# Hypoxia-ischemia and retinal ganglion cell damage

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**Abstract:** Retinal hypoxia is the potentially blinding mechanism underlying a number of sight-threatening disorders including central retinal artery occlusion, ischemic central retinal vein thrombosis, complications of diabetic eye disease and some types of glaucoma. Hypoxia is implicated in loss of retinal ganglion cells (RGCs) occurring in such conditions. RGC death occurs by apoptosis or necrosis. Hypoxia-ischemia induces the expression of hypoxia inducible factor-1α and its target genes such as vascular endothelial growth factor (VEGF) and nitric oxide synthase (NOS). Increased production of VEGF results in disruption of the blood retinal barrier leading to retinal edema. Enhanced expression of NOS results in increased production of nitric oxide which may be toxic to the cells resulting in their death. Excess glutamate release in hypoxic-ischemic conditions causes excitotoxic damage to the RGCs through activation of ionotropic and metabotropic glutamate receptors. Activation of glutamate receptors is thought to initiate damage in the retina by a cascade of biochemical effects such as neuronal NOS activation and increase in intracellular $Ca^{2+}$ which has been described as a major contributing factor to RGC loss. Excess production of proinflammatory cytokines also mediates cell damage. Besides the above, free-radicals generated in hypoxic-ischemic conditions result in RGC loss because of an imbalance between antioxidant- and oxidant-generating systems. Although many advances have been made in understanding the mediators and mechanisms of injury, strategies to improve the damage are lacking. Measures to prevent neuronal injury have to be developed.

**Keywords:** retinal hypoxia, retinal ganglion cells, glutamate receptors, neuronal injury, retina

## Introduction

The structural and functional integrity of the retina depends on a regular oxygen supply. Being one of the most metabolically active tissues, retina consumes oxygen more rapidly than other tissues (Cohen and Noell 1965) such as the brain (Anderson and Saltzman 1964; Ames 1992). The presence of a dual circulation (Osborne et al 2004) makes retinal oxygenation unique. The photoreceptors and the greater portion of the outer plexiform layer receive nourishment from the choriocapillaris indirectly whereas the inner retinal layers are supplied by the superficial and deep capillary plexuses formed by branches of the central artery of the retina. Inner layers of the retina are known to show highest sensitivity to hypoxic challenges (Janáky et al 2007), whereas the outer retina is more resistant to a hypoxic stress (Tinjust et al 2002).

Retinal hypoxia occurs in ocular conditions such as central retinal artery occlusion and ischemic central retinal vein thrombosis. Hypoxia is also implicated in the development of glaucoma (Flammer 1994; Tielsch et al 1995; Chung et al 1999; Osborne et al 1999b; Costa et al 2003; Tezel and Wax 2004), diabetes (Linsenmeier et al 1998), and is thought to underlie many of the sight-threatening complications of diabetic eye disease including retinal and optic nerve head neovascularization. Systemic causes of retinal hypoxia include the cardiovascular effects of chronic obstructive airways disease, the ocular ischemic syndrome associated with arterial obstructive conditions such as carotid artery stenosis (Brown and Magargal 1988) and Takayasu's arteritis (Shelhamer et al 1985), hyperviscosity syndromes (Ashton et al 1963) or following trauma (Purtscher's retinopathy; Purtscher 1912; Buckley and James 1997).

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Retinal hypoxia associated with the above conditions is a common cause of visual impairment and blindness (Osborne et al 2004). Retinal ganglion cells (RGCs) have been reported to be particularly sensitive to acute, transient, and mild systemic hypoxic stress (Kergoat et al 2006). Loss of RGCs occurs in many ophthalmic conditions such as glaucoma and diabetes (Sucher et al 1997; Abu-El-Asrar et al 2004), hypoxia being implicated in such a loss (Wax and Tezel 2002; Tezel and Wax 2004; Chen et al 2007). This review details some of the molecular and cellular mechanisms which may be involved in RGC death in ocular conditions associated with hypoxia-ischemia. A better understanding of the mechanisms causing hypoxic damage to RGCs may aid the development of therapies aimed at reducing blindness from retinal hypoxic-ischemic visual loss.

