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Molecular Basis of Glutamate Toxicity in Retinal Ganglion Cells

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Loss of retinal ganglion cells (RGCs) is a hallmark of many ophthalmic diseases including glaucoma, retinal ischemia due to central artery occlusion, anterior ischemic optic neuropathy and may be significant in optic neuritis, optic nerve trauma, and AIDS. Recent research indicates that neurotoxicity is caused by excessive stimulation of receptors for excitatory amino acids (EAAs). In particular, the amino acid glutamate has been shown to act as a neurotoxin which exerts its toxic effect on RGCs predominantly through the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor. NMDA-receptor-mediated toxicity in RGCs is dependent on the influx of extracellular Ca^{2+}. The increase in $[Ca^{2+}]_i$ acts as a second messenger that sets in motion the cascade leading to eventual cell death. Glutamate stimulates its own release in a positive feedback loop by its interaction with the non-NMDA receptor subtypes. Ca^{2+}-induced Ca^{2+} release and further influx of Ca^{2+} through voltage-gated Ca^{2+} channels after glutamate-induced depolarization contribute to glutamate toxicity. *In vitro* and *in vivo* studies suggest that the use of selective NMDA receptor antagonists or Ca^{2+} channel blockers should be useful in preventing or at least abating neuronal loss in the retina. Of particular importance for future clinical use of NMDA receptor antagonists in the treatment of acute vascular insults is the finding that some drugs can prevent glutamate-induced neurotoxicity, even when administered a few hours after the onset of retinal ischemia.

N-Methyl-D-Aspartate Retinal ganglion cells Neurons Excitotoxicity Glutamate Cell death

INTRODUCTION

Pathological activation of glutamate receptors is thought to be a final common pathway leading to neuronal damage in the course of many neurological diseases such as hypoxic-ischemic brain injury, epilepsy, Huntington's disease, Parkinson's disease, Alzheimer's disease, and HIV-associated dementia (for a review with original references, see Lipton & Rosenberg, 1994). Diseases of the eye and retina that are likely to involve glutamate receptor-mediated toxicity, at least to some degree, are glaucoma (including open angle glaucoma, chronic angle closure glaucoma and pseudo-exfoliative glaucoma), central and branch retinal arterial and retinal vein occlusions. Moreover, anterior ischemic optic neuropathy, optic neuritis (at least drug-induced) and optic nerve trauma possibly involve glutamate-related toxicity. Several of the above mentioned systemic diseases, such as Alzheimer's disease and the HIV-associated dementia, have been reported to affect the retinal ganglion cell layer (Hinton *et al.*, 1986; Tenhula, Xu, Madigan, Heller, Freeman, & Sadun, 1992).

Glutamate, or related excitatory amino acids (EAAs), are thought to mediate excitatory synaptic transmission at the photoreceptor/bipolar cell synapses and at the bipolar/ganglion cell synapses, acting through activation of glutamate receptors. Glutamate is probably the excitatory transmitter used by retinal ganglion cells (RGCs). EAAs acting at specific membrane receptors also influence neuronal plasticity and survival, and EAA receptor activation plays an important role in the development of the visual system (Kleinschmidt, Bear, & Singer, 1987; Lipton & Kater, 1989; Simon, Prusky, O'Leary, & Constantine-Paton, 1992; Komuro & Rakic, 1993).

Glutamate receptors have been divided according to their primary signal transduction mechanism into ionotropic and metabotropic receptor subtypes (Nakanishi, 1992). The ionotropic glutamate receptors have been named according to their preferred agonists the *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxyl-5-methyl-isoxazol-4-propionic acid (AMPA), and kainate subtypes. The latter two types are also referred to as non-NMDA receptors since the functional properties between the two groups are not totally distinct.

This article will review experimental evidence from

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our laboratories that glutamate itself can act as a major neurotoxin in the retina and that EAA-induced neurotoxicity in RGCs is predominately mediated by NMDA receptors, at least under certain conditions. Potential mechanisms of toxicity at the molecular level downstream of glutamate receptor activation are described. Finally, the significance of these findings for possible therapeutic strategies to prevent or abate loss of RGCs as a consequence of ophthalmic diseases are discussed.

PHYSIOLOGY OF RGCS

RGCs are the converging endpoint of retinal information processing (Wässle & Boycott, 1991). The result of retinal preprocessing of visual information is transmitted to subsequent areas in the brain in the form of trains of action potentials that travel along RGC axons. Mammalian RGCs receive excitatory and inhibitory input from bipolar, amacrine and other RGCs through chemical synapses. Accordingly, RGCs express a variety of neurotransmitter receptors and voltage-gated ion channels. Of particular importance for both the physiological function of RGCs and pathophysiological changes that can lead to cell death are those receptors and ion channels that influence the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) of RGCs (Lipton & Kater, 1989).

Ca^{2+} ions serve as ubiquitous intracellular second messenger molecules. The $[Ca^{2+}]_i$ determines the activity of many ion channels and enzymes either directly or indirectly through activation or inactivation of enzymes like phospholipases, phosphokinases and phosphatases. Ca^{2+} influx from the extracellular space and the Ca^{2+} flux into and out of intracellular compartments are tightly regulated, and Ca^{2+} itself can serve as both a positive and negative regulator of $[Ca^{2+}]_i$. For example, influx of Ca^{2+} has been shown to stimulate further release of Ca^{2+} from intracellular stores. This effect of Ca^{2+} has been termed Ca^{2+}-induced Ca^{2+} release and has been shown to be particularly important in RGCs Lei *et al.*, 1992; Leinders-Zufall, Rand, Waxman, & Kocsis, 1994).

RGCs from rat have been studied in great detail using the patch-clamp technique (Lipton, Aizenman, & Loring, 1987; Lipton & Tauck, 1987; Aizenman, Frosch, & Lipton, 1988; Aizenman, Lipton, & Loring, 1989; Karschin, Aizenman, & Lipton, 1988; Lipton, 1988; Tauck, Frosch, & Lipton, 1988; Karschin & Lipton, 1989; Lipton, 1989; Sucher, Aizenman, & Lipton, 1991a,b,c; Pan, Bähring, Grantyn, & Lipton, 1995). They possess receptors for excitatory transmitters such as acetylcholine (Lipton *et al.*, 1987) and glutamate or related EAA (Aizenman *et al.*, 1988; Zhang & Lipton, 1992) and receptors for the inhibitory neurotransmitters GABA and glycine (Tauck *et al.*, 1988; Lipton, 1989; Pan *et al.*, 1995). It has been shown that virtually all rat neonatal RGCs possess EAA receptors of the non-NMDA and/or the NMDA type (Aizenman *et al.*, 1988). Transcripts for many subunits of the glutamate receptor family have been found in rat RGCs by *in situ* hybridization (Hamassaki, Hermans, Heinemann, & Hughes, 1993; Brandstätter, Hartveit, Sassoe-Pognetto, & Wässle, 1994). Interestingly, individual RGCs appear to co-express transcripts for multiple subunits (Hamassaki *et al.*, 1993). NMDA, certain non-NMDA receptors, and nicotinic acetylcholine receptors are directly permeable to Ca^{2+}. Moreover, it has been demonstrated that Ca^{2+}-induced Ca^{2+} release is responsible for up to 50% of the increase in $[Ca^{2+}]_i$ following NMDA receptor activation in RGCs (Lei *et al.*, 1992a).

