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Neuroprotection in Glaucoma

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1. Introduction

Glaucoma is an age related heterogeneous group of diseases affecting 70 million people worldwide that is commonly associated with elevated intraocular pressure due to impairment of the outflow pathway of the trabecular meshwork, and characterized by pathological changes in optic nerve head, and lamina cribosa, leading to visual field defects and eventual blindness due to apoptosis of retinal ganglion cells (RGCs) (Clark and Yorio, 2003; Fraser and Wormald, 1999; and Lipton, 2003). The predominant risk factor for patients that suffer from glaucoma is elevation of intraocular pressure (IOP) (Fatma, et al. 2008). Elevated IOP imposes strain on the unmyelinated portion of axons of the RGCs, at the optic nerve head where they take a 90 degree turn to traverse to their final destination at the lateral geniculate nucleus in the thalamus of the brain, or to the superior colliculus in lower vertebrate animals (Quigley 1981). This strain on the axons of the RGCs at the optic nerve head is thought to be the initiating primary insult that damages the axons of the RGCs, inhibiting retrograde transport of neurotrophic factors produced in the brain, and eventually leading to cell death of RGCs (Quigley 1981). This primary insult is hypothesized to initiate a release of noxious secondary factors including glutamate, endothelin, and tumor necrosis-factor-α from injured RGCs and proliferating astrocytes going through reactive gliosis (Clark and Yorio 2003; Prasanna et al., 2010; Tezel and Wax, 2000; Tezel et al., 2001; Tezel and Wax 2004; Nakazawa et al. 2006). These secondary factors are also hypothesized to be associated with RGC death. Other mechanisms include ischemia/hypoxia at the optic nerve head, which could trigger release of glutamate, endothelin-1 (ET-1) and TNF-α from astrocytes, which could contribute to neurodegeneration.

Currently used treatment modalities for glaucoma are agents targeted at lowering IOP and preventing the primary insult contributing to RGC apoptosis. If the main cause of RGC death is axotomy due to pressure induced trauma to axons at the optic nerve head, then a reduction of IOP should protect the remainder of RGCs and halt the progression of this disease process. Although IOP in glaucoma patients is often held within "normal" ranges, the disease process and RGC death can still progress. Additionally, patients with IOP measurements of 6-10 mmHg (normal being 10-20 mmHg) can still develop glaucoma (normal tension glaucoma), thus suggesting that elevation of IOP is not the only predisposing factor for patients that suffer from glaucoma, and suggests that there may be

secondary causes of RGC apoptosis during the progression of this heterogeneous disease process (Bahrami 2006). However, what remains unclear are the secondary factors contributing to the continual apoptotic cell death of the RGCs after the hypothesized initial insult to axons, causing optic nerve damage at the lamina cribosa. Commonly proposed secondary factors that appear to be implicated in this self-perpetuating model of RGC death include free radical formation, glutamate excitotoxicity, and trophic factor withdrawal. Agents that can block these noxious agents need to be studied for their potential role as neuroprotective agents that can protect injured and uninjured RGCs, enhance viability and functionality after exposure to both primary and secondary causes of RGC death during this disease process. These neuroprotectants can be administered as adjunct therapies to IOP lowering agents with a view to provide better therapeutic options aimed at treating both the major risk factor (IOP elevation) as well as secondary pathological process (RGC death) of glaucoma.
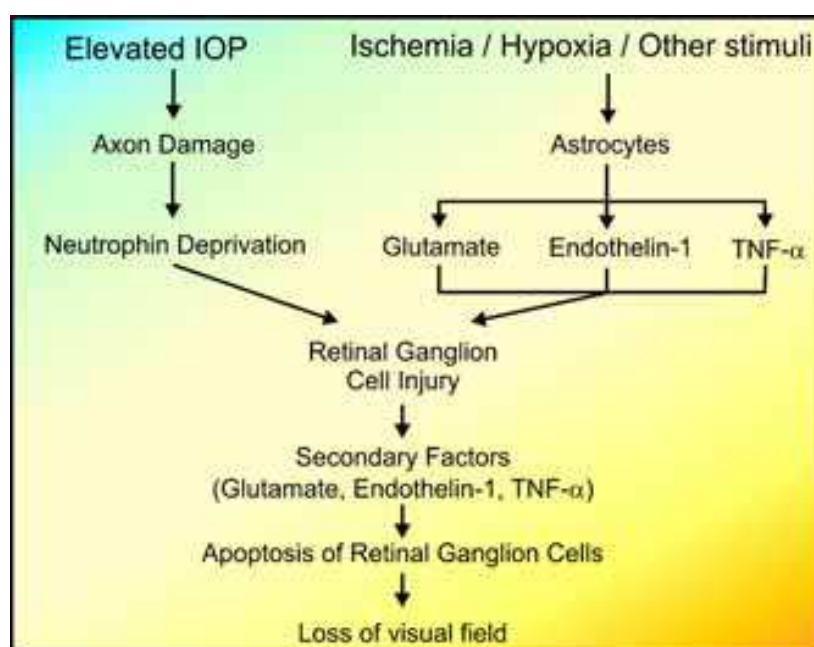