A number of systemic and cellular responses such as glycolysis, angiogenesis, vasodilation, and erythropoiesis enable the organisms to respond to hypoxia (Harris 2002). The neural tissue is capable of inducing protective mechanisms under hypoxic-ischemic conditions (Kitagawa et al 1990) which are induced within minutes and are of putative importance for limiting the damage. However, these protective mechanisms are lost within hours of the hypoxic-ischemic insult (Prass et al 2003) following which cell death and tissue damage occur. Transcriptional activator hypoxia-inducible factor-1$\alpha$ (HIF-1$\alpha$) is a master regulator of cellular $O_2$ homeostasis (Iyer et al 1998). Hypoxia is known to induce HIF-1$\alpha$ and its target genes (Bernaudin et al 2002) such as vascular endothelial growth factor (VEGF) and nitric oxide synthase (NOS) in many tissues. Overproduction of these factors has been implicated in neuronal death in hypoxic-ischemic conditions. In addition, enhanced extracellular accumulation of glutamate and inflammatory cytokines damage the neurons. Upregulated expression of HIF-1$\alpha$, VEGF, and various isoforms of NOS has been reported in the retina following hypoxic injury (Kaur et al 2006) and in the glaucomatous retina (Tezel and Wax 2004).

## Retinal ganglion cell death in hypoxia ischemia

RGC death has been reported to occur in many experimental studies using different methods to induce retinal ischemia (Adachi et al 1996; Goto et al 2002; Lafuente et al 2002; Wang et al 2002; Chidlow and Osborne 2003). Neuronal degeneration resulting from retinal hypoxia-ischemia, caused by oxygen and substrate deprivation, may be mediated by free oxygen radicals (Block and Schwarz 1997; Muller et al 1997; Szabo et al 1997), glutamate excitotoxicity (Louzada-Junior et al 1992; Osborne et al 2004; Kaur et al 2006), inflammation (Hayashi et al 1996) as well as disruption of the blood retinal barrier (Kuroiwa et al 1985; Kaur et al 2007).

Based on morphological, histochemical, and biochemical criteria, cell death has been classified as apoptotic or necrotic in hypoxic-ischemic conditions (Mehmet et al 1994; Charriaut-Marlangue et al 1996a, 1996b; Chopp and Li 1996; Macaya 1996; Yue et al 1997; MacManus and Linnik 1997; Banasiak and Haddad 1998; Pulera et al 1998; Renolleau et al 1998; Nakajima et al 2000). In necrotic death, swelling of cell body, disruption of plasma membrane, and irregularly scattered condensation of nuclear chromatin occur (Dessi et al 1993; Gwag et al 1997; Sohn et al 1998). In apoptosis, on the other hand, nuclear condensation and contraction occurs early with the membrane and organelles remaining intact until the final stages. Similar necrotic (Buchi 1992; Joo et al 1999) and apoptotic changes in RGCs have been observed in experimental hypoxic-ischemic conditions (Buchi 1992; Joo et al 1996, 1999) as well as in elevated intraocular pressure (Garcia-Valenzuela et al 1995; Quigley et al 1995) and glaucoma (Kerrigan et al 1996) where ischemia is involved in retinal damage directly or indirectly.

Ischemia is known to induce several apoptosis-regulatory genes in cells. Upregulated expression of Bax, a bcl-2 homolog that effects apoptosis in neurons destined to die, after global ischemia (Krajewski et al 1995; Chen et al 1996) and expression of antiapoptotic gene *bcl-2* in neurons that survive ischemia (Shimazaki et al 1994; Chen et al 1997) has been reported suggesting that endogenously induced apoptosis-regulatory genes may play a role in determining the fate of ischemic neurons. Caspases play a key role in cell death by apoptosis (Jacobson and Evan 1994). Among the caspases, caspase-3 is activated by many cell death signals and cleaves a variety of important cellular proteins (Jänicke et al 1998; Namura et al 1998). Caspase-3-like protease activation is likely to be relevant in neuronal apoptosis in ischemic injury (Fink et al 1998; Namura et al 1998). Caspase-2 and -3 (Kurokawa et al 1999; Lam et al 1999) and Bax (Kaneda et al 1999) have been reported to be involved in retinal cell loss after ischemic insult.