Recent studies from several laboratories have demonstrated that RGCs possess Ca^{2+}-permeable non-NMDA receptors (Rörig & Grantyn, 1993; Leinders-Zufall *et al.*, 1994; Zhang, Sucher, & Lipton, 1995). The kainate-evoked current is generally much larger than that evoked by NMDA in RGCs and it is non-desensitizing. The Ca^{2+} permeability coupled with the rate of desensitization of the non-NMDA receptors determine how much Ca^{2+} enters the cell via these channels. A large sustained response to glutamate and a higher permeability to Ca^{2+} will work synergistically to allow greater influx of Ca^{2+} into RGCs. Therefore, the amount of Ca^{2+} entering through AMPA/kainate receptor-operated channels in RGCs might be considerable, even though the $P_{Ca^{2+}}/P_{Cs^+}$ value for non-NMDA receptors has been found to be about 10-times lower than that for NMDA receptors in RGCs. In individual RGCs, the combination of multiple glutamate receptor subunits to form single channels, as well as the co-expression of the resulting multiple channel types determine the response to glutamate (Zhang *et al.*, 1995). In addition, voltage-gated Ca^{2+} channels allow further influx of Ca^{2+} after glutamate-induced depolarization.

The combined effect of NMDA/non-NMDA receptor-mediated excitatory and GABA/glycine receptor-mediated inhibitory inputs together with the activation state of voltage-gated ion channels determines the membrane potential of RGCs. Rat RGCs express a variety of voltage-gated ion channels (Lipton & Tauck, 1987; Karschin & Lipton, 1989; Sucher & Lipton, 1992; Taschenberger & Grantyn, 1995). Of particular importance for the biochemistry of RGCs are voltage-gated ion channels that are permeable to Ca^{2+} ions. RGCs possess a variety of voltage-gated Ca^{2+} channels including N-, T-, L- and possibly Q-type channels (Karschin & Lipton, 1989; Rayudu, Chen, & Lipton, 1995; Taschenberger & Grantyn, 1995). Of particular importance for the discussion of the etiology of RGC neurotoxicity and possible therapeutic prevention of cell death are the L-type calcium channels because of their prolonged action and availability of inhibitory drugs (Karschin & Lipton, 1989).

GLUTAMATE AS A MAJOR RETINAL NEUROTOXIN *IN VIVO*

Lucas & Newhouse (1957) first reported the toxic effects of glutamate on the mammalian eye. Subcutaneous injection of glutamate into young mice led to severe destruction of the inner retinal layers, most notably in the retinal ganglion cell layer. Using ultrastructural techniques, Olney demonstrated similar

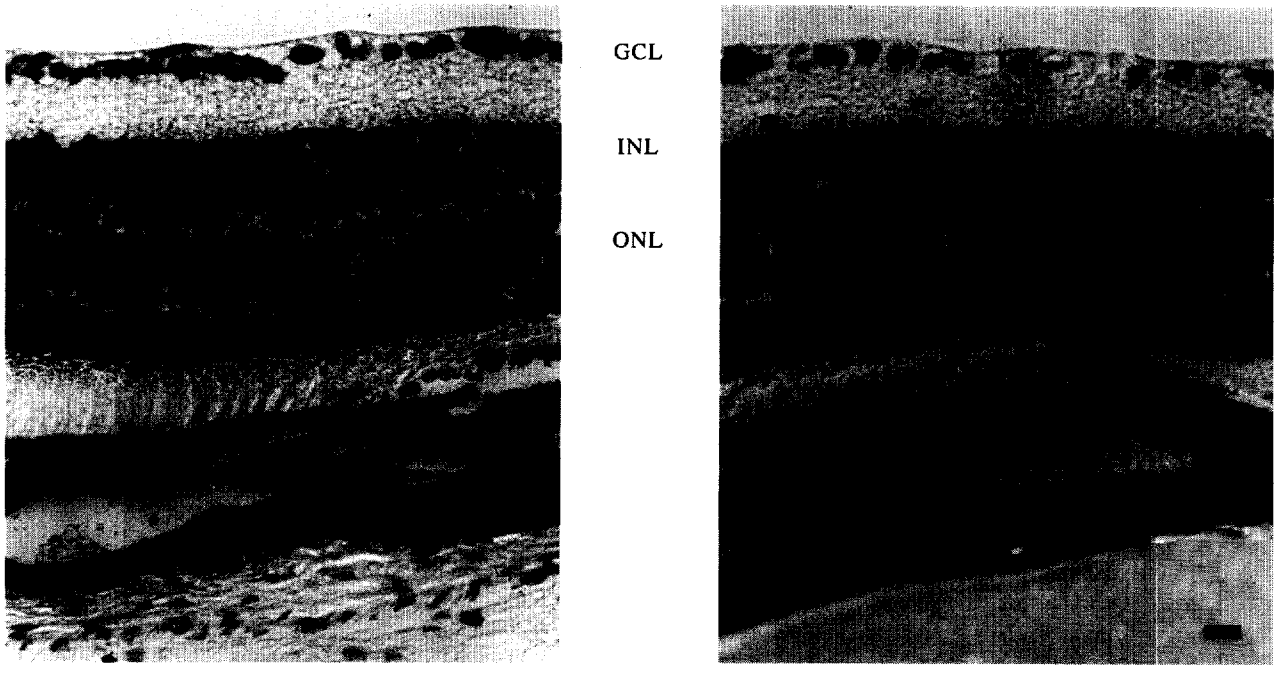

FIGURE 1. Toxicity of chronic administration of glutamate to the retinal ganglion cell layer. Adult rats were anesthetized, and one eye of each rat injected under direct visualization every 5 days with 1 ml of a 5 mM solution of glutamate. The contralateral eye was used as a control, and was injected with 1 ml of vehicle on the same days. The glutamate-treated rat retina (right) shows a diminution of the cells in the retinal ganglion cell layer (GCL) when compared with the control eye (left). Accordingly, chronic glutamate administration is toxic to cells of the retinal ganglion layer. Note the diminution of the retinal ganglion cell layer, with sparing of the other layers of the retina. This finding is strikingly similar in appearance to human glaucomatous retina (not illustrated). INL, inner nuclear layer; ONL, outer nuclear layer. Scale bar: 15 μm.

glutamate-induced retinal toxicity in neonatal mice, and coined the term "excitotoxicity" to describe these lesions (Olney, 1982). Sisk & Kuwabara (1985) injected glutamate intravitreally into adult albino rats and observed degeneration of the inner nuclear and ganglion cell layers.

These classical studies demonstrated that acute administration of intravitreal glutamate is severely toxic to the inner layers of the retina. However, the effect of a chronic elevation in vitreal glutamate on the retina had not been explored until recently (Samy, Lui, Kaiser, Lipton, & Dreyer, 1994; Vorwerk, Lipton, Zurakowski, Hyman, Sabel, & Dreyer, 1996). To do this, one eye of adult rats received an intravitreal injection of glutamate (1 μl of a 5 mM solution) every 5 days for 3 months. This injection elevated vitreal glutamate to $\sim$30 μM, compared to a baseline value of $\sim$5 μM in the control eye. The contralateral eye received an injection of control vehicle. Histologic sections were prepared from both glutamate treated and control eyes, and cells in the retinal ganglion cell layer were counted in a masked fashion. This treatment resulted in selective damage to the retinal ganglion cell layer (Fig. 1). The number of cells in the retinal ganglion cell layer in eyes treated with chronic glutamate was 56 000 $\pm$ 9600 cells per retina (n = 8). The retinal ganglion cell layer in control eyes contained 96 500 $\pm$ 8500 cells per retina (n = 8). This difference was statistically significant ($P < 0.001$; non-parametric Wilcoxon rank sum test with Bonferroni procedure). No changes were seen in eyes treated with glutamate for less than 30 days, suggesting that chronic administration was necessary for the observed effect at these low concentrations of glutamate. These results indicate that chronic glutamate administration is deleterious to the retinal ganglion cell layer. Interestingly, chronic administration results in a histologic pattern similar to that seen with an acute bolus of intravitreal glutamate, and is remarkably similar to the histopathology seen in human glaucoma (Schumer & Podos, 1994; Dreyer, Zurakowski, Schumer, Podos, & Lipton, 1995c).