Fig. 1. Mechanisms underlying neurodegeneration in glaucoma. Elevated intraocular pressure (IOP) is a well known risk factor contributing to axon loss and retinal ganglion cell death. Other factors including ischemia/hypoxia and lesser known glaucomatous stimuli have been hypothesized to contribute to release of other mediators including glutamate, endothelin-1 and TNF-α from astrocytes which produce degenerative effects on RGCs. Damage to RGCs could unleash secondary factors which contribute to apoptosis of RGCs and subsequent loss of visual field

Much of the studies conducted in the area of neuroprotection to treat glaucoma has been conducted in animal models where IOP is chronically elevated for a few weeks to months in order to monitor RGC survival when different neurotrophic agents are administered. IOP is artificially elevated in these animal models using several experimental approaches: hypertonic saline injections of the episcleral vessels in the eye causing venous congestion (Morrison et al. 1997), by injection of hypertonic saline into the limbal plexus causing sclerosis of trabecular meshwork (Johnson et al. 2009), through laser-induced damage to the trabecular meshwork causing impairment of aqueous humour outflow (Levkovitch-Verbin

et al. 2002), or by trabecular meshwork obstruction by injecting polystyrene beads (Sappington et al. 2010). Researchers also use DBA/2J mice carrying inherent genetic mutation in the glycoprotein nmb-like protein (Gpnmb) and tyrosinase-related protein 1b (Tyrp 1b) genes which causes iris atrophy late in the lifespan of these mice (John et al. 1998). This atrophy causes sloughing off of the iris pigment which clogs the meshwork and leads to elevation of IOP and RGC loss (Anderson et al. 2002). Some studies also use a more acute *in vivo* model of RGC death called optic nerve crush, in which the axons protruding from the posterior pole of the eye are physically crushed using forceps (Heacock and Agranoff, 1976; Benowitz et al., 1981). This acute *in vivo* model causes considerable RGC death after 1-2 weeks following the crush; therefore, this experimental paradigm enables researchers to quickly screen a variety of agents for neuroprotective properties (Danesh-Meyer 2011). The optic nerve crush is a traumatic optic neuropathy model producing damage to optic nerve axons, however it has provided insight into the neuroprotective ability of various test compounds administered intravitreally (Danesh-Meyer 2011). In addition, the optic nerve crush model has also been useful to study other systemic factors conferring neuroprotection. For instance, it was found that a T-cell-mediated immune response directed against self-antigens residing in the site of damage can be beneficial for the injured optic nerve or spinal cord (Schwartz 2004).

