Ganglion Cell Loss after Optic Nerve Crush Mediated through AMPA-Kainate and NMDA Receptors

Frank Schuettauf,1 Rita Naskar,1 Christian K. Vorwerk,1 David Zurakowski,2 and Evan B. Dreyer1

PURPOSE. Glutamate antagonists can block ganglion cell death due to optic nerve crush. Although most investigators have focused on blockade of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor, we have chosen to evaluate the efficacy of blockade of the AMPA-kainate (KA) receptor in this experimental paradigm.

METHODS. The optic nerves of rats were crushed, and ganglion cell survival was assessed. Groups of animals were treated with an NMDA antagonist, an AMPA-KA antagonist, or both.

RESULTS. The AMPA-KA antagonist DNQX was more effective, although not additive in preserving retinal ganglion cells after optic nerve crush than the NMDA antagonist MK801.

CONCLUSIONS. Activation of the AMPA-KA subtype of glutamate receptor may play a role in glutamate-mediated cell death after optic nerve crush. (Invest Ophthalmol Vis Sci. 2000;41:4313–4316)

Axonal damage usually induces neuronal death within the vertebrate central nervous system (CNS).1 Optic nerve crush is a graded, reproducible injury to the axons of the optic nerve that can be used to explore changes in retinal ganglion cells (RGCs) after axonal injury.2 3 Systemic administration of glutamate antagonists can protect RGCs from the effects of axotomy.4 This suggests that glutamate toxicity contributes to neuronal death after optic nerve injury. The toxic potential of glutamate to neurons is well documented. In 1957, Lucas and Newhouse5 first reported the toxic effects of glutamate on the mammalian eye. Although they were trying to block retinal degeneration, they found that s.c. injection of glutamate in young mice led to severe destruction of the inner retinal layers, most notably the ganglion cell layer. Olney6 demonstrated similar glutamate-induced retinal toxicity in neonatal mice and described this lesion as “excitotoxic”—because of excess stimulation by an excitatory amino acid through one of several ionotropic glutamate receptors. The ionotropic glutamate receptors are classified on the basis of agonist binding. The subunits of all three major ionotropic receptors share sequence homology and belong to a single superfamily.6

Among these classes of glutamate receptors, however, excitotoxic RGC loss is generally believed to be primarily mediated through the N-methyl-D-aspartate (NMDA) subtype.7 8 However, the AMPA and kainate (KA) receptor subtypes may contribute to ganglion cell loss after axotomy.7 9 10 Otori and coworkers9 have shown that low doses of glutamate are toxic to cultured RGCs through activation of the AMPA-KA receptors, with no effect on NMDA receptors. Nellgard and Wieloch11 demonstrated that application of AMPA blockers was protective against severe cerebral ischemia; NMDA antagonists were not protective in this model. Chen and coworkers12 have shown that activation of non-NMDA receptors can be toxic to retinal neurons. We, therefore, explored the AMPA-KA blocker, 6,7-dinitroquinoxaline-2,3-dione (DNQX), and compared its efficacy in this model to a well-characterized NMDA antagonist.

METHODS

All experiments were carried out in accord with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Adult Long-Evans rats were housed in a 12-hour light–dark cycle, with water and food ad libitum. Anesthesia was attained with choral hydrate (6 ml/kg body weight of a 7% solution), administered intraperitoneally. For those animals treated with DNQX or MK801, intraocular (i.o.) injections were performed with a heat-pulled glass capillary connected to a microsyringe (2 or 3 µl Microdispenser; Drummond Scientific, Broomall, PA). Injections were directed to the posterior pole of the eye to avoid any damage of the lens over a 30-second time period. Any animal with visible lens damage was euthanized and not included further.

To crush the optic nerve, adult Long-Evans rats were deeply anesthetized with chloral hydrate. The conjunctiva of one eye was incised. The optic nerve was exposed by blunt dissection. The meninges were pierced and bluntly dissected with forceps. A cross-action calibrated crush forceps was placed approximately 2 mm back from the globe, and the optic nerve was partially crushed for 20 seconds.13 For sham operations, the same procedure was followed, except that the forceps was not closed. In all cases, the blood supply to the retina was grossly intact after the crush procedure (as determined by direct visualization).