IN VITRO MODEL OF GLUTAMATE TOXICITY IN RGCS

In order to investigate glutamate toxicity in RGCs under well controlled and defined conditions, short-term *in vitro* culture of freshly dissociated RGCs was developed (Leifer, Lipton, Barnstable, & Masland, 1984; Hahn, Aizenman, & Lipton, 1988). In this model system, rat RGCs are labeled *in situ* by retrograde transport of a fluorescent dye. The dye is injected into the superior colliculus of 4–9-day-old animals under anesthesia. The animals are sacrificed 2 days after injection, enucleated, and the retinas removed. Retinas are treated with papain and mechanically dissociated for plating by trituration. Living RGCs can be identified by the presence of granular blue and by the uptake and cleavage of fluorescein diacetate to fluorescein (Fig. 2). Toxicological studies or patch-clamp recordings can be performed 4–48 hr after plating. RGCs comprise about 1% of the

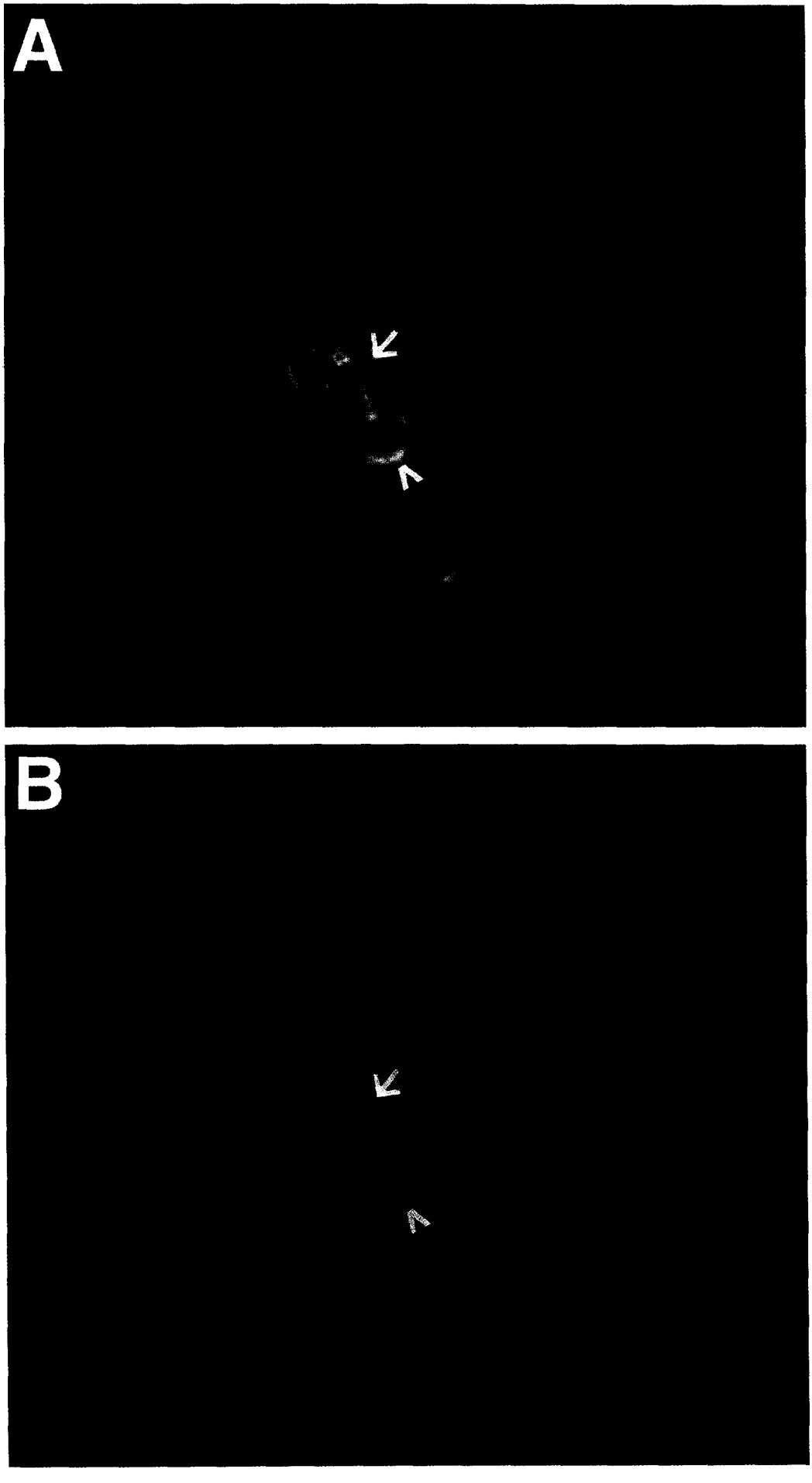

FIGURE 2. *Caption on facing page.*

cells in these short-term cultures. Eighty five to ninety percent of the RGCs are commonly found in small clusters with other retinal cells while the remaining cells are solitary RGCs (Lipton, 1986). Thus, the biophysical and pharmacological characterization of ion channels can be carried out in the same culture system that is used for toxicity studies (Levy, Sucher, & Lipton, 1990, 1991; Sucher *et al.*, 1991a,c).

Using such RGC cultures, it has been demonstrated that glutamate, NMDA, kainate, endogenous glutamate or a glutamate-like toxin kills RGCs in a Ca^{2+}-dependent manner (Hahn *et al.*, 1988; Sucher *et al.*, 1991a). The association between rises in $[Ca^{2-}]_i$ and subsequent neuronal death was reinforced by the finding that activation of NMDA receptor-operated channels via a redox modulatory site increases both NMDA-induced neuronal injury and NMDA-receptor activated Ca^{2+} flux (Levy *et al.*, 1990; Sucher, Wong, & Lipton, 1990; Levy *et al.*, 1991; Sucher & Lipton, 1991).

Excitotoxicity in RGCs caused by either endogenous toxin(s) or exogenously added NMDA or glutamate could be blocked by NMDA receptor antagonists or L-type Ca^{2+} channel blockers (Lipton, 1991, Lipton, Sucher, Kaiser, & Dreyer, 1991; Sucher *et al.*, 1991c). HPLC analysis of the culture medium indicated that RGC cultures contained $\sim 26\ \mu M$ glutamate, suggesting that this EAA itself may be the endogenous toxin (Lipton *et al.*, 1991). In order to directly test this hypothesis, RGC cultures were incubated with the enzyme glutamate-pyruvate transaminase (GPT). This enzyme catalyzes the transamination of glutamate to α-ketoglutarate while the required co-substrate pyruvate goes to alanine. GPT-treatment abrogated toxicity due to the endogenous toxin in the presence, but not absence, of pyruvate. HPLC analysis confirmed that addition of GPT and pyruvate reduced the glutamate concentration in the treated cultures to below 7 μM (Lipton *et al.*, 1991). Thus, glutamate appeared to be required for the manifestation of excitotoxicity in RGC cultures. A simple explanation consistent with these data is that glutamate itself is an endogenous toxin causing RGC death by activating NMDA receptors.

In order to assess more directly the possible role of non-NMDA receptors in glutamate-mediated toxicity, a separate set of experiments was performed (Sucher *et al.*, 1991a). Addition of kainate (125 μM) to neonatal RGC cultures increased the proportion of RGCs that die compared with sibling cultures not receiving kainate. The toxicity due to both kainate and/or the endogenous toxin was blocked by addition of the competitive NMDA receptor antagonist 2-amino-5-phosphonovalerate (APV). However, only a portion of kainate-induced toxicity was blocked by the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). These data indicate that either kainate might activate NMDA receptors or that kainate might activate AMPA/kainate receptors and stimulate the release of endogenous glutamate, which in turn would kill RGCs by activating NMDA receptors. Patch-clamp recordings demonstrated that kainate-evoked currents were not affected by NMDA antagonists, thus arguing against the first possibility. Since GPT/pyruvate blocked the toxicity due to endogenous toxin, it is likely that stimulation of non-NMDA type glutamate receptors leads to an increase in the release of endogenous glutamate. It is not known, however, if toxic glutamate levels are reached by synaptic release of this amino acid alone and/or due to glutamate release from dying cells. The blockade of electrical activity by addition of the sodium channel blocker tetrodotoxin (TTX) to these cultures also promoted the death of clustered RGCs derived from postnatal days 2–10 animals (Lipton, 1986; Kaiser & Lipton, 1990). Thus, it has not been possible to evaluate the contribution of synaptic glutamate release to excitotoxicity.