Drugs for neuroprotection to treat glaucoma have to reach the axons and somas of the RGCs. It would be ideal to administer these neuroprotectants as eyedrops; however, the eye possesses key barriers that would impede pharmaceutical agents from penetrating to the back of the eye. The most notable barriers include the precorneal tear clearance and the selective corneal epithelial barrier (Ghate and Edelhauser 2008). The traditional means available to administer pharmaceutical agents to the posterior segment of the eye include: intravenous or oral (which has poor bioavailability with increasing risks of systemic side effects), intravitreal injections (which has excellent bioavailability but carries the inherent risk of ocular infections), or periocular injections (Ghate and Edelhauser 2008). More recent drug delivery systems that could serve to administer neuroprotectants to the posterior pole of the eye include nanoparticles and viral vectors which hold much promise to prolong effective treatment to the retina and optic nerve. Nanoparticles can administer a prolonged stead flow of neuroprotective compounds to the back of the eye for a long period of time (Diebold and Calonge 2010). Additionally, both nanoparticles and viral vectors could be used to administer gene therapies in order to up-regulate potent protective genes, and down-regulate neurodegenerative genes. Specifically, gene therapy through viral vectors has gained some momentum in recent years especially after treatment of patients suffering from Leber's congenital amaurosis, using adeno-associated viral vector encoding RPE65, showed promising results of safety and efficacy (Bainbridge et al. 2008).

A number of studies have been conducted over the past 30 years to identify neuroprotective molecules to protect the neurons in central nervous system (CNS) from acute (stroke) and chronic neurological diseases (e.g. Alzheimer, Parkinson, and glaucomatous optic neuropathy), with numerous encouraging preclinical neuroprotective outcomes, however most clinical studies done to demonstrate neuroprotection in humans failed to show efficacy. Only three neuroprotective drugs have been demonstrated to improve outcomes in human clinical trails: riluzole for amytrophic lateral sclerosis, memantine for moderate to severe Alzheimer disease (Bensimon et al. 1994, Lacomblez et al. 1996, Reisberg et al. 2003) and brimonidine for low pressure glaucoma (Krupin et al. 2011). Memantine and riluzole have failed to have a dramatic impact on the progression of these neurological diseases

(Danesh-Meyer and Levin 2009). From the perspective of clinical trails aimed at developing neuroprotective therapies in glaucoma, the memantine trial was disappointing and failed to demonstrate efficacy in two large multicenter clinical trails at sites worldwide (Allegran 2008). Brimonidine (α2 adrenergic agonist) was another drug given to a small group of patients suffering from ischemic optic neuropathy, where the treated and untreated groups did not show any statistical significant difference in visual field tests (Wilhelm 2006). However, a recent promising study of brimonidine treatment in patients suffering from low-pressure glaucoma, suggests that patients treated with brimonidine are less likely to have worsening of visual fields compared to patients treated with timolol (Krupin et al., 2011). Further studies are required to determine the long term neuroprotective and clinical efficacy of brimonidine in glaucoma patients.

A review article written by Levin and Danesh Meyer (2010) go on to describe the disparity between pre-clinical neuroprotective data and clinical trails. They emphasize lack of appropriate animal models for each neurological disease, appropriate dose of the neuroprotective compounds, timing of the neuroprotective agent administration, poor pre-clinical study designs, premature initiation of clinical trails, problems in execution of neuroprotection clinical trails, and choice of clinical end points. Some or all of these factors may result in the disparity that is observed between pre-clinical experimental results, and clinical trials.

The difficulty in finding an appropriate animal model for neurological diseases is that for most neurological diseases, it is unclear why and how certain specific subpopulations of neurons die. For example in glaucoma, increasing levels of IOP is the major risk factor for patients suffering from glaucoma. In fact, when an individual's IOP reaches 21 mm Hg or higher (normal values being close to 16 mm Hg), IOP lowering agents are administered as a preventative measure so that patients do not develop glaucoma. However, the Baltimore Eye Survey published in 1991 demonstrated that more than half of all the glaucomatous eyes tested in this prospective cohort had IOPs under 21mmHg, whether they were being treated with IOP lowering agents or not (Sommer et al. 1991). Yet, the only animal models used to mimic the disease of glaucoma are those that artificially elevate IOP. Therefore, there is a paucity of animal models that accurately mimic the disease process of primary open angle glaucoma.