## Hypoxia-ischemia, retinal edema, and vascular endothelial growth factor

Hypoxia-ischemia underlies various blinding ocular conditions such as diabetic retinopathy and may play a role in the wet form of age-related macular degeneration and in the visual

loss from retinal detachment (Tso 1982; Yanoff et al 1984; Marmor 1999; Bressler et al 2001; Davis and Blodi 2001; Jackson et al 2003). It is associated with fluid accumulation in the extracellular spaces (vasogenic edema) or intracellulary (cytotoxic edema) in the neural retina (Yanoff et al 1984; Marmor 1999). The extracellular spaces in the inner retina consist of the narrow clefts between the tightly packed cellular elements (Hamann 2002). Fluid leaking out from damaged capillaries in the inner retina accumulates in the extracellular spaces displacing the retinal cellular elements and disrupting the normal anatomy of the neuronal connections (Hamann and La Cour 2005). Factors implicated in pathogenesis of macular edema are retinal ischemia, oxidative stress, and inflammation (Bresnick 1983; Guex-Crosier 1999; van Dam 2002; Miyake and Ibaraki 2002). Increased permeability of blood-retinal barrier (BRB) resulting in fluid accumulation has been reported to contribute to retinal neuronal degeneration by compression (Cunha-Vaz and Travassos 1984; Antcliff and Marshall 1999; Marmor 1999; Reichenbach et al 2007). Excess production of VEGF, nitric oxide (NO) and aquaporin-4 in hypoxic-ischemic insults causes dysfunction of the BRB in the inner retina resulting in serum leakage into the retinal tissues (Marmor 1999; Kaur et al 2007) and retinal edema (Hamann and La Cour 2005). In addition to an increase in vascular permeability, hypoxia has also been correlated with endothelial cell death, leukocyte plugging of vessels, and microaneurysms (Linsenmeier et al 1998).

VEGF, also known as vascular permeability factor (Senger et al 1983), is a key player of angiogenesis in health and disease (Ferrara 2001; Carmeliet 2003). VEGF binds to two tyrosine kinase receptors, VEGFR-1 or fms-like tyrosine kinase Flt-1 and VEGFR-2 or fetal liver kinase receptor Flk-1 to exert its actions (De Vries et al 1992; Quinn et al 1993; Neufeld et al 1999; Shibuya 2001). VEGF is inducible by hypoxia-ischemia *in vitro* and *in vivo* and has been suggested as a likely candidate for the development of vasogenic brain edema (Schoch et al 2002). A 3–12-fold increase in VEGF gene expression has been reported in hypoxia (Ikeda et al 1995; Levy et al 1995; Stein et al 1995).

Increased expression of VEGF has been reported in hypoxic brains (Schoch et al 2002; Kaur et al 2006), and astrocytes were identified as the cells expressing VEGF (Kaur et al 2006). Upregulation of endogenous VEGF in astrocytes in hypoxia-ischemia is believed to interact with receptors for VEGF on the vessels and contribute to the disruption of blood-brain barrier (BBB) resulting in vascular leakage (Zhang et al 2000, 2002). Inhibition of VEGF is known to reduce the BBB permeability (Zhang et al 2000). Similar to the brain, increased production of VEGF and enhanced permeability of BRB was recently reported in the hypoxic retina and inhibition of VEGF production with melatonin reduced BRB permeability (Kaur et al 2006, 2007).

In addition to its role in increasing vascular permeability, VEGF has also been described as an inflammatory mediator which contributes to inflammatory responses observed in cerebral ischemia (Croll et al 2004). The disruption of BBB by VEGF allows contact of normally sequestered central nervous system antigens with blood-borne immune mediators altering the immune privileged status of the brain (Proescholdt et al 1999). VEGF enhances the adhesion of leukocytes to vascular walls and increases intercellular cell adhesion molecule-1(ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) expression in the brain and retina (Melder et al 1996; Lu et al 1999; Min et al 2005). Overexposure of normal neural tissue to VEGF has been shown to enhance ICAM-1 and major histocompatibility complex class I and II expression (Proescholdt et al 2004). Many changes induced by diabetes such as ICAM-1 up-regulation, leukocyte adhesion and increased vascular permeability in the retina (Murata et al 1996; Amin et al 1997) have been reported to occur in nondiabetic retinas with intravitreous VEGF injections (Tolentino et al 1996; Lu et al 1999) supporting the role of VEGF in inflammation. Suppression of inflammation in retina after VEGF inhibition has been reported (Joussen et al 2002).

Intracellular edema has been reported to occur in ischemia through damage to the cell membrane ionic channels (Marmor 1999). Neuronal and/or glial swelling has been considered as a component of retinal edema. The neuronal cells have been reported to become edematous during ischemia and degenerate eventually in the post-ischemic period (Johnson 1974).

