Neuroprotective Effects of Eliprodil in Retinal Excitotoxicity and Ischemia

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PURPOSE. To evaluate whether eliprodil (SL82.0715), a NR2B-selective N-methyl-D-aspartate (NMDA) antagonist, is protective of retina subjected to an excitotoxic or ischemic insult.

METHODS. To evaluate protection against retinal excitotoxicity, eliprodil was administered intraperitoneally before and after the injection of NMDA (5 μL, 20 nmol) into the vitreous of rats. Integrity of the retina was assessed by counting cells in the retinal ganglion cell layer (GCL) and measuring choline acetyltransferase (ChAT) activity. In a subsequent experiment, total retinal ischemia, as measured by a cessation of electroretinographic (ERG) activity, was induced in anesthetized rabbits by elevating intraocular pressure above systolic blood pressure for 65 minutes. After ischemia, recovery of ERG activity was assessed at 24 and 48 hours in animals treated with vehicle or eliprodil (1.0–10.0 mg/kg).

RESULTS. Intravitreal NMDA injection resulted in a dose-related decrease in cells of the GCL and in ChAT activity. Eliprodil administered intraperitoneally at 10 mg/kg completely prevented the loss of ChAT and the loss of cells in the GCL. Twenty-four hours after retinal ischemia, A and B waves of vehicle-treated animals were suppressed by 60% to 70%. Eliprodil administered intraperitoneally at 10 mg/kg ameliorated the A- and B-wave depression throughout the 48-hour experiment.

CONCLUSIONS. Eliprodil is neuroprotective of retinace subjected to either an excitotoxic or ischemic challenge and may be useful for treating a variety of retinal and optic nerve head disorders. (Invest Ophthalmol Vis Sci. 1999;40:1177-1182)

To date, there are no neuroprotective agents indicated for the treatment of retinopathies or optic neuropathies, including glaucoma. Characteristic of these disorders is the development of visual field deficits concomitant with inner retina or optic nerve head pathology. Numerous lines of evidence now support the view that retinal dysfunctions brought about by ischemia or trauma may cause neuronal cell death due, at least in part, to a process termed excitotoxicity.

Excitotoxicity has been extensively implicated in disorders of the retina inclusive of glaucoma. In glaucoma, the pathologic changes of the optic nerve head (i.e., cupping, excavation, thinning of neural rim) are linked to the loss of retinal ganglion cells, toxicity that may be caused by glutamate. To date, numerous lines of evidence have demonstrated the sensitivity of cultured retinal ganglion cells to exogenously administered glutamate, perturbations of isolated or intact retinal tissue after application of a variety of excitatory amino acid (EAA) agonists, and distinct pathologies of retinal tissue after intravitreal injection of excitotoxins. Recently, a role for glutamate as a causative factor of glaucoma has been discussed by Dreyer and coworkers after a demonstration that patients with open-angle glaucoma or nonhuman primates with pressure-induced optic neuropathy have higher concentrations of glutamate in the vitreous.

It is well established that blockade of specific EAA receptors can impart resistance to neuronal tissues in the face of ischemia, trauma, or other metabolic disturbances. Numerous studies have shown protection of retinal tissue by selective EAA antagonists, particularly those that target the NMDA receptor. Thus, antagonists of the NMDA receptor are neuroprotective; however, not all antagonists of diversely distributed EAA receptors are neuroprotective to the inner retina, and many of these EAA antagonists have significant central nervous system (CNS) side effects, making them unsuitable for treating degenerative diseases of the eye.

In the present study, we evaluated the retinal neuroprotective effects of the NMDA antagonist eliprodil. This agent, which acts at the polyamine binding site of the NMDA receptor (NR2B subunit), has been shown to be potentially neuroprotective in a variety of CNS trauma and ischemia models and, unlike many other NMDA antagonists, is devoid of CNS side effects. Currently, eliprodil is being evaluated in the clinic for ameliorating neuronal dysfunction associated with head trauma. Because ischemia and excitotoxicity may be implicated in a variety of retinopathies and optic neuropathies, we tested the ability of eliprodil to attenuate injury to the inner retina produced by such stresses.
MATERIALS AND METHODS

All animal experiments were performed in accordance with the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research.