Other impediments in the discovery of neuroprotective compounds in clinical trails from promising pre-clinical data is trying to identify the correct therapeutic dose for these neurological diseases in humans (Danesh-Meyer and Levin 2009). Knowing how much of a drug reaches its therapeutic target in the retina is a challenge. Also, most pre-clinical studies looking at the neuroprotective compounds often administer these compounds before the glaucomatous insult (Danesh-Meyer and Levin 2009). This never happens in clinical practice, as most patients enrolled in these neuroprotective clinical trials have advanced disease pathology. Lastly, many investigators carrying out pre-clinical animal studies looking at neuroprotection often are not blinded to the animal getting the therapeutic agent (Danesh-Meyer and Levin 2009). This could inadvertently bias the researcher that is trying to demonstrate neuroprotective efficacy in an animal model. If researchers can adopt a set standard of rules that more closely mimics the rules applied to patients going through clinical trials, perhaps fewer drugs will show promising pre-clinical data (Bebarta 2003). However, those drugs which show pre-clinical efficacy in animal models with standardized protocols could be more closely investigated in order to discover a more effective neuroprotective compound in humans that can treat glaucoma. Moreover, interventions in

different steps of the neurodegenerative pathways could have an additive effect to bolster neuroprotective effects.

The failure of the memantine clinical trial for treating glaucoma has generated skepticism over the discovery of neuroprotective agents to treat glaucoma. Memantine is a drug that blocks the toxic effects of glutamate excitoxicity by antagonizing N-methyl-D-aspartate (NMDA) receptors (Chen et al. 1992 and Parsons et al. 1993). Glutamate excitoxicity and its effects on RGCs are not without controversy. Studies have demonstrated that glutamate excitoxicity has toxic effects on primary hippocampal neurons but not on primary RGCs. A thorough investigation by Ullian et al. (2004) which showed that primary RGCs cultured in the presence of 500 µM glutamate for 1 hour did not cause apoptosis of these cells. However, hippocampal neurons treated with glutamate for 1 hour produced almost 100% death of these cultured cells. While this is one report of inability of glutamate to kill RGCs, it does demonstrate that perhaps there may be different levels of susceptibility to the same noxious stimuli between different types of neurons in the CNS. If excitotoxicity does not occur in RGCs, then there is less likelihood of memantine to work as a neuroprotective compound in patients suffering from glaucoma. It is not completely clear how RGCs are dying in the disease process of glaucoma. Without fully understanding the mechanisms of cell death in neurodegenerative diseases, it is difficult to develop strategies for neuroprotection. Perhaps, the lack of a comprehensive understanding of the pathways leading to degeneration in the CNS is a stumbling block in the development of a neuroprotective compound in humans.

As mentioned earlier, most of the molecular studies done on neurons have been performed on cortical neurons, not RGCs. Additionally, researchers have identified up to 22 different morphological distinct RGC subtypes in a mouse retina (Sun et al., 2002; Badea and Nathans, 2004, Kong et al., 2005, Coombs et al., 2006; Völgyi et al, 2009). Even though it is assumed that all these RGCs behave in a similar fashion, it is unclear if each subtype of RGCs has its own set of distinct rules for survival and functionality. Besides, it is not known if some of these subtypes of RGCs are the ones that are consistently dying in glaucoma. This adds even more complexity to the research that is being conducted in neuroprotection. Lastly, an emerging area of research being conducted is the investigation of neuronal cell death in the lateral geniculate nucleus (LGN) in glaucoma (Gupta et al., 2006). The LGN is a subcortical structure that receives the axons of the RGCs, and relays that information to the visual cortex. The LGN has been demonstrated to show apoptotic changes after intravitreal NMDA injections (Shimazawa et al., 2007; Suemori et al., 2006; Ito et al., 2008). Additionally, it is one of the hypothesized reasons for why nearly 50% RGC loss is needed to cause visual field loss, and why the initial loss of RGCs does not cause any noticeable defect in visual function (Quigley et al. 1989). Perhaps there is a need to also look at the neuroprotective capabilities of various compounds in the retina and the brain. This is another emerging area of interest that is poised to draw a lot of attention in the coming years, and will probably help to unveil some of the key mechanisms underlying apoptosis of RGCs in the disease process of glaucoma.