It has been shown that glaucoma preferentially damages larger RGCs (Glovinsky, Quigley, & Dunkelberger, 1991; Chaturvedi, Hedley-Whyte, & Dreyer, 1993; Glovinsky, Quigley, & Pease, 1993), and this has also been suggested in Alzheimer's disease (Hinton *et al.*, 1986) and possibly in AIDS (Tenhula *et al.*, 1992). Previous experiments suggested that, *in vitro*, larger RGCs show greater susceptibility to glutamate-induced cell stress, as evidenced by increased production of heat shock proteins. Recently, a study was undertaken to explore directly whether larger RGCs might be more sensitive to glutamate-mediated neuronal cell death *in vitro* and *in vivo* (Dreyer, Pan, Storm, & Lipton, 1994). When cultured RGCs were scored based on viability and size in the face of incubation with glutamate, it was found that the smaller RGCs were apparently not very sensitive to the addition of exogenous glutamate. Glutamate (100 μM) had little effect on RGC viability for cells smaller than 7.7 μm in diameter. Only larger RGCs were killed in this experimental paradigm (960 RGCs were scored by size in four experiments; significant by paired *t*-test, $P < 0.001$). Results similar to these in culture were also seen in the intact animal. Rodent eyes were injected with NMDA, and the animals sacrificed 5 days later. When viable cells were scored by size ($n = 1200$), the mean cell size in the control eyes was statistically larger than in eyes treated with an intravitreal injection of

FIGURE 2. *(opposite)* Photomicrographs of RGCs in cell culture following a ~20 hr incubation with 1 mM glutamate (with permission from Hahn *et al.*, 1988). Photographs were taken from the same field under standard UV and fluorescein filter sets. (A) RGCs are shown under a combination of phase contrast and UV epifluorescence. Two RGCs are identified by their blue fluorescence because of prior labeling and retrograde transport of the fluorescent dye granular blue. Other retinal cells are not labeled. Cellular debris can be seen in the picture as a consequence of disintegrating cells due to glutamate-induced cytotoxicity. (B) Live RGCs can be distinguished from dead RGCs by the uptake and cleavage of the green fluorescent dye fluorescein diacetate (arrow). Dead RGCs are not fluorescent (arrow head). Other retinal cells visible in this field are alive by this criterion.

NMDA ($n = 3$; significant by paired t-test, $P < 0.05$). NMDA had little effect on RGCs smaller than 7.7 μm in diameter. RGCs larger than this diameter were sensitive to NMDA-induced death.

The ability of RGCs to survive an NMDA excitotoxic challenge *in vitro* was scored as a function of the presence of neurites, and the number of neurite branch points (Heng, Moscaritolo, & Dreyer, 1995). It has been found that RGCs with processes were far more sensitive to NMDA-mediated excitotoxicity than RGCs without such neurites. Susceptibility to NMDA toxicity increased with process length. For example, NMDA was toxic to 29% of the RGCs with a total neurite length of between 25 and 50 microns, but the same dose of NMDA killed 58% of the cells with a process length between 101 and 125 microns. NMDA toxicity was abrogated by the addition of 12 μM of the NMDA antagonist MK-801 (dizocilpine). MK-801 had no effect on ganglion cell survival alone. Those cells with longer neurites were more likely to have an increased number of neurite branch points; consequently, cells with more branch points were also more susceptible to NMDA toxicity. However, this finding is dependent on the primary result, i.e., sensitivity to NMDA toxicity was increased in cells with longer neurites.

"DOWNSTREAM" MECHANISMS OF GLUTAMATE TOXICITY

NMDA-receptor mediated excitotoxicity in RGCs has been shown to be dependent on the influx of extracellular Ca^{2+} (Hahn *et al.*, 1988) and the block of L-type Ca^{2+} channels by dihydropyridines has been found to attenuate toxicity in RGCs due to either the endogenous EAA-related neurotoxin(s) or exogenous glutamate and NMDA (Dreyer, Kaiser, Offermann, & Lipton, 1990; Lipton, 1991; Sucher *et al.*, 1991c). It is thought that the increase in $[Ca^{2+}]_i$ acts as a second messenger that leads to changes in the baseline activity of a number of enzymes and in turn sets in motion the cascade leading to eventual cell death (Fig. 3). Interestingly, however, elevation of $[Ca^{2+}]_i$ due to depolarization of RGCs by high concentrations of extracellular K^+ appears to cause less neuronal damage than similar $[Ca^{2+}]_i$ increases induced by glutamate (S. A. Lipton, unpublished observation). It has not been established yet if the location or mode of entry of Ca^{2+} are critical or if activation of glutamate receptors leads to the generation of additional, as yet unidentified messengers that contribute to neurotoxicity.

A possible contributor to the glutamate-activated cascade leading to cell death is neuronal nitric oxide synthase (nNOS). The critical role of nNOS in neuronal function has been demonstrated in a plethora of studies over the past half dozen years. Intense interest has focused on the role of nitric oxide in excitotoxicity since the publication of a report linking NMDA receptor-mediated neurotoxicity and nitric oxide production. In several subsequent studies, however, apparently contradictory results were obtained when nitric oxide was found to be neurodestructive in some studies but neuroprotective in others (Dawson, Dawson, London, Bredt, & Snyder, 1991; Lei *et al.*, 1992; Moncada, Lekieffre, Arvin, & Meldrum, 1992; Dawson, Dawson, Bartley, Uhl, & Snyder, 1993; Lipton *et al.*, 1993). These apparently contradictory findings could be explained, at least in part, by a redox-based mechanism taking into account that nitric oxide proper is only one of three possible redox forms of nitrogen monoxide (NO) (Lipton *et al.*, 1993). NO-related neurotoxicity is probably mediated by the generation of peroxynitrite ($ONOO^-$) by the reaction of NO (true nitric oxide with an unpaired electron) with superoxide anion ($O_2\cdot^-$). The NO group in the NO^+ form (with one less electron than NO·) exerts its neuroprotective effects by down-regulating NMDA receptor activity via reaction with a redox modulatory site consisting of critical cysteine sulfhydryl groups. Among the modulatory sites of the NMDA receptor, these so-called redox modulatory sites of the NMDA receptor are of particular interest since manipulation of the redox state influences NMDA receptor-mediated neurotoxicity (Levy *et al.*, 1990, 1991).

The redox modulatory sites of the NMDA receptor are operationally defined by the effect of the reducing and oxidizing agents dithiothreitol (DTT) and 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) (Aizenman *et al.*, 1989). Extracellular application of DTT increases ionic currents through the NMDA receptor-gated ion channel; DTNB decreases this current. Up-regulation of the NMDA receptor-gated ion channel current by DTT is reversed by application of DTNB, and downregulation following DTNB application is reversed by subsequent exposure to DTT. The sulfhydryl group alkylating agent *N*-ethylmaleimide (NEM) irreversibly blocks the effect of both drugs (Lei *et al.*, 1992). Thus, sulfhydryl groups are likely to be important for the formation of the redox modulatory site. This modulatory site acts like a gain switch controlling channel activity. Neither the channel pore itself nor the receptor binding sites for glutamate or glycine appear to be affected by redox modulation (Sucher *et al.*, 1990). Recent experiments with mutant subunits in which cysteines were changed by site-directed mutagenesis identified at least two cysteines in the NMDAR1 subunit that appear to be required for redox modulation (Sullivan, Traynelis, Chen, Escobar, Heinemann, & Lipton, 1994).

