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REVIEW

Glutamate in Life and Death of Retinal Amacrine Cells*

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ABSTRACT. 1. Glutamate is the neurotransmitter released by bipolar cells at their synapses with amacrine cells. The amacrine cells express ionotropic (NMDA, AMPA and kainate) and metabotropic (mGluR1, mGluR2, mGluR4 and mGluR7) glutamate receptors and may take up glutamate from the synaptic cleft.

2. Activation of the ionotropic glutamate receptors increases the intracellular free calcium concentration ($[Ca^{2+}]_i$), owing to Ca^{2+} entry through the receptor-associated channels as well as through voltage-gated Ca^{2+} channels. The $[Ca^{2+}]_i$ response to glutamate may be amplified by Ca^{2+}-induced Ca^{2+} release from intracellular sources.

3. Activation of NMDA and non-NMDA glutamate receptors stimulates the release of GABA and acetylcholine from amacrine cells. GABA is released by a Ca^{2+}-dependent mechanism and by reversal of the neurotransmitter transporter.

4. Excessive activation of glutamate receptors during ischemia leads to amacrine cell death. An increase in $[Ca^{2+}]_i$ due to Ca^{2+} influx through NMDA and AMPA/kainate receptor channels is related to cell death in studies *in vitro*. In other studies, it was shown that nitric oxide may also take part in the process of cell damage during ischemia. GEN PHARMAC 30;3:289–295, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. Glutamate, retina, amacrine cells, ischemia, GABA, acetylcholine

INTRODUCTION

In the radial pathways of the retinal circuitry, photoreceptors, the light-sensitive cells, pass information to the ganglion cells, which form the optic nerve, through "ON"- and "OFF"-type bipolar cells. The flow of visual information along these glutamatergic pathways is modulated by interaction of the cells with horizontal cells and amacrine cells (Barnstable 1993; Ehinger, 1989; Masland, 1988; Masland and Tauchi, 1986; Massey, 1991; Wässle and Boycott, 1991). The AII amacrine cells constitute one of the pathways from rods to ganglion cells because the rod-driven bipolar cells do not contact ganglion cells directly (DeVries and Baylor, 1995; Famiglietti, 1983a; Kolb and Famiglietti, 1974; Sterling, 1983; Strettoi *et al.*, 1992).

The amacrine cells form a dense network in the inner plexiform layer (IPL) of the retina, and their cell bodies may be located in the inner nuclear layer (INL) or in the ganglion cell layer of the retina. They may contain acetylcholine (Baughman and Bader, 1977; Brecha *et al.*, 1988; Famiglietti, 1983a, 1983b; Hayden *et al.*, 1980; Masland and Mills, 1979; Masland and Tauchi, 1986; Masland *et al.*, 1984; O'Malley and Masland, 1989; Tauchi and Masland, 1984; Voigt, 1986), GABA (Barnstable, 1993; Brecha *et al.*, 1988; Davanger *et al.*, 1991; Mosinger *et al.*, 1986; O'Malley and Masland, 1989), glycine (Davanger *et al.*, 1991; Pourcho and Goebel, 1987), dopamine (Djamgoz and Wagner, 1992; Ehinger, 1983; Pourcho, 1982; Voight and Wässle, 1987; Wässle and Chun, 1988; Witkovsky and Schütte, 1991), serotonin (Brunken *et al.*, 1993; Vaney, 1986; Watt, 1992), and possibly adrenaline (Hadjiconstantinou *et al.*, 1983). Some subpopulations of the amacrine cells contain more than one type of neurotransmitter (Brecha *et al.*, 1988; Massey *et al.*, 1992; O'Malley and Masland, 1989; Pourcho, 1980; Vaney and Young, 1988; Wässle and Chun, 1988; Watt, 1992).

Amacrine cells express receptors for glutamate released from bipolar cells, and this neurotransmitter plays an important role in the physiology of amacrine cells. However, overactivation of glutamate receptors under pathological conditions, such as during retinal ischemia, causes amacrine cell death. We will review here the role of glutamate in the physiology of amacrine cells as well as the excitotoxic effects of the neurotransmitter in these cells.