Many neuroprotective agents targeting various proposed pathological mechanisms associated with RGC death in glaucoma have been tested. These include calcium channel blockers, trophic factors, and anti-apoptotic factors (Danesh-Meyer 2011). Other neuroprotective strategies include, blocking the toxic effects of TNF-α and endothelin, and providing immunomodulation (Frank et al. 2009, Krishnamoorthy et al. 2008, Nakazawa et al. 2006, Schober et al. 2008, Schwartz, 2004). Other studies have demonstrated that

mesenchymal stem/stromal cell transplantation can also provide robust neuroprotective effects in rat models of glaucoma (Bull et al. 2009 and Johnson et al. 2010). This chapter focuses on those therapies which have demonstrated pre-clinical neuroprotective effects against excitotoxicity, blocking pathological influxes of calcium, and neurotrophin withdrawal. Additionally, a section will discuss emerging neuroprotectants including endothelin antagonists, TNF antagonists and sigma-1 receptor agonists as potential therapeutic targets in treating RGC degeneration.

2. Neurotrophins and their role in neuroprotection

This section will discuss the role of neurotrophins in promoting neuroprotection under conditions of neurotrophin deprivation observed in several models of glaucomatous optic neuropathy. The various types of neurotrophins and their receptors as well as their downstream signaling pathways will be briefly described. Results obtained after different strategies for neurotrophin delivery in animal models of glaucoma will be discussed.

2.1 Neurotrophins their function and structure

Neurotrophins (NTs) are a family of proteins that promote survival (Hempstead BL, 2006), development and function (Reichardt LF, 2006) of neurons and maintenance of the nervous system. They could be considered as the growth factors of the nervous system and are secreted primarily by the target tissue innervated by neurons. Typically, during development, only those neurons that make synaptic contact with target cells releasing neurotrophins survive, while others neurons that unable to gather trophic support are eliminated *via* apoptosis. The term trophic is widely used to point to a pro-survival action towards target cells by signaling molecules, including neurotrophins (NTs). Neurotrophins are the member of the neurotrophic factors (NTFs) family (Table 1). The NT family is composed of Nerve Growth Factor (NGF), brain-derived neurotrophic factor (BDNF), Neurotrophin-3 (NT-3) and Neurotrophin-4/5 (NT-4/5). Other related class of proteins include several neurotrophic factors which provide trophic support to RGCs.

Neurotrophic factor group	Neurotrophic factor member	Main receptors
Neurotrophins	Nerve Growth Factor (NGF)	TrkA, p75
	Brain Derived Neurotrophic Factor (BDNF)	TrkB, p75
	Neurotrophin-3 (NT3)	TrkC, TrkA, TrkB, p75
	Neurotrophin-4/5 (NT4/5)	TrkB,TrkA, TrkC, p75
Other NTFs	Ciliary Neurotrophic Factor (CNTF), Leukemia Inhibitory Factor (LIF), Transforming Growth Factor β 1-3 (TGFβ1 -3), Transforming Growth Factor α(TGFα), Glial Cell Line-Derived Neurotrophic Factor (GDNF), Neurturin (NTN), Persephin (PSP), Artemin (ARTN), Fibroblast Growth Factor (FGF), Neuritin -1, Insulin-like Growth Factors 1-2 (IGF-1, IGF-2), Stem Cell Factor (SCF), Platelet-Derived Growth Factor (PDGF), Erythropoietin (Epo)	

Table 1. Neurotrophic factor groups, members and main receptors

In addition to synthesis of neurotrophins in the brain, all of them are locally produced in the retina. NGF's (Liu et al 2010) and NT-3's (Seki et al, 2004) mRNA were found in the retina. BDNF and NT4/5 are expressed by RGCs (Vecino et al., 2002, Spalding et al., 2004) and by Muller cells in the retina (Seki et al., 2005). Other sources of neurotrophins in the eye include the lamina cribrosa cells and optic nerve head astrocytes which were found to express both NTs and Trk receptors (Lambert et al., 2001). BDNF is synthesized primarily in the brain and together with its receptor TrkB is taken up by RGC axon terminals and transported retrogradely to the somas of RGCs. Table 2 shows chromosomal localization of NT receptors.

