>Aspartate</td>
<td>5.49 ± 0.81</td>
<td>2.34 ± 0.67</td>
<td>2.36 ± 0.48</td>
</tr>
<tr>
<td>Glutamate</td>
<td>100.46 ± 5.80</td>
<td>40.96 ± 5.86</td>
<td>46.29 ± 4.89</td>
</tr>
<tr>
<td>Glycine</td>
<td>336.60 ± 14.41</td>
<td>323.40 ± 15.17</td>
<td>363.68 ± 22.89</td>
</tr>
<tr>
<td>Histidine</td>
<td>21.28 ± 1.35</td>
<td>23.89 ± 1.34</td>
<td>19.78 ± 0.51</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>20.54 ± 1.44</td>
<td>18.81 ± 0.57</td>
<td>17.56 ± 3.89</td>
</tr>
<tr>
<td>Leucine</td>
<td>26.32 ± 1.97</td>
<td>26.12 ± 2.60</td>
<td>23.64 ± 3.08</td>
</tr>
<tr>
<td>Lysine</td>
<td>89.10 ± 3.69</td>
<td>79.38 ± 3.30</td>
<td>67.45 ± 1.03</td>
</tr>
<tr>
<td>Methionine</td>
<td>27.24 ± 1.27</td>
<td>27.99 ± 2.03</td>
<td>27.28 ± 1.83</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>12.99 ± 1.18</td>
<td>11.60 ± 0.90</td>
<td>11.52 ± 0.11</td>
</tr>
<tr>
<td>Serine</td>
<td>60.34 ± 4.06</td>
<td>51.14 ± 3.26</td>
<td>49.38 ± 7.00</td>
</tr>
<tr>
<td>Threonine</td>
<td>32.15 ± 3.27</td>
<td>27.38 ± 1.23</td>
<td>21.58 ± 1.17</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>41.05 ± 2.87</td>
<td>44.55 ± 1.99</td>
<td>34.43 ± 5.99</td>
</tr>
</tbody>
</table>

*Values are given as mean ± SEM.
†Indicates statistically significant difference in comparison with corresponding control by Wilcoxon signed rank test (P < .05).
Comparison of amino acid concentrations in the vitreous body of endothelin-1 (ET-1)-administered eyes and fellow control eyes. Each bar represents the mean for 5 animals.

excitotoxicity. These agents are regarded as potentially useful in the future management of glaucoma. We suggest that an ET-1–administered model may be useful in screening neuroprotective drugs for glaucoma. This model simulates glaucoma caused by microcirculatory compromise in terms of the optic nerve blood flow reduction and neural rim loss. Current demonstration of the elevation of glutamate and aspartate levels in the vitreous further justifies its use in studies aimed at understanding and arresting the mechanism of neurotoxicity on retinal ganglion cells.

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