GLUTAMATE RECEPTORS

Glutamate activates ionotropic receptors and receptors coupled to guanine nucleotide–binding proteins [for reviews, see Hollmann and Heinemann (1994), Mori and Mishina (1995), Bettler and Mulle (1995), and Pin and Bockaert (1995)]. On the bases of ligand-binding studies and molecular biological and electrophysiological properties, the ionotropic glutamate receptors were classified in three categories: the *N*-methyl-D-aspartate (NMDA), S-α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and kainate receptors. These receptors are permeable to Na^+ and, in some cases, to Ca^{2+} as well, being responsible for the rapid synaptic effects of glutamate. With the use of molecular cloning techniques, four distinct subunits of AMPA receptors (GluRA– GluRD), five kainate

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receptor subunits (GluR5–GluR7, KA1 and KA2) and five NMDA receptor subunits (NR1 and NR2A–NR2D) were identified. These receptors are thought to have pentameric structures, and different combinations of receptor subunits may generate receptor subspecies with different selectivities for permeating ions, different pharmacological profiles and distinct properties regarding modulation by protein kinases (Bettler and Mulle, 1995; Duarte *et al.*, 1995; Hollmann and Heinemann, 1994; Mori and Mishina, 1995).

The metabotropic glutamate receptors modulate, rather than mediate, synaptic transmission by modulating the activity of membrane enzymes and ion channels through G proteins (Pin and Bockaert, 1995). On the basis of molecular cloning studies, eight different metabotropic glutamate receptors were reported (mGluR1–mGluR8), and they were assigned to three distinct groups: the first group consists of subtypes mGluR1 and mGluR5, which activate phospholipase C, whereas members of the second (mGluR2 and mGluR3) and third (mGluR4, mGluR6, mGluR7 and mGluR8) groups are negatively coupled to adenylyl cyclase (Pin and Bockaert, 1995).

EXPRESSION OF IONOTROPIC GLUTAMATE RECEPTORS BY AMACRINE CELLS

In situ hybridization studies revealed that mammalian amacrine cells express mRNAs for NMDA (NR1, NR2A, NR2B and NR2C), AMPA (GluRA–GluRD) and kainate (GluR5–GluR7 and KA2) receptor subunits (Brandstätter *et al.*, 1994; Hamassaki-Britto *et al.*, 1993; Hartveit *et al.*, 1994; Hughes *et al.*, 1992; Müller *et al.*, 1992; Watanabe *et al.*, 1994). Labeling with probes for NR1, GluRA–GluRD, KA2 and GluR7 was homogeneous in the INL of the rat retina, suggesting that those receptor subunits may be expressed by all amacrine cells. The transcripts for NR2A, NR2B, NR2C, GluR5 and GluR6 had a more restricted distribution, indicating that those subunits may be expressed by only a subpopulation of amacrine cells. In these studies, no evidence was found for the expression of NR2D or KA1 in the rat retina.

The localization of the retinal ionotropic glutamate receptor subunits was also investigated by immunocytochemistry, using specific antisera. In the rat retina, anti-GluR1 antibodies stained certain amacrine cells in the INL, and GluR6 and GluR7 immunoreactivity was found in some amacrine cells located along the inner border of the INL (Peng *et al.*, 1995). Immunocytochemical localization of AMPA receptor subunits in the cat retina showed staining for the GluRA subunit in a limited number of amacrine cells and displaced amacrine cells, and a number of amacrine cells were also stained with GluRD antiserum. An antibody that recognizes both GluRB and GluRC subunits labeled about 50% of the total amacrine cell population in the cat retina (Qin and Pourcho, 1996).

The presence of NMDA and kainate receptors in cultures enriched in chick amacrine-like neurons also was reported, on the basis of ligand-binding studies. The intact cells were shown to bind [^{3}H]MK-801 ([+]-[3-^{3}H]5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine maleate), a noncompetitive antagonist of the NMDA receptors (Wong *et al.*, 1986), with a K_D of 6.1 nM, and [^{3}H]kainate, a specific radioligand for kainate receptors (Bettler and Mulle, 1995; Hollmann and Heinemann, 1994), with a K_D of 66.4 nM (Agostinho *et al.*, 1996). These dissociation constants are close to those estimated for the binding of the ligands to mammalian brain receptors (Bettler *et al.*, 1992; Laurie and Seeburg, 1994; Lomeli *et al.*, 1992). However, the subunit composition of the ionotropic glutamate receptors expressed by cultured chick amacrine-like neurons has not been determined.