Receptor	chromosome	Reference
p75	17q21-q22	(Huebner et al., 1986)
TrkA	1q21-q22	(Weier et al., 1995)
TrkB	9q22.1	(Nakagawara et al., 1995)
TrkC	15q25	(Valent et al., 1997)

Table 2. Chromosomal localizations of neurotrophin receptor genes. The BDNF protein is coded by the bdnf gene. In humans, this gene is located on chromosome 11p13 (Maisonpierre et al., 1991) and codes for a 247 aa protein. NT-3 gene *nt-3* is located on human chromosome 12p13 (Maisonpierre et al., 1991) codes for a 257 aa protein. Human *nt-4/5* gene encodes the NT-4/5 protein comprising of 210 aa and is localized to chromosome 19 band q13.3 (BERKemeier et al., 1992). NGF (299 aa protein) is coded by a gene located on chromosome 1p13.1 (www.kegg.com)

All neutrophins are synthesized as large precursors and they have analogous biochemical characteristics (Sariola et al., 1994; Barbacid 1995). They contain nerve growth factor family signature ([GSRE]-C-[KRL]-G-[LIVT]-[DE]-x(3)-[YW]-x-S-x-C). BDNF, NT-3 and NT-4/5 have been found to be structurally and functionally related to NGF (Lo DC, 1992). NGF is a protein of about 120 residues. It contains six cysteines which are involved in intra-chain disulphide bonds. Representation of the structure of NGF is shown on Figure 2. All neutrophins in modified mature forms are secreted and act as dimers
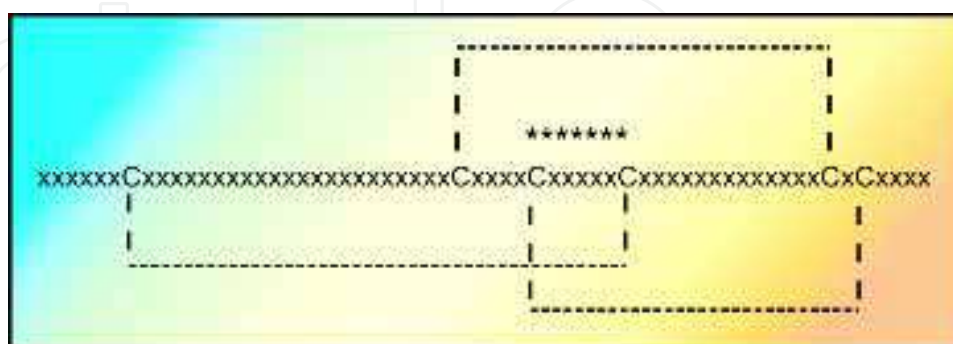

Fig. 2. Schematic representation of the structure of NGF. The signature pattern for the NGF family contains the middle region which includes two of the six cysteines involved in formation of disulfide bonds. (Stucture taken from http://www.expasy.org/prosite/PDOC 00221). Where: 'C': conserved cysteine involved in a disulfide bond, '*' position of the pattern

2.2 Neutrophin receptors TrkA, TrkB, TrkC and p75. Signaling through NT receptors

The neurotrophins in general are able to bind to two types of receptors. The high-affinity binding occurs *via* Tropomyosin Receptor Kinase (TrkA, TrkB or TrkC) and low affinity
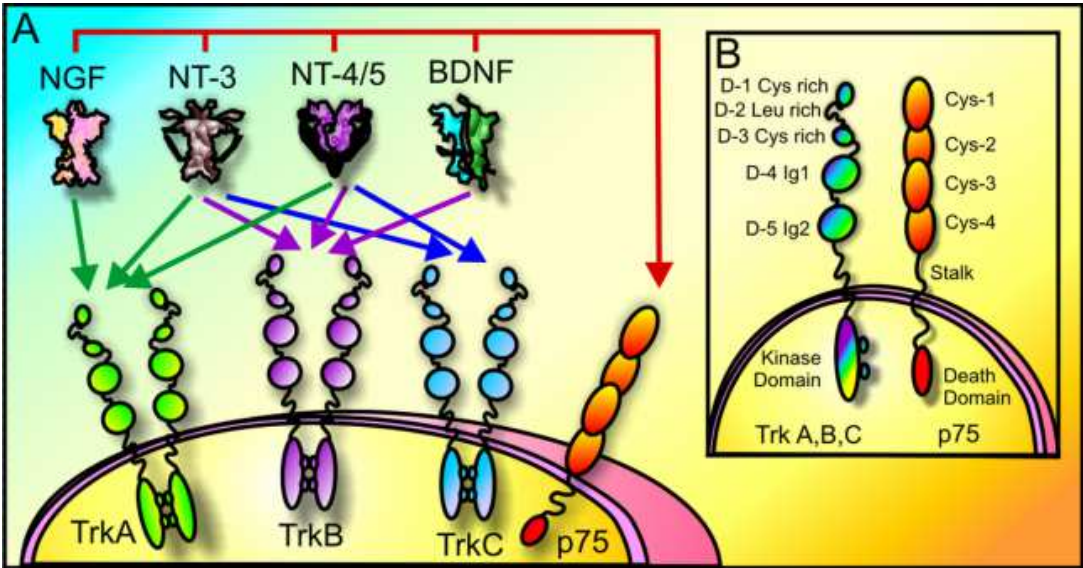