DNQX (105 nm, n = 7) was dissolved in solution of 50% DMSO and PBS and injected i.o. in a total volume of 3 µl.

From the 1Department of Ophthalmology, Veterans Administration and the University of Pennsylvania, Philadelphia; and the 2Department of Biostatistics, Children’s Hospital, Boston, Massachusetts.

Supported in part by grants from the Veteran’s Administration, the Potts Foundation, Research to Prevent Blindness, Inc., and the Paul and Evanna Mackall Foundation Trust.

Submitted for publication June 16, 2000; accepted July 19, 2000.

Commercial relationships policy: N.

Corresponding author: Evan B. Dreyer, Department of Ophthalmology, University of Pennsylvania, 51 North 39th Street, Philadelphia, PA 19104. ebd@mail.med.upenn.edu

Copyright © Association for Research in Vision and Ophthalmology
Animals were injected i.o. once on 2 consecutive days; the second injection was followed by optic nerve crush.

For MK801 injections, the NMDA antagonist (40 nm, n = 4) was dissolved in Hanks and given as a single i.o. injection in a total volume of 2 μl. This injection was followed by optic nerve crush.

Additional animals were injected with DNQX as described above, whereas on the 2nd day, 3 μl was aspirated from the vitreous, and DNQX and MK801 were injected, followed by optic nerve crush.

To determine RGC densities, cells were labeled retrogradely 5 days later with the fluorescent tracer Fluorogold (Molecular Probes, Eugene, OR) by stereotactic injections into the contralateral colliculus as described previously. Two days after the superior colliculus was injected with Fluorogold, animals were killed by chloral hydrate overdose (7 days after injection). Retinas were dissected, flat-mounted on cellulose nitrate filters (pore size 60 μm; Sartorius, Long Island, NY) and fixed in 2% PFA for 30 minutes. Cells were visualized under fluorescence microscopy. Three areas per retinal quadrant at three different eccentricities of one-sixth, one-half, and five-sixths of the retinal radius were counted. Labeled cells were thereby counted in 12 distinct areas of 62,500 μm² each in each retina.

**Statistical Analysis**

A two-level, nested analysis of variance (ANOVA) mixed model was used to determine group differences in RGCs/mm² with each group treated as the mean square error term, eyes nested within groups, and 12 individual replicates for each eye. A significant F ratio was followed by the post hoc Fisher least significant difference (LSD) procedure with a two-tailed Bonferroni adjusted α level. Because nine group comparisons were tested, P < 0.05/9 or P < 0.006 was considered statistically significant. Analysis of the data was performed with the SPSS software package (version 10.0; SPSS Inc., Chicago, IL). All P values are two-tailed.

**RESULTS**

Representative sections indicating ganglion cell survival are presented in Figure 1; survival is quantified in Figure 2.

RGC survival was assessed as described above. In eyes subjected to sham crush, 1796 ± 522 cells were noted per mm² (means ± SD, n = 6). Eyes subjected to optic nerve crush followed by injection with vehicle had 891 ± 437 (n = 11, DMSO) or 832 ± 472 (Hanks, n = 4) cells surviving. Eyes subjected to optic nerve crush and treated with MK801 had 1195 ± 493 RGCs surviving (n = 4). Eyes treated with DNQX had 1623 ± 431 (n = 7) cells surviving after crush. Eyes treated with both DNQX and MK801 after crush had 1434 ± 329 cells surviving (n = 4). In all cases, the concentrations of DNQX and MK801 used were based on maximal survival; higher and lower concentrations led to diminished ganglion cell survival (not shown). Only these optimal concentrations were explored in additive experiments.

Optic nerve crush led to a significant loss of ganglion cells, irrespective of vehicle (P < 0.001 in both cases). Both DNQX and MK801 were both protective against crush (P < 0.001 in both cases). However, these were not additive; that is, no more cells survived with both drugs than with either alone. In addition, more cells survived with DNQX treatment than with MK801 treatment (P < 0.001).