Physiological experiments indicated that rabbit starburst amacrine cells, which are cholinergic, express both NMDA and non-NMDA receptors (Cunningham and Neal, 1985; Linn and Massey, 1991; Linn *et al.*, 1991; Zhou and Fain, 1995). Both receptors are also expressed by cultured chick GABAergic amacrine-like cells, as determined by patch clamp (Huba and Hofmann, 1991; Yamashita *et al.*, 1994). In the salamander retina, transient amacrine cells are endowed with both NMDA and non-NMDA receptors, whereas sustained amacrine cells have only non-NMDA receptors (Dixon and Copenhagen, 1992). The AII amacrine cells of the rat retina express only non-NMDA ionotropic glutamate receptors (Boos *et al.*, 1993), indicating that the distribution of NMDA receptors among amacrine cells depends on cell type and perhaps on species.

EXPRESSION OF METABOTROPIC GLUTAMATE RECEPTORS BY AMACRINE CELLS

Some, but not all, amacrine cells in the INL of the rat retina also express mRNAs for mGluR1, mGluR2, mGluR4 and mGluR7 [Hartveit *et al.*, (1995), but see Nakajima *et al.* (1993) and Azakawa *et al.* (1994)]. However, whether there is a subpopulation(s) of amacrine cells that expresses all four receptor subtypes or, alternatively, whether each cell expresses a more limited set of metabotropic glutamate receptors remains to be established. Immunocytochemistry revealed that mGluR2 is localized mainly in the processes of rat amacrine cells, postsynaptic to cone and rod bipolar cells. The localization of mGluR4 immunoreactivity in the rat retina was similar to that of mGluR2 but was more widely distributed through the IPL (Koulen *et al.*, 1996). MGluR7 receptors were also localized in processes of amacrine cells, postsynaptic to "OFF" and "ON" cone bipolar cell synapses (Brandstätter *et al.*, 1996). In agreement with *in situ* hybridization studies, an antibody against mGluR1α was found to label the processes of amacrine cells, the bodies of which are located in the middle of the INL (Peng *et al.*, 1995). Metabotropic glutamate receptors, activated by (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) and coupled to the activation of phospholipase C, are also present in cultures enriched in amacrine-like neurons (Duarte *et al.*, 1996). However, to our knowledge, there is no direct electrophysiological evidence for the expression of metabotropic glutamate receptors in amacrine cells.

EFFECT OF GLUTAMATE ON THE $[Ca^{2+}]_i$ IN CULTURES ENRICHED IN AMACRINE-LIKE NEURONS

Stimulation of cultures enriched in amacrine-like neurons (Agostinho *et al.*, 1996; Gleason *et al.*, 1993; Huba and Hofmann, 1990), with NMDA, under depolarizing conditions, or with kainate evokes a sustained increase of the intracellular free sodium concentration ($[Na^+]_i$), as determined by the fluorescent indicator SBFI (sodium-binding benzofuran isophthalate) (Agostinho *et al.*, 1996). The membrane depolarization, mainly due to Na^+ entry through the glutamate receptor–associated channels, activates voltage-gated Ca^{2+} channels (VGCCs), leading to an increase in the intracellular free calcium concentration (Duarte *et al.*, 1993, 1996). Activation of NMDA receptors in cultures enriched in chick amacrine-like neurons increases the $[Ca^{2+}]_i$ due to Ca^{2+} entry through L-type VGCCs sensitive to nitrendipine, as well as through the receptor-associated channel. The $[Ca^{2-}]_i$ response to AMPA in the same preparation is mostly due to Ca^{2+} entry through L-type VGCC, but a small component is due to Ca^{2+}-permeating the receptor-associated channels (Duarte *et al.*, 1996). Surprisingly, the ionotropic glutamate receptors sensitive to kainate in cultured chick amacrine-like neurons are highly Ca^{2+} permeable, and stimulation with kainate also opens up

nitrendipine-sensitive VGCCs, as well as P- or Q-type Ca^{2+} channels or both (Duarte *et al.*, 1996). However, whether the activation of P- and Q-type VGCCs by kainate, but not by NMDA or AMPA, is due to a distinct localization of the receptors in the same cells or to the fact that the receptors are expressed by different cells, with different populations of VGCCs remains to be determined. Furthermore, the functional significance of the high Ca^{2+} permeability of the AMPA/kainate receptors present in cultured amacrine-like cells is not known.

Depletion of the intracellular Ca^{2+} stores, with the microsomal Ca^{2+} ATPase inhibitor thapsigargin (Thastrup *et al.*, 1990), was found to decrease the initial $[Ca^{2+}]_i$ responses to NMDA or AMPA in cultured chick retina cells (Duarte *et al.*, 1996), suggesting that calcium-induced calcium release may increase the $[Ca^{2+}]_i$ signals triggered by the two agonists. The Ca^{2+} entry through AMPA/kainate receptors also amplifies the $[Ca^{2+}]$ signals in the cytoplasm and nucleus of cultured rat retinal neurons, by releasing Ca^{2+} from intracellular stores (Kocsis *et al.*, 1993).