Fig. 3. A) Preferred receptors for neurotrophins are shown by color coding: Green for TrkA, purple for TrkB and Blue for TrkC. All Neutrophins bind to p75 (Red arrow). B) Schematic representation of the Trk and p75 receptors showing individual domains
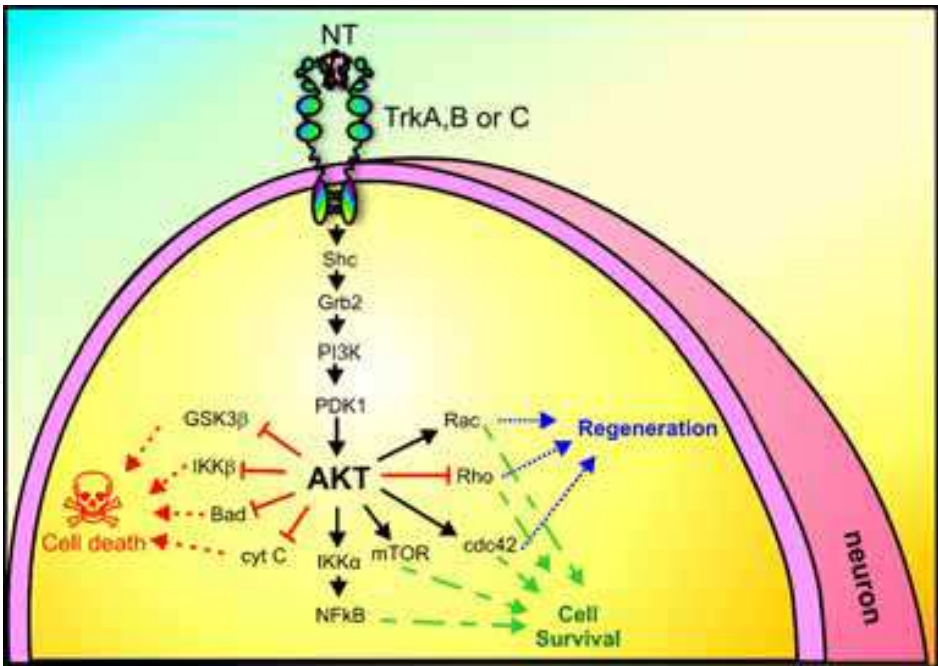

Fig. 4. AKT survival pathway activated by neurotrophins. Binding of neurotrophins to Trk receptors activates signal transduction pathway leading to activation of AKT which has an effect on several effectors. By inhibiting GSK3β, IKKβ, Bad and cytochrome C release, AKT prevents cell death. On the other hand cell survival responses are also activated by AKT through NFκB, mTor, Rac and cdc42 mediated mechanisms

binding through common neurotrophin receptor p75 (p75NTR know also as LNGFR). Receptor p75 has no tyrosine kinase domain (Greene and Kaplan, 1995; Chao and Hempstead, 1995). Preferentially, NGF binds to TrkA, BDNF and NT-4/5 bind to TrkB and NT-3 binds to TrkC (Figure 3). However, NT-3 and NT-4/5 have the ability to also bind to all the Trk receptors with different affinities. All NTs bind to the p75 receptor, which is a member of tumor necrosis factor superfamily (Figure 3).