## Hypoxia-ischemia and nitric oxide

NO is known to play an important role in the pathogenesis of neuronal injury during hypoxia-ischemia. NO is synthesized by the enzyme NOS from L-arginine. NOS exists in three isoforms: neuronal (nNOS) and endothelial (eNOS) which are constitutively expressed and inducible (iNOS). The activities of nNOS and eNOS are stimulated by increases in intracellular calcium whereas iNOS is calcium-independent, and NO generated from this isoform is known to mediate immune functions. Enhanced nNOS, eNOS, and iNOS expression has been reported in the retina in response to hypoxia (Kaur et al 2006).

NO has been described to have neuroprotective and neurotoxic roles (Iadeacola 1997). NO produced by the eNOS isoform is a protective response as it maintains retinal perfusion in hypoxic-ischemic conditions (Toda et al 2007). Vasodilation occurring after hypoxic-ischemic episodes is mediated by eNOS (Bolanos and Almeida 1999) leading to increased blood flow. Blood vessels in retina showed enhanced expression of eNOS following a hypoxic insult (Kaur et al 2006). However, it has been proposed that besides its beneficial effects of producing vasodilatation and increasing the blood flow, eNOS is also involved in VEGF-induced vascular hyperpermeability (Fukumura et al 2001).

All three types of NOS are produced in the retina in hypoxic-ischemic conditions (Kaur et al 2006) and glial cells have been suggested as the major cell types producing them (Kobayashi et al 2000; Kashiwagi et al 2003). In addition to glial cells, infiltrating leukocytes may also be an important source of iNOS production. The RGCs were also reported to express iNOS and nNOS in the hypoxic retina (Kaur et al 2006). NO production from nNOS and iNOS contributes to cytotoxicity resulting in cell death and axonal damage. Other than generation of free radicals, a number of pathways such as N-methyl-D-aspartate (NMDA)-mediated intracellular $Ca^{2+}$ influx and CREB-mediated transcription of apoptotic proteins such as Bax, Bad, and Bcl-xl are triggered by NO resulting in neuronal death (Mishra et al 2002, 2006; Zubrow et al 2002a, 2002b). Increased expression of Bax but not Bcl-2 in hypoxic cerebral tissue thus increasing the Bax/Bcl-2 ratio in favor of hypoxia-induced apoptosis has been reported (Mishra et al 2004). In retinal ischemia, RGCs death has been reported to be due to involvement of iNOS as it has been observed that iNOS-positive leukocytes enter the ganglion cell layer and surround the RGCs and cause their degeneration which could be prevented with an inhibitor of iNOS (Neufeld et al 2002).

NO induces the proapoptotic cascade in hypoxic neural tissues by increasing phosphorylation of Bcl-2 (Mishra et al 2004). NO-mediated inactivation of MAPK phosphatases has been described as a potential mechanism of activation of ERK and JNK which leads to phosphorylation of the antiapoptotic protein Bcl-2 (Mishra et al 2004). The anti-apoptotic potential of phosphorylated Bcl-2 is lost due to its inability to heterodimerize with the proapoptotic protein Bax, resulting in Bax-mediated activation of caspases and initiation of apoptosis (St. Clair et al 1997, Haldar et al 1996; Hu et al 1998). Other mechanisms by which NO contributes to cytotoxicity may be peroxynitrite-mediated oxidative damage, DNA damage, and energy failure (Beckman et al 1990; Nguyen et al 1992; Zhang et al 1994; Gross et al 1996). This observation is supported by recent studies which have suggested that peroxynitrite produced by iNOS is a highly reactive oxidant capable of inducing injury to a number of cell types (Li et al 2005).

## Hypoxia-ischemia and excitotoxicity

Excitatory amino acids have been reported to play an important role in the development of hypoxic-ischemic retinal injury. Glutamate, the excitatory neurotransmitter in the retina, is released by photoreceptors, bipolar cells and ganglion cells and mediates the transfer of visual signals from the retina to the brain (Massey 1990). However, augmented release of glutamate and its accumulation in extracellular spaces in hypoxic-ischemic conditions leading to activation of glutamate receptors has been implicated in hypoxic/ischemic neuronal death (Benveniste et al 1984; Lu et al 1993). Glutamate neurotoxicity is considered as the underlying problem in retinal neuropathies and neurodegenerative conditions such as glaucoma (Dreyer 1998). Elevation of extracellular glutamate concentration in the retina has been shown to mimic hypoxia induced changes in the electroretinogram (Ikeda et al 1995). Over-activation of glutamate receptors due to excess glutamate accumulation in the retina can contribute to retinal dysfunction (Dreyer 1998; Pang et al 1999).