GLUTAMATE STIMULATES NEUROTRANSMITTER RELEASE BY AMACRINE CELLS

Acetylcholine release by amacrine cells

Starburst amacrine cells are the only cells in the retina that synthesize acetylcholine, and they receive input from cone bipolar cells [Wässle and Boycott, (1991); see also the section on glutamate receptors for other references]. Bipolar cells are thought to be glutamatergic (Barnstable, 1993; Ehinger, 1989; Massey, 1991), and both NMDA and kainate stimulate the release of acetylcholine from the rabbit retina (Linn and Massey, 1991; Linn *et al.*, 1991). However, NMDA receptors do not mediate the release of acetylcholine evoked by diffuse light (Linn and Massey, 1991). The release of acetylcholine from the rat retina is triggered by Ca^{2+} entry through P- or Q-type Ca^{2+} channels or both, and by as yet uncharacterized Cd^{2+}-sensitive channels (Tamura *et al.*, 1995).

L-Homocysteate (L-HCA) is a sulfur-containing amino acid that interacts with neurons predominantly by binding to the NMDA receptors (Thompson and Kilpatrick, 1996). L-HCA stimulates the release of acetylcholine from the rabbit and rat retina, as well as inhibiting light-evoked acetylcholine release in the rat retina, in a dose-dependent manner (Linn and Massey, 1996; Neal and Cunningham, 1992; Neal *et al.*, 1989). However, L-HCA is not expected to be the neurotransmitter released by bipolar cells at the synapses with starburst amacrine cells, owing to the relatively low affinity of L-HCA for postsynaptic receptors and the absence of a specific uptake system in the retina to terminate the synaptic activity (Linn and Massey, 1996).

GABA release by amacrine cells

In the retina, GABA is the neurotransmitter of subpopulations of amacrine cells, as well as of horizontal cells (Barnstable, 1993; Wässle and Boycott, 1991). This has made it difficult to investigate the mechanisms controlling GABA release from amacrine cells in the intact retina. With the use of immunocytochemistry and antisera against GABA, it was possible to visualize the release of GABA from amacrine cells upon stimulation with NMDA, AMPA and kainate (Osborne and Herrera, 1994). In cultures enriched in chick amacrine-like neurons, [^{3}H]GABA is released by two different processes: (1) a Ca^{2+}-dependent mechanism, possibly by exocytosis, due to Ca^{2+} entry through L-type VGCCs, and (2) a Ca^{2+}-independent mechanism, due to reversal of the GABA transporter (Agostinho *et al.*, 1997; Alfonso *et al.*, 1994; Carvalho *et al.*, 1995; Duarte *et al.*, 1992, 1993; Ferreira *et al.*, 1994; Hofmann and Möckel, 1991). Glutamate was first proposed to stimulate the release of [^{3}H]GABA from cultured chick retina cells by a glutamate– GABA exchange mechanism (de Mello *et al.*, 1988; do Nascimento and de Mello, 1985), but this hypothesis was ruled out on the basis of the observation that the effect of glutamate was antagonized by blockers of the NMDA and non-NMDA glutamate receptors and by an inhibitor of the GABA transporter (Carvalho *et al.*, 1995; Duarte *et al.*, 1992, 1993; Hofmann and Möckel, 1991). The agonists of ionotropic glutamate receptors, NMDA and AMPA, stimulate the release of [^{3}H]GABA from cultures enriched in amacrine-like neurons by Ca^{2+}-independent processes, whereas the release evoked by kainate is exclusively due to reversal of the GABA carrier (Ferreira *et al.*, 1994). Interestingly, domoic acid, a relatively selective agonist of the kainate receptors (Boulter *et al.*, 1990; Lerma *et al.*, 1993; Sommer *et al.*, 1992), stimulates the release of [^{3}H]GABA in Na^+-free medium (Alfonso *et al.*, 1994), suggesting that Ca^{2+} entry through the receptor-associated channel may also trigger neurotransmitter release in cultured chick amacrine-like neurons.