NMDA receptor-mediated neurotoxicity in RGCs increased with DTT treatment, and this effect was blocked by DTNB (Levy *et al.*, 1990). These findings suggest that the redox state of the NMDA receptor is crucial for the survival of neurons facing glutamate-related injury. Endogenous redox agents, such as free radicals, glutathione, ascorbate and others, have been shown to directly or indirectly influence this redox site(s). Both oxidized and reduced glutathione have been found to be protective and to attenuate RGC damage mediated by NMDA receptor activation (Levy *et al.*, 1991; Sucher & Lipton, 1991). Consistent with these findings, oxidized glutathione was effective as a downregulator at the

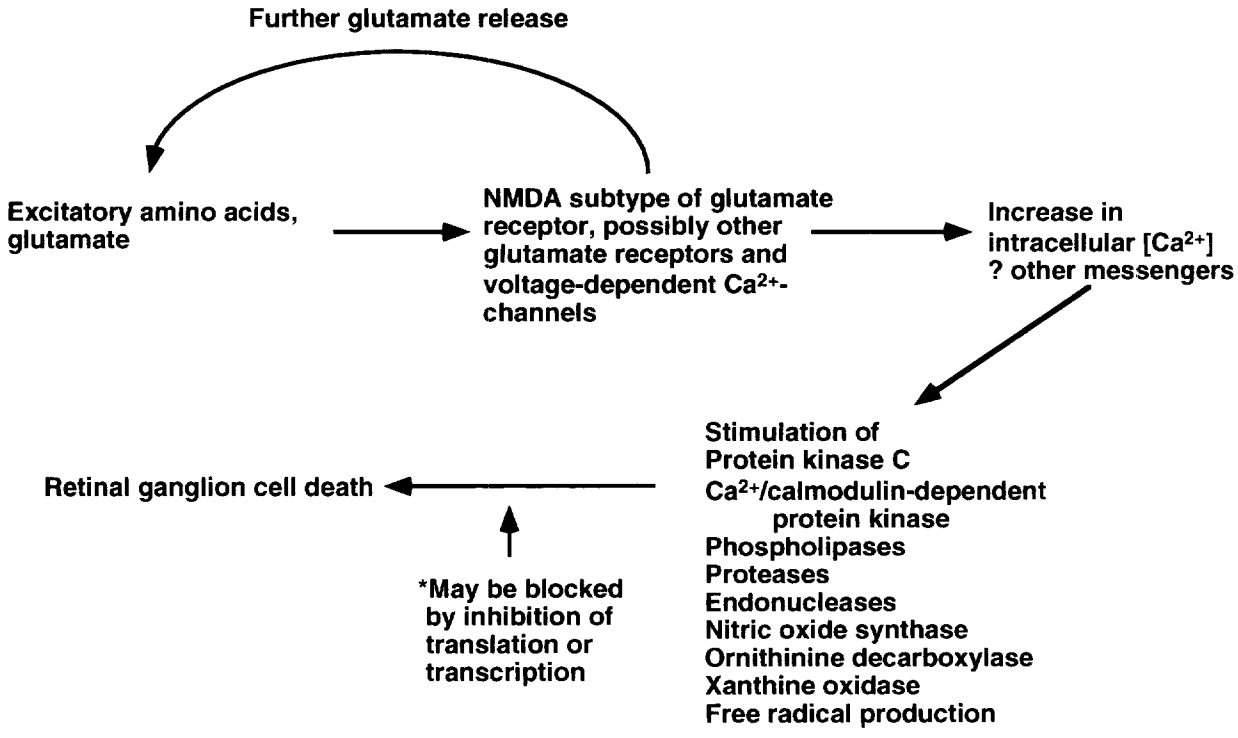

FIGURE 3. Diagram of the pathophysiology of glutamate-induced toxicity in RGCs. *Mild insults (low-dose glutamate or NMDA) lead to RGC death through apoptosis while intense insults (high-dose glutamate or NMDA) are more likely to produce RGC necrosis.

NMDA receptor redox sites. Interestingly, reduced glutathione was ineffective as a redox modulator of NMDA receptors but instead enhanced inhibitory GABA currents in RGCs (Pan *et al.*, 1995).

The role of NOS in the eye, and in particular the retina has been investigated recently by using mice deficient in neuronal and endothelial NOS. (Dreyer *et al.*, 1995a). The nNOS and eNOS deficient mouse (Huang, Dawson, Bredt, Snyder, & Fishman, 1993) and a control cohort were subjected to (a) intravitreal injections of NMDA; or (b) multiple retinal arterial occlusions induced either by thermal photocoagulation or photo-thrombosis. RGC survival was evaluated by retrograde transport of horseradish peroxidase (HRP) and stereological analysis of retinal whole mounts. In the wild type mouse, 63% of the RGCs were killed by a single injection of 10 nmol of NMDA. In comparison, only 35% of the NOS-knockout RGCs were lost at this dose. Furthermore, with either retinal arterial occlusion model, the NOS-knockout mouse lost only half as many RGCs as the wild type mouse. Pre-injection with 100 μM of the NOS inhibitor, L-nitro-arginine also protected RGCs from both NMDA and arterial-occlusions.

These data indicate that neuronal nitric oxide synthase appears to be critically involved in the full expression of retinal ganglion cell excitotoxicity mediated through NMDA receptors. These findings parallel the previously demonstrated relative resistance of the nNOS-mouse to cerebral ischemia. However, some residual NMDA toxicity can be seen in the nNOS-mouse, indicating that some RGCs are lost to an excitotoxic insult by a different pathway. One possibility is that other isoenzymes substitute for neuronal nitric oxide synthase (perhaps endothelial or inducible NOS) in the nNOS-mouse (Huang *et al.*, 1993). However, there was no detectable difference between the response of the endothelial NOS deficeint mouse and control animals. Consequently, at least in this experimental paradigm, endothelial NOS is not critical for the expression of excitotoxicity. Alternatively, as has been previously suggested (Dreyer, Zhang, & Lipton, 1995b), NMDA excitotoxicity in RGCs may develop through more than a single pathway, and only one of these potential pathways may require nNOS for full expression. Another potential factor for the lack of complete protection in the nNOS-deficient mice is that NO^- equivalent as well as NO· should be decreased. Hence, in the nNOS knockout mice the beneficial effect of NO^+ in down-regulating NMDA receptor overactivity would be lost, leading to enhanced NMDA receptor-mediated neurotoxicity via pathways not involving NO·.

Cell death has been classified as either necrotic or apoptotic. Necrosis and apoptosis are morphologically distinct mechanisms of cell death with very different features (Ankarcrona *et al.*, 1995; Bonfoco, Krainc, Ankarcrona, Nicotera, & Lipton, 1995). Necrosis has been associated with passive cell swelling, injury to cytoplasmic organelles including mitochondria, and the rapid collapse of cellular homeostasis. Necrosis leads to membrane lysis, release of cellular contents, and resulting inflammation and consequent damage to neighboring cells. On the other hand, apoptosis has been regarded as an "active" process of neuronal cell

destruction with unique morphologic and molecular features. Apoptosis seen in both physiologic and pathologic conditions is characterized by cell shrinkage (often resulting in characteristic "apoptotic" bodies), membrane blebbing, chromatin condensation, and, if a nucleus is present, nuclear pyknosis and extensive genomic fragmentation. To prevent leakage of proteolytic enzymes, DNA, and oxidized lipids with a potential inflammatory response, apoptotic cells in some cases shield their intracellular milieu by cross-linking membrane proteins (Bonfoco *et al.*, 1995). Interestingly, during normal development of the visual system, approximately one-third of immature retinal ganglion cells die by apoptosis (Young, 1984; Penfold & Provis, 1986; Ilschner & Waring, 1992). It is conceivable that apoptosis may play a role not only during development, but may underlie the cell loss in a broad spectrum of disease states. It has also been reported that optic nerve transection causes retinal ganglion cell apoptosis in both adult and neonatal rats (Garcia-Valenzuela, Gorczyca, Darzynkiewicz, & Sherma, 1994; Rabacchi, Bonfanti, Liu, & Maffei, 1994). However, the exact mechanism underlying cell death after axotomy is not clear. Quigley and coworkers have recently demonstrated a role for apoptosis both in experimental primate glaucoma and axotomy (Quigley, Nickells, Pease, Kerrigan, Thibault, & Zack, 1995).