Glutamate exerts its action through ionotropic (amino-methyl-propionic-acid [AMPA], NMDA, and kainate glutamate receptors) and metabotropic receptors (Brandstätter et al 1998; Gründer et al 2001). The metabotropic glutamate receptors (mGluRs) have been grouped into three main classes, group I (mGluR1 and 5), Group II (mGluR2, 3), and Group III (mGluR 4, 6, 7, 8) according to their amino acid sequence, pharmacological properties and transduction mechanisms (Conn and Pin 1997). RGCs express ionotropic receptors (Hartveit et al 1994; Brandstätter et al 1998) as well as mGluRs (Hartveit et al 1995). Glutamate receptor-mediated damage has been reported to occur in glaucoma, central, and branch retinal arterial and retinal vein occlusions resulting in loss of retinal ganglion cells (Sucher et al 1997).

Neurotoxic effects of glutamate are reported to occur predominantly through activation of ionotropic glutamate receptors (GluR) (Levy et al 1991). NMDA receptors are highly permeable to $Ca^{2+}$ (MacDermott et al 1986; Hollmann et al 1991; Rörig and Grantyn 1993), their activation resulting in an increase in the intracellular calcium levels (Siliprandi et al 1992; Sucher et al 1990, 1991, 1997). $Ca^{2+}$ overload has been reported to be a central event in neuronal death during ischemia (Nicotera and Orrenius 1998; Sattler and

Tymianski 2001). Many cellular functions such as regulation of enzymes require calcium. Abnormal higher concentrations of calcium lead to inappropriate activation of enzymes such as proteases, nucleases, and lipases which are harmful to the cellular constituents, generate free radicals as well as cause mitochondrial failure which results in energy depletion and further free radical production (Dugan et al 1995).

Depolarization of neuronal membranes due to energy failure results in $Ca^{2+}$ influx through the voltage-dependent $Ca^{2+}$ channels followed by $Ca^{2+}$-dependent glutamate release (Katsura et al 1994) which further increases the extracellular accumulation of glutamate. Activation of ionotropic glutamate receptors also results in influx of $Na^{+}$ and $Cl^{-}$ ions, inducing osmotic swelling. Glutamate acting via NMDA receptors activates nNOS (Garthewaite and Garthewaite 1991) and the production of NO (Kiss and Vizi 2001). Expression of ionotropic glutamate receptors (GluR2/3 and NMDA) has been reported to be upregulated in the RGCs in hypoxic-ischemic conditions (Kaur et al 2006).

Glutamate has also been reported to induce and exacerbate cell death by activating group I mGluRs (Allen et al 2001; Hilton et al 2006). Neuronal excitation and excitotoxicity is thought to be potentiated by Group I mGluRs (Buisson and Choi 1995; Pin and Duvoisin 1995; Buisson et al 1996), possibly through their interaction with NMDA receptors (Fitzjohn et al 1996; Bordi and Ugolini 1999). It has been reported that mGluR5 are coexpressed with, and functionally coupled to, NMDA receptors and that activation of mGluR5 enhances NMDA responses in neurons (Jia et al 1998; Awad et al 2000; Salt and Binns 2000) contributing to neuronal death (Bruno et al 2000).

Glutamate is also known to be involved in the production of inflammatory cytokines such as tumor necrosis factor-α (TNF-α) (De et al 2005). Glutamate-induced activation of AMPA and NMDA receptors has been shown to enhance the production of TNF-α (Noda et al 2000; Matute et al 2001) and interleukin-1 β (IL-1β) (Hagan et al 1996) significantly. Co-operation between glutamate receptors and inflammatory cytokines may be one of the mechanisms involved in cell damage.

Glutamate toxicity also results in glutathione depletion and oxidative stress (Ratan et al 1994). Glutathione is a major cellular antioxidant which protects the cells against oxidative stress (Meister and Anderson 1983; Mizui et al 1992; Bobyn et al 2002). Increase in intracellular reactive oxygen species (ROS) generation in response to glutathione depletion has been reported in several studies (Coyle and Puttfarcken 1993; Tan et al 1998).