Intravitreal NMDA Injection

Long-Evans rats were anesthetized with isoflurane and injected intravitreally with 20 nmoles of NMDA. All animals received eliprodil intraperitoneally (IP) at one of three doses (0.3 mg/kg, 1 mg/kg, or 10 mg/kg) given 1 hour before NMDA injection, 1 hour after NMDA injection, and twice a day for the next 7 days. Animals were subsequently euthanatized by CO2.

For histology, enucleated eyes were fixed in Bouin’s fixative for 24 hours, sectioned (10 µm thickness), and stained with hematoxylin and eosin. Cells greater than 5 µm in diameter were counted in the ganglion cell layer 200 µm from the edge of the optic nerve head in the superior, inferior, and temporal quadrants. This size criterion excludes the majority of glial cells, the remaining cells being ganglion or displaced amacrine cells. In some animals, retinae were assayed for ChAT activity according to the method modified by Fonnum. Briefly, retinae were excised, rinsed in phosphate-buffered saline, and placed on ice in 10 × 75 mm glass tubes containing 500 µl of 25 mM phosphate buffer with 0.2% Triton X-100. Retinae were then sonicated (10X, 0.5 second), and the homogenates were transferred to microfuge tubes and spun at 20,000g for 1 hour at 4°C. To 50 µl of the supernatant, 50 µl incubation buffer and 0.2 mM 14C acetyl CoA were added, and tubes were incubated for 20 minutes at 37°C. Five milliliters ice-cold sodium phosphate buffer was then added to the scintillation vials, followed by 2 ml acetonitrile containing 10 mg sodium tetraphenylboron. Finally, 30 µl of the reaction mixture and 10 ml toluene solution containing 500 mg naphthalene were added. The vials were lightly shaken for 1 minute and then counted in a scintillation counter.

Retinal Ischemia

Total retinal ischemia was induced in pentobarbital-anesthetized (30 mg/kg, intravenously [IV]) Dutch Belted rabbits by elevating intraocular pressure to 140 mm Hg for 65 minutes. Pressure elevation was achieved by cannulating the anterior chamber of the eye with a tube connected to an elevated reservoir containing saline (BSS; Alcon Laboratories). Severe hypoxia was confirmed by a complete disappearance of the electroretinographic (ERG) waveforms for the duration of the ischemia. After ischemia, retinal functional status was evaluated by examining the ERG waveforms measured at 24 and 48 hours. To evaluate the effect of eliprodil, animals were administered a preschematic loading dose of eliprodil at 1 mg/kg IV, followed by 1 mg/kg IP twice a day for 2 days, or a 5 mg/kg IV loading dose, followed by 10 mg/kg IP twice a day for 2 days.

Electroretinograms were recorded using a JET contact lens electrode and a gold cup forehead electrode, using the brightest flash available from a Grass Instruments RS-22 xenon flash unit. Each recording was the computer-averaged mean of four flashes given 1 minute apart. Animals were dark-adapted overnight before recordings, and the pupils were dilated with 0.5% tropicamide (Mydriacyl; Alcon Laboratories).

RESULTS

NMDA-Induced Retinal Injury in the Rat

The excitotoxic action of glutamate that results in inner retinal degeneration is thought to be mediated in part by NMDA receptor activation. Antagonists of NMDA receptors have been demonstrated to provide significant retinal protection in a number of experimental paradigms. In rats, intravitreal injection of NMDA has been shown to attenuate ganglion cell number, to reduce inner plexiform layer thickness, and to depress ChAT activity, a measure of cholinergic amacrine cell function. In this study, the ability of eliprodil to attenuate the NMDA-mediated decline in ChAT activity was evaluated in Long-Evans rats at 7 days after intravitreal injection of 20 nmoles NMDA. The data in Figure 1 corroborate the results of Siliprandi et al. by demonstrating that intravitreal NMDA causes a dose-dependent decline in ChAT activity (IC50 = 22 nmoles) compared to the noninjected contralateral eye. No significant differences were measured between BSS-injected eyes compared to contralateral noninjected eyes (mean ± SEM, 1193 ± 114 versus 1082 ± 203 pmol/min per retina for BSS-injected versus noninjected, respectively). Figure 2 shows data from animals injected intraperitoneally with vehicle or eliprodil (0.3 mg/kg, 1.0 mg/kg, and 10.0 mg/kg, IP) before a 20-nmole NMDA injection and twice each day for 7 days after NMDA injection. Eliprodil attenuated, in a dose-dependent fashion, the loss of ChAT activity, with a complete block observed at the 10 mg/kg level (P < 0.05, Student’s t-test).

