Elevation of Intraocular Glutamate Levels in Rats With Partial Lesion of the Optic Nerve

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**Background:** Acute partial lesion of the rat optic nerve, although not a model for glaucoma, mimics some of the features of the disease.

**Objective:** To learn whether degeneration of rat optic nerve fibers and death of their retinal ganglion cells induced by an acute partial lesion are associated with elevated levels of glutamate, known to occur in the eyes of humans and monkeys with glaucoma.

**Materials and Methods:** Rat optic nerve was subjected to a partial crush injury. Aqueous humor samples were aspirated from the anterior and posterior aqueous chambers at specified times and their amino acid contents were determined by means of high-performance liquid chromatography.

**Results:** Three and 7 days after injury, intraocular glutamate and aspartate levels were found to be significantly higher than in normal or sham–operated-on eyes, and returned to normal by day 14.

**Conclusions:** Degeneration of the optic nerve, induced by a mechanical injury of the axons, leads to intraocular elevation of glutamate and aspartate levels. These results illustrate that the model of the partial optic nerve lesion exhibits another feature typical of a long-term optic neuropathy, such as glaucoma.


GLAUCOMA is a leading cause of blindness.1 The loss of vision in patients with glaucoma results from damage to optic nerve axons, with ensuing death of their retinal ganglion cells.2,3 The pathogenesis of optic neuropathy in glaucoma is still a matter of debate. Increased intraocular pressure (IOP) is probably the most important risk factor in primary open-angle glaucoma,4,5 and this has led to the widely held view that IOP plays a central role in the initiation and development of glaucomatous neuropathy. Increased IOP may act directly on optic nerve axons at the level of the lamina cribrosa or cause a change in the posterior microcirculation of the eye that could affect the optic nerve head.4,5 Alleviation of IOP is currently the treatment of choice in attempting to minimize the propagation of optic neuropathy and ganglion cell loss in patients with glaucoma. However, many of these patients continue to experience visual field loss long after their IOPs have been restored to normal.5 Moreover, as many as one sixth of all patients with glaucomatous damage show no evidence of elevated IOP, even on repeated testing.7,8

In view of the above and other observations, it is now commonly accepted that additional primary risk factors and/or secondary factors are involved in the pathogenesis of glaucoma.5,9 Secondary factors might include mediators produced by or associated with the axons that fell victim to primary risk factors (eg, increased IOP). According to this view, the progression of visual field loss in patients with glaucoma could be at least partly caused by secondary degeneration10,11 and might therefore be amenable to neuroprotection.

To study the possible mediators of neurotoxicity contributing to continuing optic nerve degeneration, and in an attempt to screen potential neuroprotective drugs for glaucoma, we have developed an animal model of an acute partial lesion of the optic nerve of the adult rat. Using this model, we have previously shown that a well-controlled crush injury of moderate severity causes acute damage to only a certain percentage of optic nerve fibers (approximately 58%). With time, however, the fibers that escaped the
MATERIALS AND METHODS

ANIMALS

Animals were used according to the Association for Research in Vision and Ophthalmology resolution on the use of animals in research. Male Sprague-Dawley rats weighing 300 to 400 g, from The Weizmann Institute of Science animal house, Rehovot, Israel, were anesthetized with ketamine hydrochloride, 50 mg/kg, and xylazine hydrochloride, 0.5 mg/kg, both administered intraperitoneally. Before tissue excision, the rats were killed by an overdose of pentobarbital sodium (170 mg/kg intraperitoneally).

CRUSH INJURY

With the aid of a binocular operating microscope, lateral canthotomy was performed in the right eyes of anesthetized rats. Optic nerves of the nonoperated-on contralateral eyes served as controls. An additional control group consisted of sham-operated-on animals, which were subjected to the surgical procedure but without the crush injury. Surgery was performed by incising the conjunctiva laterally to the cornea and exposing the optic nerve. Using calibrated cross-action forceps, we inflicted a moderate crush injury on the nerve 2 mm from the eyeball, taking special care not to interfere with the retinal blood supply.

FLUID COLLECTION

Animals were deeply anesthetized and their right (injured) and left (noninjured) eyes were excised 2 hours and 1, 3, 7, or 14 days after the crush injury, washed quickly 3 times in phosphate-buffered saline, dried carefully, and placed on clean, dry Petri dishes. The sclera was punctured with a scalpel and a large incision was made in the sclera and cornea, allowing the fluid to drain out of the eye into the dish. With the aid of a micropipette, fluid specimens (16 µL) were collected and placed in Eppendorf tubes. They were immediately frozen on dry ice and maintained at −70°C until use.

GLUTAMATE ANALYSIS

Each specimen was dissolved in 100 µL of double-distilled water and stirred for 1 minute. To separate proteins from the specimen, we ran the specimen through a solid-phase extraction device column (Sep-Pak C-18 cartridge; Waters Corp, Milford, Mass), prewashed with acetonitrile and double-distilled water, using a 43-µm pore size filter. Specimens were dried and then reconstructed in 20 µL of double-distilled water. Aliquots (5 µL) of the solution were subjected to amino acid analysis by means of a reverse-phase column (C-18, HPL, HP100), a precolumn derivation procedure, and detection by UV light. The amount of each amino acid was expressed as the mean percentage of the total amino acids recovered by the column.

RESULTS

Because of the extremely small volume of the vitreous body in the rat and the difficulty in measuring it accurately, our measurements were carried out in aqueous humor rather than in vitreous.