Removal of excess glutamate from the extracellular space by glutamate transporters is crucial to terminate glutamate excitotoxicty. Glutamate transporters are responsible for the removal of glutamate from the extracellular fluid in the retina (Danbolt 2001). It has been suggested that excess glutamate accumulation in the extracellular spaces may result from a failure of the glutamate transporters, such as GLAST, in the vicinity of RGCs (Harada et al 2007). Glutamate transporters have been described as necessary to prevent excitotoxic retinal damage and to synthesize glutathione and their deficiency has been reported to result in RGC degeneration (Harada et al 2007).

## Hypoxia-ischemia and reactive oxygen species

Hypoxia-ischemia results in perturbation of the cellular prooxidant-antioxidant balance by accumulation of ROS, known as oxidative stress, which has been implicated as an important mechanism of cytotoxicity. *In vitro* studies have shown that ROS generation in hypoxic-ischemic conditions in neurons occurs from three sources: mitochondria generating an initial burst of ROS followed by a second phase of ROS generation due to xanthine oxidase activation and a third phase of $Ca^{2+}$-dependent ROS generation (Abramov et al 2007).

ROS are known to cause lipid peroxidation, protein oxidation, and DNA oxidation, which contributes to neurodegeneration (Hall and Braugher 1989; Chan 1994, 1996). ROS can also stimulate ischemic cells to secrete inflammatory cytokines and chemokines which induce cell damage and disruption of BBB (Wang et al 2007). ROS have been reported to be cytotoxic to RGCs (Tezel and Yang 2004) causing necrotic cell death by direct oxidative damage to cellular constituents and apoptotic death by participating in the signal transduction pathway for apoptosis (Kortuem et al 2000; Levkovitch-Verbin et al 2000; Lieven et al 2003, 2006; Nguyen et al 2003).

NO, a free radical is produced by the endothelial cells and serves as a vasodilator (Garthwaite et al 1988; Lamas et al 1992; Southam and Garthwaite 1993; Iadecola et al 1994). However, NO, as mentioned above, can also be neurotoxic causing neuronal death in hypoxic and excitotoxic insults (Dawson et al 1991; Moncada et al 1991; Boje and Arora 1992; Lees 1993). It has been shown that NO can react with the superoxide anion ($O_2^-$) to form peroxynitrite ($OONO^-$) (Beckman et al 1990) which is neurotoxic (Lipton et al 1993). NO alone, even at high levels, has been reported as nontoxic to cortical neurons, but becomes neurotoxic after its reaction with $O_2^-$ to form $ONOO^-$ (Lipton et al 1993). *In vitro*

studies have shown that formation of $OONO^-$ increases the VEGF-induced permeability of retinal microvascular endothelial cells (Marumo et al 1999) and tissue damage through DNA damage, lipid peroxidation, and reduced cellular antioxidant defenses (Salgo et al 1995; Salvemini et al 1998).

## Hypoxia-ischemia and inflammation

Hypoxia is known to regulate expression of many genes modulating inflammation (Hedtjärn et al 2004). An acute inflammatory reaction, characterized by increased expression of proinflammatory mediators (Szaflarski et al 1995; Bona et al 1999), a rapid microglial/monocytic response (Ivako et al 1996) and gliosis (Burtrum et al 1994), have been reported to be elicited in the brain by hypoxia-ischemia (Cowell et al 2002). Many cell types including injured neurons have been reported as a major source of chemokines such as monocyte chemoattractant protein (MCP-1) (Ivako et al 1997) whereas expression of macrophage inflammatory protein-α has been reported in monocytes and activated microglial cells (Cowell et al 2002) in hypoxic-ischemic brain injury. Chemokine receptors CCR2 and CCR5 have also been reported to be upregulated (Hedtjärn et al 2004). Chemokine expression may play a role in leukocyte recruitment and infiltration in the inner retina, leading to RGC damage (Jo et al 2003). Leukocytes are known to play a central role in post-ischemic tissue damage (del-Zoppo et al 1991; Heinel et al 1994; Zhang et al 1994) by producing free radicals (Werns et al 1985) and inflammatory cytokines (Ghezzi et al 1991).