**DISCUSSION**

AMPA receptors mediate much of the rapid synaptic excitatory neurotransmission. The functional properties of the receptor, as in the nicotinic receptors, are dependent on the subunit composition, with the receptor composed of a combination of five of these subunits. Four closely related subunits have been cloned thus far, named GluR1 through GluR4 (also termed GluR-A through GluR-D). Furthermore, each of these subunits exists in two different forms—termed “flip” and

![Image](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933215/)
“flop,”—due to alternate splicing of a 115-bp region immediately preceding one of the transmembrane regions. Each of the eight possible splice variants of the four subunit types shows different expression patterns within the brain, both spatially and temporally. The alternate forms confer different properties to the receptors: flip channels continue to open in the face of repeated binding of glutamate, whereas the flop channel shows a gradual decrease in response. It has been suggested that this difference in desensitization and that change from flop to flip may play a role in long-term potentiation, the relatively long-lived strengthening of synaptic connectivity believed to be associated with memory.

AMPA receptors, again depending on the composition of subunits, have a variable selectivity to different ions, specifically Ca$^{2+}$ and Na$^+$. The GluR2 subunit is responsible for much of the difference: inclusion of this subunit in the channel substantially reduces the ability of the channel to pass Ca$^{2+}$ ions. AMPA channels are found throughout the brain—including the retina and specifically on RGCs.

KA receptors can be further subdivided into two classes based on the subunits cloned thus far: GluR5 through GluR7 and KA1 to -2 (also termed gamma 1 and gamma 2). Channels (which also have five subunits) that are composed of the GluR5 through GluR7 subunits are often referred to as low-affinity KA channels, with binding constants for KA approximately 10 times lower than for those channels containing KA1 and -2 subunits.

GluR5 to 7 subunits are expressed in many regions of the brain, including the retina and retinal ganglion cells, but in comparison to GluR1 to 4 are more restricted, and the distribution appears to be developmentally regulated. The KA-1 and -2 subunits are not found as functional homomeric KA channels but rather are found in combination with the GluR5 through GluR7 subunits. KA-1 subunits are found in the CA3 and dentate gyrus of the hippocampus, which is the classical KA high-affinity-binding site in the CNS. Both these subunits are found in the retina. No splice variants have yet been reported for these subunits.

The NMDA receptors are the most widely studied of the three subtypes of glutamate receptors, partly because they have been implicated in many CNS functions and dysfunctions, which are discussed below in the context of excitotoxicity. NMDA channels, unlike certain AMPA-KA channels, show very high selective permeability to Ca$^{2+}$ compared with that of other cations.

Five subunits have been cloned and are named NMDAR1, NMDAR2A through NMDAR2D. Functional channels can be formed completely from NMDAR1. This is not true of the 2A-2D subunits, which must be expressed in concert NMDAR1 to make a functioning channel. Inclusion of the 2A-2D subunits in functional NMDA channels alters the pharmacokinetics of the channel considerably. In particular, heteromeric channels containing these subunits increase the amplitude of the Ca$^{2+}$ flow through the receptor by 5- to 60-fold. NMDAR1 and 2A subunits are found throughout the brain whereas 2B is expressed selectively within the forebrain, 2C is found predominately in the cerebellum, and 2D is most prominently expressed in the brain stem, cerebellum, and olfactory bulb. All the subunits have been found in the retina.

The major interest in limiting excitotoxic damage in the past several decades has been directed at blockade of the NMDA receptor. We have previously shown (manuscript submitted) that optic nerve crush leads to release of glutamate into the vitreous of a rat eye, and that NMDA antagonists can limit damage from crush. Yoles and Schwartz have shown similar results, exploring elevation of glutamate in the vitreous. They and others have proposed the concept of secondary degeneration, whereby the initial insult of crush leads to loss of a population of ganglion cells; however, this primary insult also triggers additional death, perhaps through the release of toxic levels of glutamate. Although, as note above, NMDA antagonists are partially protective against this insult, we demonstrate here that AMPA-KA antagonists are if anything more protective, although not additive in this model system.

In summary, these data suggest that activation of the AMPA-KA receptors in the face of optic nerve crush may be critical in regulating neuronal death; their blockade may deserve additional consideration in limiting ganglion cell loss from glutamate-mediated damage.

References