We assessed the ability of eliprodil to attenuate the NMDA-mediated decline in cell number in the ganglion cell layer in Long-Evans rats. Treatment with NMDA and eliprodil followed the paradigm described in the ChAT experiments. Cells with a diameter of 5 µm or greater were counted, which includes both displaced amacrine and ganglion cells. The left side of Figure 3 depicts the two non-NMDA-injected controls. Cell counts for the BSS-injected (OD) eyes and the noninjected contralateral eyes were not significantly different (P > 0.05, Student’s t-test). This indicates that the placement of a needle into the vitreous was without measurable injurious effect on the ganglion cell layer.

Figures 3 and 4 also show that intravitreal injection of NMDA produced a loss of cells in the ganglion cell layer that
was attenuated by the administration of eliprodil ($P < 0.05$, ANOVA/least significant difference test). In animals that were injected with NMDA, but not eliprodil, the loss of cells was 24% and 30% below noninjected and BSS injected controls, respectively. Complete protection against loss of cells was observed after the administration of 10 mg/kg eliprodil.

**Pressure-Induced Retinal Ischemia**

During ischemia, the A- and B-waves both went to zero amplitudes in all rabbits. Figure 5 shows examples of typical ERG waveforms. Because there was significant interanimal variation in baseline amplitudes, data were calculated as percent of baseline amplitude for each animal before statistical analyses were performed.

All animals exhibited some degree of recovery of A- and B-wave amplitudes after the ischemia was terminated. However, there were residual deficits in both A- and B-wave amplitudes (i.e., the ERGs showed evidence of persistent damage to retinal function). As can be seen in Figure 6, in control (vehicle-treated) animals, the A-wave was at 41% ± 8% and 42% ±...
8% of baseline at 24 and 48 hours, whereas the B-wave was at 32% ± 5% and 35% ± 6% of baseline at the same times. In contrast, animals receiving a loading dose of 3 mg/kg eliprodil IV followed by 10 mg/kg IP showed more postsischemic recovery, with the A-wave recovering to 85% ± 10% and 67% ± 8% of baseline at 24 and 48 hours, and with the B-wave recovering to 55% ± 4% and 63% ± 5% of baseline, respectively, at these times. Recoveries in the eliprodil-treated groups were significantly higher than in the control groups (P < 0.05, repeated measures ANOVA).

**DISCUSSION**

Eliprodil is a neuroprotective agent with activities at several sites. Apart from its well-documented action at the polyamine site of the NMDA receptor,28 eliprodil has also been shown to block L-, N-, and P-type voltage-dependent calcium channels.30 Eliprodil has low nanomolar affinity for the sigma opiate receptor, although it remains unclear whether this activity contributes to its overall neuroprotective profile.31 The ability of eliprodil to protect cultured neurons of the brain and the retina from an excitotoxic insult has been demonstrated elsewhere.28,29,32 The drug has been shown to potentiate the recovery of excitatory postsynaptic potentials and to enhance hippocampal slices’ eventual neuronal survival, after an hypoxic insult.32 In vivo studies have clearly established a neuroprotective profile for this agent that is based on a variety of acute focal cerebral ischemia paradigms in the rat,37,35 cat,35 and mouse.43 Additionally, eliprodil has been found to significantly reduce the extent of cortical damage after fluid percussion injury in the rat.35