Recent experiments suggest that the addition of low doses of NMDA to RGCs initiates a cascade of cellular events including both translational and transcriptional events leading to cell death (Dreyer *et al.*, 1995b). Blockade of either translation with cycloheximide or transcription with actinomycin D effectively prevented retinal ganglion cell death resulting from exposure to low concentrations of NMDA, consistent with the prevention of apoptosis. These agents could be added 2 hr after the initial NMDA insult and could still protect against excitotoxicity. However, these agents were not protective at high doses of NMDA. The presence of actinomycin D or cycloheximide had no effect on NMDA-induced rises in intracellular calcium as monitored by fura-2 calcium imaging.

A similar distinction between the effect of low and high doses of NMDA was recently described in cultures of cortical neurons (Bonfoco *et al.*, 1995). It was found that low doses of NMDA or free radicals (including nitric oxide, superoxide anion and peroxynitrite) induced delayed death of neurons with the typical features of apoptosis, while high doses of NMDA led to immediate cell swelling and lysis with the features commonly found in necrosis. It is thus conceivable that actinomycin D and cycloheximide block the expression of genes that are part of the pathway(s) leading to apoptosis in RGCs.

THERAPEUTIC IMPLICATIONS

Using rat RGCs in our *in vitro* culture system, the uncompetitive NMDA receptor antagonist MK-801 protected these neurons from NMDA receptor-mediated excitotoxicity even when administered 1–4 hr after the initial insult (Levy & Lipton, 1990). Importantly, the drug memantine has also been shown to be effective in preventing the NMDA receptor-mediated neurotoxicity when administered up to 4 hours after the insult (Pellegrini & Lipton, 1993). This drug has been used clinically in the treatment of Parkinson's disease for more than a decade and its mild side-effect profile is well known. Memantine has been shown to be an open-channel blocker of the NMDA receptor. Its action is, therefore, similar to that of MK-801 and Mg^{2+}. The kinetics of the NMDA receptor channel block by memantine are, however, significantly different from both of MK-801 and Mg^{2+} with blocking and unblocking rates that are intermediate between the two (Chen *et al.*, 1992). In fact, the more rapid kinetics of channel blocking and un-blocking by memantine compared with MK-801 may be responsible for its lack of serious neurological side-effects (Lipton, 1993).

The finding that actinomycin D and cycloheximide could be added 2 hr after an initial mild NMDA insult and still protected against RGC excitotoxicity, points the way to the development of therapeutic strategies aimed at gene transcription or protein translation.

Ca^{2+} channel antagonists of the dihydropyridine group have been shown to attenuate NMDA-receptor mediated neurotoxicity in RGCs both *in vitro* and *in vivo*. The dihydropyridines nimodipine and nifedipine were tested in the *in vitro* RGC culture system. The two drugs were found to attenuate the early rise in $[Ca^{2+}]_i$ and the subsequent toxic effects of endogenous glutamate-related toxin or exogenous glutamate (Dreyer *et al.*, 1990; Lipton, 1991; Sucher *et al.*, 1991c).

The effectiveness of the Ca^{2+} channel blocker flunarizine was tested *in vitro* (Lipton, 1991) and *in vivo* following optic nerve axotomy (Eschweiler & Bahr, 1993). In this model, axotomy of the optic nerve leads to the death of RGCs within a few weeks, although their somata are not injured directly (Villegas, Vidal, Rasminsky, Bray, & Aguayo, 1993). Flunarizine was given daily to rats (5 mg/kg body weight) after unilateral axotomy of the optic nerve. The density of retrogradely labeled RGCs was determined 14 days after axotomy. It was found that flunarizine significantly enhanced RGC survival after axotomy in adult rats ($P < 0.001$; 1065 ± 142 vs 922 ± 237 RGCs mm^2). It remains to be established if flunarizine blocks the increase in $[Ca]_i$ that is induced by the axotomy *per se* and/or if flunarizine prevents secondary excitotoxic RGC death (induced by glutamate that is released from damaged or dying cells).

These findings should be of particular relevance for therapeutic strategies aimed at combating ganglion cell loss in ophthalmic and neurologic diseases. RGCs are relatively resistant to calcium-dependent toxicity compared with many other types of central neurons. This results in a therapeutic advantage in that it takes many hours for the RGCs to die. There may, therefore, be more time to intervene to save RGCs from the consequences of ischemic or hypoxic insults. Ca^{2+} channel antagonists are widely used in clinical practice for the treatment of

hypertension and their clinical safety and side-effect profiles are well established. The NMDA receptor antagonist memantine is another drug that has been used extensively in clinical practice in Europe. It should therefore be relatively easy to plan and execute controlled studies as to the therapeutic efficacy of these drugs in diseases affecting RGCs.

CONCLUSIONS

RGCs co-express multiple NMDA and non-NMDA receptors with distinct functional properties. The functional diversity of these receptors plays an important role in controlling Ca^{2+}-entry into RGCs. Glutamate has been shown to act as a endogenous neurotoxin which exerts its toxic effect on RGCs directly through the NMDA subtype of glutamate receptor. NMDA receptor-mediated toxicity in RGCs is dependent on the influx of extracellular Ca^{2+}. The increase in $[Ca^{2+}]_i$ acts as a second messenger that sets in motion the cascade leading to eventual cell death. Glutamate stimulates its own release in a positive feedback loop by its interaction with the non-NMDA receptor subtypes. Ca^{2+}-induced Ca^{2+} release and further influx of Ca^{2+} through voltage-gated Ca^{2+} channels after glutamate-induced depolarization contribute to glutamate toxicity. Larger RGCs (10 μm) appear to be more sensitive to NMDA receptor-mediated neurotoxicity both *in vivo* and *in vitro*. NMDA receptor antagonists or Ca^{2+} channel blockers should be useful in preventing or at least abating neuronal loss in the retina. Some drugs can prevent glutamate-induced neurotoxicity even when administered a few hours after the onset of the insult. Further experiments may help to identify other aspects of the cellular pathways stimulated by toxic levels of excitatory amino acids

REFERENCES

Aizenman, E., Frosch, M. P. & Lipton, S. A. (1988). Responses mediated by excitatory amino acid receptors in solitary retinal ganglion cells from rat. *Journal of Physiology, 396*, 75–91.

Aizenman, E., Lipton, S. A. & Loring, R. H. (1989). Selective modulation of NMDA responses by reduction and oxidation. *Neuron, 2*, 1257–1263.

Ankarcrona, M., Dybukt, J. M., Bonfoco, E., Zhivotovsky, B., Orrenius, S., Lipton, S. A. & Nicotera, P. (1995) Glutamate-induced neuronal death: a successioon of necrosis or apoptosis depending on mitochondrial integrity. *Neuron*, in press.

Bonfoco, E., Krainc, D., Ankarcrona, M., Nicotera, P. & Lipton, S. A. (1995). Apoptosis and necrosis: two distinct events induced respectively by mild and intense insults with NMDA or nitric oxide/superoxide in cortical cell cultures. *Proceedings of the National Academy of Sciences USA, 92*, 7162–7166.

Brandstatter, J. H., Hartveit, E., Sassoe-Pognetto, M. & Wässle, H. (1994). Expression of NMDA and high-affinity kainate receptor subunit mRNAs in the retina. *European Journal of Neuroscience, 6*, 1100–1112.

Chaturvedi, N., Hedley-Whyte, E. T. & Dreyer, E. B. (1993). Lateral geniculate nucleus in glaucoma. *American Journal of Ophthalmology, 116*, 182–188.