The GABAergic amacrine cells may also contain other neurotransmitters, such as acetylcholine, glycine, dopamine, or serotonin (Brecha *et al.*, 1988; Marc, 1988; O'Malley and Masland, 1989; Pourcho, 1980; Vaney and Young, 1988; Wässle and Chun, 1989; Watt, 1992). However, whether different neurotransmitters present in the same cell are released differentially or whether more than one of these transmitters can be released at the same time in each synapse is not known.

Putative roles of metabotropic glutamate receptors in amacrine cells

Although the role of metabotropic glutamate receptors in amacrine cells has not been investigated, several putative roles were proposed, on the basis of the observation that mGluR2, mGluR4 and mGluR7 are present on the cell processes. Group II and Group III metabotropic glutamate receptors are coupled to the inhibition of adenylyl cyclase, and therefore mGluR2, mGluR4 and mGluR7 receptors, activated by glutamate released from bipolar cells, may decrease the release of GABA from amacrine cells; the inhibition of GABA release may lead to a disinhibition of the bipolar cells (Brandstätter *et al.*, 1996; Koulen *et al.*, 1996). Because the cholinergic amacrine cells also express mGluR2 on the processes, and taking into account that these cells make feed-forward synapses to ganglion cells (Famiglietti, 1983a), Koulen *et al.* (1996) suggested that activation of those receptors could decrease the release of GABA or acetylcholine or both, modulating the firing rate of the postsynaptic ganglion cells. This process might play a role in directional selectivity.

Metabotropic glutamate receptors coupled to the activation of phospholipase C are present in chick retina cells in culture and are expected to activate protein kinase C, through the generation of diacylglycerol, in addition to the production of inositol phosphates (Duarte *et al.*, 1996). In the same cultures, protein kinase C activation with phorbol esters increases Ca^{2+} entry through the AMPA/kainate receptor–associated channels and decreases the $[Ca^{2+}]_i$ response to KCl depolarization (Carvalho *et al.*, 1995). However, whether activation of metabotropic glutamate receptors modulates the activity of AMPA/kainate receptors and of VGCCs in those cells remains to be determined. Furthermore, the effect of protein kinase C on the activity of VGCCs also suggests that metabotropic glutamate receptors coupled to the activation of phospholipase C may also control neurotransmitter release.

EXPRESSION OF GLUTAMATE TRANSPORTERS BY AMACRINE CELLS

Although the amacrine cells are not glutamatergic, they have been found to express EAAC-1 glutamate transporters (Rauen *et al.*, 1996). EAAC-1 immunoreactivity was observed in amacrine cells with large cell somata, which are probably GABAergic (Chun *et al.*, 1992). Therefore, it is expected that amacrine cells may participate in the clearance of glutamate from the synaptic cleft. The glutamate taken up by amacrine cells may be used to synthesize GABA.

GLUTAMATE INDUCES AMACRINE CELL DEATH

Occlusion of the central artery or vein, obstructive retinopathies, venous capillary insufficiency, anomalous vascular shunts, hypertensive retinopathy, diabetic retinopathy, retinal neovascularization, retinal detachment and vitreous hemorrhage are all conditions that can lead to ischemic damage of the retina (Liu and Chiou, 1996). The mechanisms responsible for cellular damage and death after ischemia in the retina, as in other areas of the CNS, are not fully understood. However, it is known that ischemic neuronal damage entails overactivation of excitatory amino acid receptors (Choi, 1988; Obrenovitch and Urenjak, 1997; Siesjö, 1992). Accordingly, retinal ischemia due to an increased pressure in the anterior eye chamber was shown to increase the release of glutamate, and the release of the excitatory amino acid was further increased during the reperfusion period, when the pressure was released (Louzada- Junior, *et al.*, 1992). Chemical ischemia, caused by incubation of isolated retinas in glucose-free medium gassed with 95% N_2-5% CO_2 also significantly increases the release of glutamate (Neal *et al.*, 1994).

The retinal amacrine cells are particularly sensitive to ischemic or to excitotoxic insults. The amacrine cells of rabbit retinas subjected to ischemia, by increasing the intraocular pressure, followed by reperfusion, show a dramatic reduction of their GABA immunoreactivity and an inhibition of serotonin uptake. The effect of ischemia on GABA immunoreactivity was antagonized by NMDA and non-NMDA receptor antagonists (Osborne and Herrera, 1994; Osborne *et al.*, 1995a, 1996), indicating that at least part of the effects of ischemia on amacrine cells is due to overactivation of ionotropic glutamate receptors. Accordingly, NMDA, AMPA and kainate cause changes in GABA immunoreactivity of GABAergic amacrine cells of the rabbit retina, which can be compared with the changes observed under ischemic conditions (Osborne and Herrera, 1994; Osborne *et al.*, 1994; Perez and Davanger, 1994). Raising the rat's intraocular pressure for 45 min, followed by 3 days of reperfusion, also changed calretinin immunoreactivity associated with amacrine cells, and this effect was reduced by the ionotropic glutamate receptor antagonists MK-801 and CNQX (6- cyano-7-nitroquinoxaline-2,3-dione) (Osborne and Larsen, 1996).