Excitotoxicity has been linked to the pathologies associated with a variety of acute and chronic neurodegenerative disorders of the brain. Evidence now supports excitotoxicity as a causative stress in retinopathies (e.g., central/branch artery or vein occlusion) inclusive of glaucoma.15,21 Typical of these disorders are the pathologic changes of the inner retina that may include loss or dysfunction of ganglion and amacrine cells. In glaucoma, evidence is accumulating to suggest that the pathologic process leading to ganglion cell death may involve local changes at the cell soma or dendritic arbor, potentially brought about by excessive neuronal stimulation by EAAs. In this context, toxicity to the inner retina has been observed after intravitreal injection of EAAs4,14–17 and after application of EAAs to the isolated retina.4,15–17 Similarly, exogenously applied glutamate is toxic to retinal ganglion cells in culture.6–7 Although these studies do not directly implicate glutamate in the glaucomatous process, the finding that glutamate is elevated by 30% in the vitreous of nonhuman primates with pressure-induced optic neuropathy compared to ocular normotensive controls suggests a possible role.18 Moreover, vitreal samples collected from open-angle glaucoma patients undergoing cataract operations showed a twofold elevation in glutamate concentration compared to nonglaucomatous individuals.18 Although peak elevations in glutamate concentration in open-angle glaucomatous eyes were not as great as those measured in nonhuman primates, there is evidence to suggest that a chronic doubling of the vitreal glutamate concentration may over time produce retinal ganglion cell death.30 Elevations of vitreal glutamate to the range reported in these studies are consistent with concentrations previously reported to be toxic to neurons including retinal ganglion cells.6,20,37 Our data further document the marked sensitivity of the inner retina to NMDA receptor-mediated damage and cell loss. EAA antagonists, particularly of the NMDA subtype, have been shown to be neuroprotective after an ischemic or excitotoxic insult in cultured retinal ganglion cells5–7 or retinas.15,21,22 Many of these antagonists have also been shown to protect neuronal tissues subjected to ischemia, trauma, or other metabolic disturbances.19,20 Pharmacologically, the development of clinically useful NMDA antagonists has been difficult, because many competitive and noncompetitive antag-
In this article we demonstrate the neuroprotective effects of eliprodil in animal models of global retinal ischemia and excitotoxin-induced retinal injury. Our findings that eliprodil is retinoprotective after global ischemia (as measured by cessation of A- and B-wave activity) corroborates the findings of Yoon and Marmor, whereby systemic administration of the weak NMDA antagonist dextromethorphan enhanced the recovery of B-wave activity in the pigmented Dutch rabbit. Similar to their results, we observed recovery of ERG activity beginning 10 minutes after reperfusion. This duration of ischemia has been shown to result in an irregular distribution of damage involving all retinal layers and retinal pigment epithelium.

The dose range over which eliprodil was protective of retinal function (1-10 mg/kg) was consistent with the dose ranges found to be protective with this drug in acute focal ischemia and head trauma. Pharmacokinetic studies in the rabbit showed, that at neuroprotective doses described within, that retinal eliprodil concentrations were 0.5 μM to 7 μM. These levels fell within the proposed concentration range of 0.1 μM to 1 μM to provide in vivo efficacy in the CNS (Bernard Scallon, unpublished observations), and the concentration range reported to be neuroprotective in vitro (1-10 μM).

Apart from the excitotoxin-mediated loss of retinal ganglion cells, Siliprandi et al. observed that intravitreally injected EAAs also cause a loss of cells consistent with amacrine cell pathology. Amacrine cell pathology, particularly of the cholinergic subtype, has been confirmed by the loss of ChAT activity. We corroborated these findings in the rat by demonstrating that intravitreal NMDA causes a loss of both ChAT activity and cells in the ganglion cell layer. Siliprandi et al. showed a protection of ChAT activity and cell number by the NMDA antagonist MK-801. We showed similar protection with eliprodil starting at 0.5 mg/kg and complete protection at 10 mg/kg.

In summary, eliprodil is a neuroprotective agent with activities at multiple sites. The compound is neuroprotective in the CNS and free of CNS side effects that are characteristic of many other EAA antagonists. Eliprodil protects cultured retinal ganglion cells from an excitotoxic challenge and preserves retinal subjected to ischemic or excitotoxic insult. These findings make eliprodil potentially useful for treating a variety of ischemic or traumatic retinopathies potentially inclusive of glaucoma.

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