Injury of the rat optic nerve was accompanied by some changes in the intraocular content of individual amino acids. Comparison between the amino acid contents of samples taken from eyes 7 days after optic nerve injury and from eyes of nonoperated-on nerves showed that the excitatory amino acids, glutamate and aspartate, were the only ones whose levels were significantly increased in the injured eyes (Figure 1). Figure 2 shows glutamate levels from 2 hours to 14 days after injury. As shown, glutamate levels were elevated 3 days after injury, were still high on day 7, and had returned to normal by day 14. In the nonoperated-on contralateral eyes, glutamate levels were similar at all times examined, and they differed significantly from those in the injured eyes on days 3 and 7 (Table). The concentrations of glutamate and aspartate in the aqueous humor of the noninjured adult rat eye were 74.3±14.2 µmol/L and 23.4±5.1 µmol/L, respectively. The corresponding levels 7 days after injury were 147.6±15.8 µmol/L and 41±4.4 µmol/L. It should be emphasized that these levels do not represent the vitreous levels and therefore cannot be compared with levels found in humans and monkeys.

Examination of eyes of sham–operated-on nerves on day 7 after surgery disclosed no significant differences in glutamate levels from those in the nonoperated-on eyes. This finding rules out the possibility that the increase in glutamate level may have been caused by any procedure other than the nerve crush injury.

COMMENT

The results of this study show that mechanical injury of optic nerve axons results in an increase in the intraocu-
After Injury

Moreover, high levels of glutamate were shown to have toxic effects on mammalian retinal ganglion cells via N-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors.22-25 Kainic acid, a glutamate analog, was shown to induce retinal cell loss through apoptosis in both the inner nuclear layer and the ganglion cell layer.26

Although the elevated intraocular glutamate levels found in our optic nerve crush model may explain the secondary neuronal loss, the question of where and why it originates remains unanswered. The fact that intraocular glutamate levels in this study were already elevated 3 days after injury, preceding the reported death of cell bodies of the crush-injured axons,27,28 suggests that glutamate elevation is not necessarily a consequence of glutamate leakage from the dying ganglion cells. Glutamate is the neurotransmitter of neuronal elements, particularly photoreceptors and bipolar cells,29 but also ganglion cells, that transfer information vertically through the retina. Aspartate is colocated with glutamate in neuronal cell bodies but is present at lower levels than glutamate.30 Since enzymatic degradation does not occur extracellularly, glutamate released from presynaptic terminals is taken up intracellularly by at least 1 of the 3 known transporters and metabolized by a glutamate-degrading enzyme.31 Excitatory amino acid transporters are distributed in Müller cells, astrocytes, horizontal cells, amacrine cells, ganglion cells, and bipolar cells.32 Injury-induced changes in the retina, such as depolarization or oxidative stress, may affect glutamate and aspartate transporter activity. Aspartate uptake was shown to be inhibited by about 40% under oxidative stress conditions.33 Billups and Attwell34 demonstrated that with increasing extracellular K+ in brain ischemia, there is a reversal in glutamate uptake, and glutamate is released into the extracellular space.

The extracellular elevation in excitatory amino acids may result not only from changes in the activities of...
the transporters but also from excessive release of these neurotransmitters by the retinal cells. In vitro studies have shown that oxidative stress, hypoxia, and ischemia-like conditions promote the release of endogenous amino acids in cultured retinal cells. Moreover, glutamate agonists themselves stimulated the release of aspartate from monolayer cultures of chick retinal cells. In view of the above information, it seems reasonable to suggest that the initial perturbation of the retina after axonal injury may interfere with the normal neurotransmitter release and reuptake machinery and that, once initiated, this abnormal process will become self-perpetuating, with resulting neurotoxic effects on the retinal cells. This assumption is in line with the observation that the NMDA-receptor antagonist MK-801, when administered systematically immediately after the injury, protects optic neurons from secondary degeneration. We recently showed that local application of MK-801 to the vitreous body of the injured eye immediately after optic nerve crush had a similar protective effect. The fact that neuroprotection by an NMDA-receptor antagonist is effective when given immediately after injury suggests that glutamate elevation starts much earlier than 3 days after injury, but may be diluted by the local volume of vitreous and aqueous humors. Glutamate elevation becomes significant only when it exceeds a certain threshold, which depends on the dilution factor.

The partial crush injury model of the rat optic nerve, while not simulating human glaucoma, nevertheless has some features in common with it. For example, degeneration in both the animal model and the human disease continues to progress even after removal of the primary cause of optic nerve damage. In addition, cell body death is apoptotic in both cases. In the present study we demonstrated yet another common feature: intraocular glutamate elevation. The finding of elevated glutamate levels in our model is in line with the recently reported increase in glutamate levels in the vitreous bodies of humans, monkeys, and dogs with glaucoma. 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 intraocular amino acid concentration. Long-term exposure to elevated glutamate levels was found to be toxic to mammalian retinal ganglion cells. It should be noted that glutamate elevation in the animal model, unlike in humans, is transient. One possible reason for this difference is that the animal model may undergo more than 1 phase of degeneration after the primary injury, with changes in glutamate levels occurring in waves rather than continuously. This possibility should be examined by measuring postinjury glutamate levels during a longer period. Waves of degeneration may occur in the human disease as well, but careful monitoring would be required to check this possibility.

We suggest that this model may be useful in the screening of potential neuroprotective drugs for glaucoma. The model, though not simulating glaucoma itself, provides a system in which the primary mechanical loss can be demonstrated and the secondary loss can easily be quantified. The fact that the model exhibits behavioral features similar to those seen in glaucoma, such as continuous loss of neurons after removal of the external insult, apoptotic death of cell bodies, and elevation of intraocular glutamate level, further justifies its use in studies aimed at understanding and arresting the mechanism of propagation of optic neuropathy.

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References


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