Chen, H. S., Pellegrini, J. W., Aggarwal, S. K., Lei, S. Z., Warach, S., Jensen, F. E. & Lipton, S. A. (1992). Open-channel block of *N*-methyl-D-aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity. *Journal of Neuroscience, 12*, 4427–4436.

Dawson, V. L., Dawson, T. M., Bartley, D. A., Uhl, G. R. & Snyder, S. H. (1993). Mechanisms of nitric oxide-mediated neurotoxicity in primary brain cultures. *Journal of Neuroscience, 13*, 2651–2661.

Dawson, V. L., Dawson, T. M., London, E. D., Bredt, D. S. & Snyder, S. H. (1991). Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proceedings of the National Academy of Sciences USA, 88*, 6368–6371.

Dreyer, E. B., Kaiser, P. K., Offermann, J. T. & Lipton, S. A. (1990). HIV-1 coat protein neurotoxicity prevented by calcium channel antagonists. *Science, 248*, 364–367.

Dreyer, E. B., Pan, Z. H., Storm, S. & Lipton, S. A. (1994). Greater sensitivity of larger retinal ganglion cells to NMDA-mediated cell death. *NeuroReport, 5*, 629–631.

Dreyer, E. B., Zhang, D. & Lipton, S. A. (1995b) Transcriptional or translational inhibition blocks low dose NMDA-mediated cell death. *NeuroReport, 6*, 942–944.

Dreyer, E. B., Zurakowski, D., Schumer, R. A., Podos, S. M. & Lipton, S. A. (1995c) Elevated glutamate in the vitreous of humans and monkeys with glaucoma. *Arch. Ophthalmol.*, in press.

Eschweiler, G. W. & Bahr, M. (1993). Flunarizine enhances rat retinal ganglion cell survival after axotomy. *Journal of Neurological Science, 116*, 34–40.

Garcia-Valenzuela, E., Gorczyca, W., Darzynkiewicz, Z. & Sherma, S. (1994). Apoptosis in adult retinal ganglion cells after axotomy. *Journal of Neurobiology, 25*, 431–438.

Glovinsky, Y., Quigley, H. A. & Dunkelberger, G. R. (1991). Retinal ganglion cell loss is size dependent in experimental glaucoma. *Investigative Ophthalmology and Visual Science, 32*, 484–491.

Glovinsky, Y., Quigley, H. A. & Pease, M. E. (1993). Foveal ganglion cell loss is size dependent in experimental glaucoma. *Investigative Ophthalmology and Visual Science, 34*, 395–400.

Hahn, J. S., Aizenman, E. & Lipton, S. A. (1988). Central mammalian neurons normally resistant to glutamate toxicity are made sensitive by elevated extracellular Ca^{2+}: toxicity is blocked by the *N*-methyl-D-aspartate antagonist MK-801. *Proceedings of the National Academy of Sciences USA, 85*, 6556–6560.

Hamassaki, B. D., Hermans, B. I., Heinemann, S. & Hughes, T. E. (1993). Expression of glutamate receptor genes in the mammalian retina: the localization of GluR1 through GluR7 mRNAs. *Journal of Neuroscience, 13*, 1888–1898.

Heng, J. E., Moscaritolo, K. & Dreyer, E. B. (1995). NMDA sensitivity is neurite enhanced. *NeuroReport, 6*, 1890–1892.

Hinton, D. R. (1986). Optic-nerve degeneration in Alzheimer's disease. *New England Journal of Medicine, 315*, 485–487.

Huang, P. L., Dawson, T. M., Bredt, D. S., Snyder, S. H. & Fishman, M. C. (1993). Targeted disruption of the neuronal nitric oxide synthase gene. *Cell, 75*, 1273–1286.

Ilschner, S. U. & Waring, P. (1992). Fragmentation of DNA in the retina of chicken embryos coincides with retinal ganglion cell death. *Biochemical and Biophysical Research Communications, 183*, 1056–1061.

Karschin, A., Aizenman, E. & Lipton, S. A. (1988). The interaction of agonists and noncompetitive antagonists at the excitatory amino acid receptors in rat retinal ganglion cells *in vitro*. *Journal of Neuroscience, 8*, 2895–2906.

Karschin, A. & Lipton, S. A. (1989). Calcium channels in solitary retinal ganglion cells from post-natal rat. *Journal of Physiology, 418*, 379–396.

Kaiser, P. A. & Lipton, S. A. (1990). VIP-mediated increase in cAMP prevents tetrodotoxin-induced retinal ganglion cell death *in vitro*. *Neuron, 5*, 373–381.

Kleinschmidt, A., Bear, M. F. & Singer, W. (1987). Blockade of "NMDA" receptors disrupts experience-dependent plasticity of kitten striate cortex. *Science, 238*, 355–358.

Komuro, H. & Rakic, P. (1993). Modulation of neuronal migration by NMDA receptors. *Science, 260*, 95–107.

Lei, S. Z., Pan, Z. H., Aggarwal, S. K., Chen, H. S., Hartman, J., Sucher, N. J. & Lipton, S. A. (1992a) Effect of nitric oxide

production on the redox modulatory site of the NMDA receptor-channel complex. *Neuron, 8*, 1087–1099.

Lei, S. Z., Zhang, D., Abele, A. E. & Lipton, S. A. (1992b) Blockade of NMDA receptor-mediated mobilization of intracellular Ca^{2+} prevents neurotoxicity. *Brain Research, 598*, 196–202.

Leifer, D., Lipton, S. A., Barnstable, C. J. & Masland, R. H. (1984). Monoclonal antibody to Thy-1 enhances regeneration of processes by rat retinal ganglion cells in culture. *Science, 224*, 303–306.

Leinders-Zufall, T., Rand, M. N., Waxman, S. G. & Kocsis, J. D. (1994). Differential role of two Ca^{2+}-permeable non-NMDA glutamate channels in rat retinal ganglion cells: kainate-induced cytoplasmic and nuclear Ca^{2+} signals. *Journal of Neurophysiology, 72*, 2503–2516.

Levy, D. I. & Lipton, S. A. (1990). Comparison of delayed administration of competitive and uncompetitive antagonists in preventing NMDA receptor-mediated neuronal death. *Neurology, 40*, 852–855.

Levy, D. I., Sucher, N. J. & Lipton, S. A. (1990). Redox modulation of NMDA receptor-mediated toxicity in mammalian central neurons. *Neuroscience Letters, 110*, 291–296.

Levy, D. I., Sucher, N. J. & Lipton, S. A. (1991). Glutathione prevents *N*-methyl-D-aspartate receptor-mediated neurotoxicity. *NeuroReport, 2*, 345–347.

Lipton, S. A. (1986). Blockade of electrical activity promotes the death of mammalian retinal ganglion cells in culture. *Proceedings of the National Academy of Sciences, 83*, 9774–9778.

Lipton, S. A. (1988). Spontaneous release of acetylcholine affects the physiological nicotinic responses of rat retinal ganglion cells in culture. *Journal of Neuroscience, 8*, 3857–3868.

Lipton, S. A. (1989). GABA-activated single channel currents in outside-out membrane patches from rat retinal ganglion cells. *Visual Neuroscience, 3*, 275–279.

Lipton, S. A. (1991). Calcium channel antagonists and human immunodeficiency virus coat protein-mediated neuronal injury. *Annals of Neurology, 30*, 110–114.

Lipton, S. A. (1993). Prospects for clinically tolerated NMDA antagonists: open-channel blockers and alternative redox states of nitric oxide. *Trends in Neuroscience, 16*, 527–532.

Lipton, S. A., Aizenman, E. & Loring, R. H. (1987). Neural nicotinic acetylcholine responses in solitary mammalian retinal ganglion cells. *Pflügers Archives of the European Journal of Physiology, 410*, 37–43.

Lipton, S. A., Choi, Y. B., Pan, Z. H., Lei, S. Z., Chen, H. S., Sucher, N. J., Loscalzo, J., Singel, D. J. & Stamler, J. S. (1993). A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. *Nature, 364*, 626–632.