Phototrombic lesions of the rat retina, used as a model to investigate the ischemic damage of the retina, reduce the activity of choline acetyltransferase, indicating that cholinergic amacrine cells are also affected in this model of ischemia (Lombardi *et al.*, 1994; Moroni *et al.*, 1993). The reduction of choline acetyltransferase activity was antagonized by intravitrial injection of excitatory amino acid receptor antagonists, indicating that excessive activation of NMDA and non-NMDA glutamate receptors was responsible for cholinergic amacrine cell death (Lombardi *et al.*, 1994). Activation of the AMPA/kainate receptors with kainate or severe ischemic insults, such as those induced by raising the intraocular pressure or by preventing blood flow through the central artery, also significantly reduce the choline acetyltransferase immunoreactivity in the retina (Osborne *et al.*, 1995b).

In the rat retina, subjected to ischemia induced by increasing the intraocular pressure, three different patterns of cell death were identified in the INL: type I, with characteristics consistent with necrosis; type II, resembling apoptosis; and type III, showing features of "nonlysosomal vesiculate" cell death (Büchi *et al.*, 1992). Cell damage in the ganglion cell layer, under the same experimental conditions, appears to exclusively follow the first two patterns (Büchi *et al.*, 1992).

Exposure of the chick embryo retina, or of the rat retina to anoxic conditions (95% N_2/5% CO_2) results in many pyknotic profiles in the amacrine cell layer, and some of the observed effects were antagonized by the NMDA receptor antagonist MK-801 (David *et al.*, 1988; El-Asrar *et al.*, 1992). Glutamate, NMDA or kainate produces acute swelling of many of the somata in the inner half of the INL of the chick embryo retina, as well as an edematous swelling on the IPL (Romano *et al.*, 1995; Zeevalk and Nicklas, 1994; Zeevalk *et al.*, 1989), and similar results were obtained in the rat retina (El-Asrar *et al.*, 1992). When the excitotoxic insult with NMDA is removed and the chick embryo retina is allowed to recover for 24 hr, much of the swelling is eliminated; however, during this period, many amacrine cells die (Zeevalk and Nicklas, 1994).

The ionotropic glutamate receptor agonists also damage cultured chick amacrine-like neurons, as determined by the reduction of 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide (MTT) (Ferreira *et al.*, 1996). In these cells, activation of NMDA and AMPA/kainate receptors can cause toxicity by mechanisms not involving Na^+ influx, but rather depending on the permeation of Ca^{2+} through the receptor-associated channels. For small Ca^{2+} loads, the entry of Ca^{2+} through the NMDA receptor–associated channel is more efficient in triggering cell death than is the influx of Ca^{2+} through the AMPA/kainate receptors (Ferreira *et al.*, 1996).

Although it is clear that glutamate plays an important role in amacrine cell death during retinal ischemia and that overactivation of glutamate receptors leads to a Ca^{2+} overload, the intracellular events that ultimately lead to cell death have not been determined. Interestingly, pretreatment of rats with N^{ω}-nitro-L-arginine, a nitric oxide synthase (NOS) inhibitor, has been reported to almost completely abolish the ischemic damage to the retina induced by elevation of the ocular pressure (Geyer *et al.*, 1995). Under the experimental conditions used, most of the damage occurred on the IPL, but a clear disorganization of cells in the INL also was observed. Aminoguanidine, an inhibitor of the inducible isoform of NOS (iNOS), partly abolished the ischemic damage (Geyer *et al.*, 1995), suggesting that both iNOS and the constitutive Ca^{2+}-sensitive NOS (cNOS) contribute to the synthesis of NOS that leads to neuronal injury in ischemia due to an increased intraocular pressure. These observations are in agreement with the reported rise in the expression of iNOS by nonneuronal cells during reperfusion, after transient retinal ischemia (Hangai *et al.*, 1996). The activation of cNOS under ischemia may be secondary to the stimulation of ionotropic glutamate receptors.

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