Hypoxia-ischemia is known to attract macrophages to hypoxic areas through expression of MCP-1. The hypoxia-activated macrophages and microglia, the immune effector cells in the retina, release TNF-α which has been reported as a triggering factor to activate production of interleukin-8 (IL-8), VEGF, or MCP-1 in retinal vascular cells and/or glial cells adjacent to microvessels (Yoshida et al 2004). Expression of TNF-α and cyclooxygenase-2 (COX-2) was reported recently in the ischemic retina (Zheng et al 2007). Several inflammatory molecules including ICAM-1, TNF-α, IL-1β, iNOS, and COX-2 released by activated inflammatory cells and glial elements play a major role in degeneration of retinal capillaries (Joussen et al 2004; Zheng et al 2007) and subsequently the RGCs.

Expression of adhesion molecules, ICAM-1 and VCAM-1, on the endothelial cells facilitating leukocyte adhesion and infiltration into the areas of damage has been reported to be induced by TNF-α (Wong and Dorovini, 1992; Hess et al 1994; McHale et al 1999). *In vitro* studies have shown that IL-1β and TNF-α induce ICAM-1 expression in endothelial cells (Feuerstein et al 1998). ICAM-1 is important for establishing adhesion of leukocytes before their movement across the endothelium into the tissue (Wang et al 1994).

IL-1β and TNF-α may also be involved in transcriptional activation of the iNOS gene (Lopez-Figueroa et al 2000; Kadhim et al 2006). Endothelial cells of brain microvessels are known to express iNOS and produce large amounts of NO under inflammatory conditions as IL-1β has an important role in iNOS expression and NO generation (Betz et al 1996; Bonmann et al 1997). Induction of IL-1 β gene expression in the vascular wall, accompanied by perivascular induction of

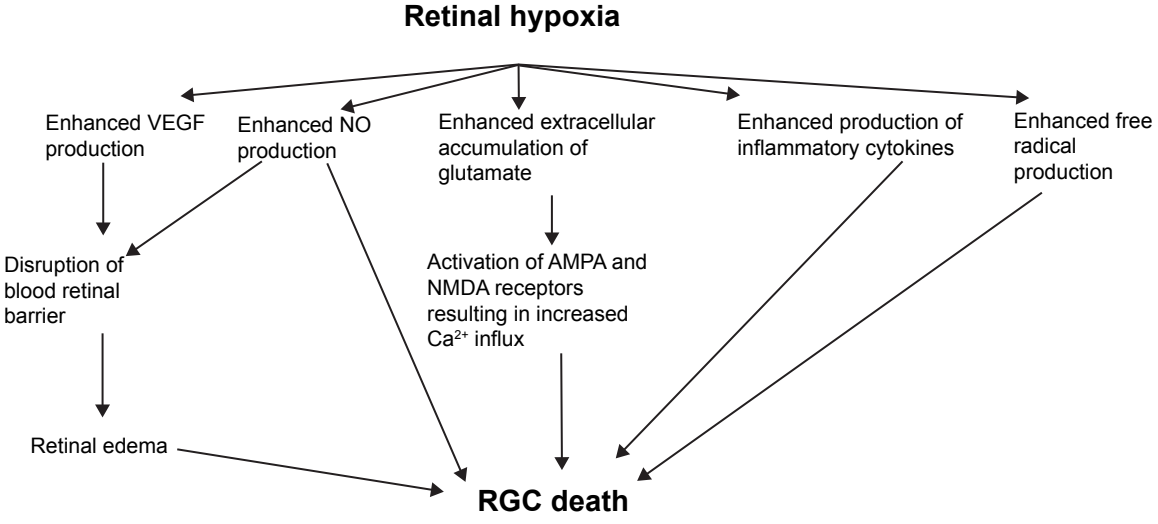


**Figure 1** Potential mediators of RGC death in retinal hypoxia-ischemia.

iNOS mRNA was observed in the rat brain during systemic inflammation (Wong et al 1996). Increased release of IL-1β and TNF-α in the retina in hypoxic-ischemic conditions may have a similar action.

## Conclusion

Retinal hypoxia results in increased release VEGF, NO, glutamate, inflammatory cytokines and ROS (Figure 1). These processes result in RGC loss through various mechanisms such as disruption of BRB, excitotoxicity and increased accumulation of intracellular $Ca^{2+}$. Understanding of the processes outlined in this review may provide new strategies to minimize RGC loss and possibly counteract or prevent it.

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