Lipton, S. A. & Kater, S. B. (1989). Neurotransmitter regulation of neuronal outgrowth, plasticity and survival. *Trends in Neuroscience, 12*, 265–270.

Lipton, S. A. & Rosenberg, P. A. (1994). Excitatory amino acids as a final common pathway for neurologic disorders. *New England Journal of Medicine, 330*, 613–622.

Lipton, S. A., Sucher, N. J., Kaiser, P. K. & Dreyer, E. B. (1991). Synergistic effects of HIV coat protein and NMDA receptor-mediated neurotoxicity. *Neuron, 7*, 111–118.

Lipton, S. A. & Tauck, D. L. (1987). Voltage-dependent conductances of solitary ganglion cells dissociated from the rat retina. *Journal of Physiology, 385*, 361–391.

Lucas, D. R. & Newhouse, J. P. (1957). The toxic effect of sodium L-glutamate on the inner layers of the retina. *American Medical Association of the Archives of Ophthalmology, 58*, 193–201.

Moncada, C., Lekieffre, D., Arvin, B. & Meldrum, B. (1992). Effect of NO synthase inhibition on NMDA- and ischaemia-induced hippocampal lesions. *NeuroReport, 3*, 530–532.

Nakanishi, S. (1992). Molecular diversity of glutamate receptors and implications for brain function. *Science, 258*, 597–603.

Olney, J. W. (1982). The toxic effects of glutamate and related compounds in the retina and the brain. *Retina, 2*, 341–359.

Pan, Z.-H., Bähring, R., Grantyn, R. & Lipton, S. A. (1995). Differential modulation by sulfhydryl redox agents and glutathione of GABA- and glycine-evoked currents in rat retinal ganglion cells. *Journal of Neuroscience, 15*, 1384–1391.

Pellegrini, J. W. & Lipton, S. A. (1993). Delayed administration of memantine prevents *N*-methyl-D-aspartate receptor-mediated neurotoxicity. *Ann. Neurol., 33*, 403–407.

Penfold, P. L. & Provis, J. M. (1986). Cell death in the development of the human retina: phagocytosis of pyknotic and apoptotic bodies by retinal cells. *Graefes Archives of Clinical and Experimental Ophthalmology, 224*, 549–553.

Quigley, H. A., Nickells, R. W., Pease, M. E., Kerrigan, L. A., Thibault, D. J. & Zack, D. J. (1995). Retinal ganglion cell death in experimental monkey glaucoma and axotomy occurs by apoptosis. *Investigative Ophthalmology and Visual Science, 36*, 774–786.

Rabacchi, S., Bonfanti, L., Liu, X.-H. & Maffei, L. (1994). Apoptotic cell death induced by optic nerve lesion in the neonatal rat. *Journal of Neuroscience, 14*, 5294–5301.

Rayudu, P. V., Chen, H.-S. V. & Lipton, S. A. (1995). High-voltage activated calcium currents sensitive to w-CTx-MVIIC in rat retinal ganglion cells. *Society of Neuroscience Abstracts, 21*, 398.

Rörig, B. & Grantyn, R. (1993). Rat retinal ganglion cells express $Ca^{(2+)}$-permeable non-NMDA glutamate receptors during the period of histogenetic cell death. *Neuroscience Letters, 153*, 32–36.

Samy, C. N., Lui, C. J., Kaiser, P. K., Lipton, S. A. & Dreyer, E. B. (1994). Toxicity of chronic glutamate administration to the retina. *Investigative Ophthalmology and Visual Science, 35*, 497.

Schumer, R. A. & Podos, S. M. (1994). The nerve of glaucoma. *Archives of Ophthalmology, 112*, 37–44.

Simon, D., Prusky, G., O'Leary, D. & Constantine-Paton, M. (1992). *N*-methyl-D-aspartate receptor antagonists disrupt the formation of a mammalian neural map. *Proceedings of the National Academy of Sciences USA, 89*, 10593–10597.

Sisk, D. R. & Kuwabara, T. (1985). Histologic changes in the inner retina of albino rats following intravitreal injection of monosodium L-glutamate. *Graefe's Archives of Clinical Experimental Ophthalmology, 223*, 250–258.

Sucher, N. J., Aizenman, E. & Lipton, S. A. (1991a) *N*-methyl-D-aspartate antagonists prevent kainate neurotoxicity in rat retinal ganglion cells *in vitro*. *Journal of Neuroscience, 11*, 966–971.

Sucher, N. J., Chen, T. P. & Lipton, S. A. (1991b) Cryopreservation of postnatal rat retinal ganglion cells: persistence of voltage- and ligand-gated ionic currents. *Neuroscience, 43*, 135–150.

Sucher, N. J., Lei, S. Z. & Lipton, S. A. (1991c) Calcium channel antagonists attenuate NMDA receptor-mediated neurotoxicity of retinal ganglion cells in culture [published erratum appears in Brain Res 1991 Sep 13, 559(1), 179]. *Brain Research, 551*, 297–302.

Sucher, N. J. & Lipton, S. A. (1991). Redox modulatory site of the NMDA receptor-channel complex: regulation by oxidized glutathione. *Journal of Neuroscience Research, 30*, 582–591.

Sucher, N. J. & Lipton, S. A. (1992). A slowly inactivating K + current in retinal ganglion cells from postnatal rat. *Visual Neuroscience, 8*, 171–176.

Sucher, N. J., Wong, L. A. & Lipton, S. A. (1990). Redox modulation of NMDA receptor-mediated Ca^{2+} flux in mammalian central neurons. *NeuroReport, 1*, 29–32.

Sullivan, J. M., Traynelis, S. F., Chen, H. S. V., Escobar, W., Heinemann, S. F. & Lipton, S. A. (1994). Identification of two cysteine residues that are required for redox modulation of the NMDA subtype of glutamate receptor. *Neuron, 13*, 929–936.

Taschenberger, H. & Grantyn, R. (1995). Several types of Ca^{2+} channels mediate glutamatergic synaptic responses to activation of single Thy-1-immunolabeled rat retinal ganglion neurons. *Journal of Neuroscience, 15*, 2240–2254.

Tauck, D. L., Frosch, M. P. & Lipton, S. A. (1988). Characterization of GABA- and glycine-induced currents of solitary rodent retinal ganglion cells in culture. *Neuroscience, 27*, 193–203.

Tenhula, W. N., Xu, S. Z., Madigan, M. C., Heller, K., Freeman, W. R. & Sadun, A. A. (1992). Morphometric comparisons of optic nerve axon loss in acquired immunodeficiency syndrome. *American Journal of Ophthalmology, 113*, 14–20.

Villegas, P. M., Vidal, S. M., Rasminsky, M., Bray, G. M. & Aguayo, A. J. (1993). Rapid and protracted phases of retinal ganglion cell loss

follow axotomy in the optic nerve of adult rats. *Journal of Neurobiology, 24*, 23–36.

Vorwerk, C. K., Lipton, S. A., Zurakowski, D., Hyman, B. T., Sabel, B. A. & Dreyer, E. B. (1996) Chronic low dose glutamate is toxic to retinal ganglion cells; toxicity blocked by memantine, submitted.

Wässle, H. & Boycott, B. B. (1991). Functional architecture of the mammalian retina. *Physiological Review, 71*, 447–480.

Young, R. W. (1984). Cell death during differentiation of the retina in the mouse. *Journal of Comparative Neurology, 229*, 362–373.

Zhang, D. & Lipton, S. A. (1992). L-Homocysteic acid selectively activates *N*-methyl-D-aspartate receptors of rat retinal ganglion cells. *Neuroscience Letters, 136*, 173–177.

Zhang, D., Sucher, N. J. & Lipton, S. A. (1995). Co-expression of AMPA/kainate receptor-operated channels with high and low Ca^{2+} permeability in single rat retinal ganglion cells. *Neuroscience, 67*, 177–188.

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