Binding of neurotrophin to Trk type receptor induces dimerization and transphosphorylation (MacPhee et al, 1999) followed by activation of signaling cascades leading in general to pro-survival cellular responses (Figure 5). However, specific cell responses to NT-dependent Trk activation, depend upon the cell context and the balance of associated adaptors and kinase proteins. A common feature of neurotrophin signaling through Trk has been activation of MAP kinases (Marshall, 1995). Trk dependent antiapoptotic and growth pathways are mediated by cytoplasmic adaptors proteins Shc (SHC), fibroblast growth factor receptor substrate 2 (FRS-2) and others effectors including phosphatidyl inositol 3-kinase (PI3-K) and phospholipase C gamma (PLCγ1) (Chao, 2003; Huang and Reichardt, 2003). PI3-kinase activates the RAC serine/threonine-protein kinase (AKT) pathway that supports cell survival and protein synthesis. Binding of NT to Trk receptors induces the PI3-K→AKT→mTOR pathways evoking pro-survival and/or pro-growth cellular responses. Trk receptors are receptor tyrosine kinases which activate the prototypical Ras→Raf→MEK→ERK1/2 growth promoting pathway and PLCγ1 supports activation of the PKC pathway. Trk dimers promotes also phosphorylation of ankyrin-rich membrane spanning protein (ARMS) causes formation of CrkL-C3G complex, resulting in Rap1-dependent sustained ERK activation leading to pro-survival events (Arevalo et al, 2004).
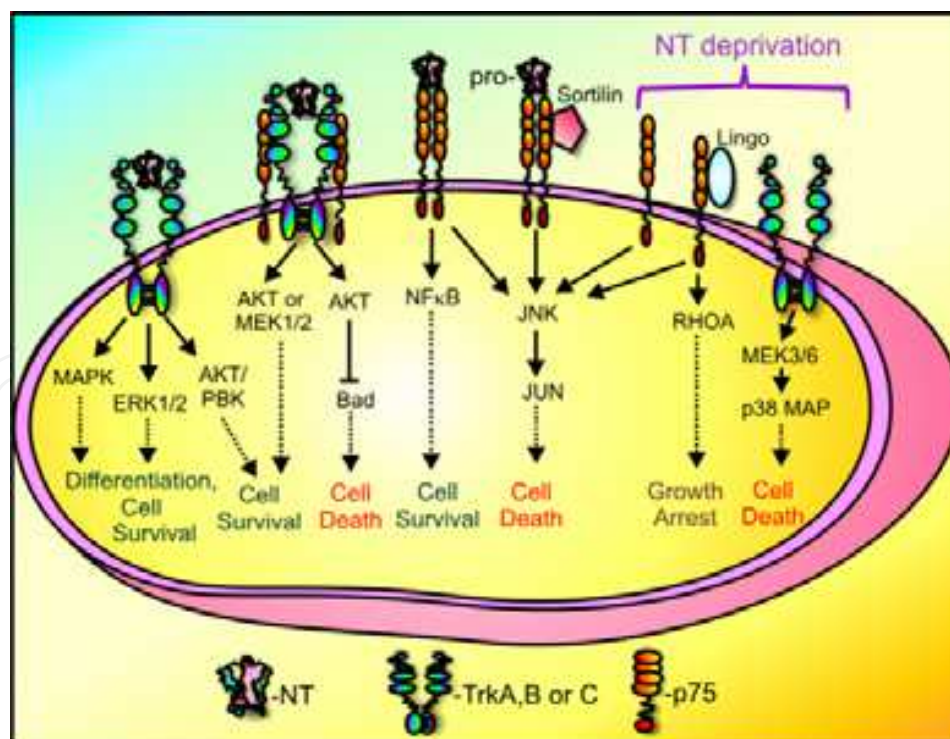

Fig. 5. A simplified summary of the NT signaling pathways. NTs binding to Trk receptors activate primarily a cell survival response. p75 receptors have predominantly cell death and growth arrest promoting effects

Trk receptors have different isoforms, some of which are deficient in the catalytic tyrosine kinase intracellular domain and their role is so far unknown (Barbacid 1995). Trk receptors may switch from pro-survival to pro-apoptotic action in the absence of its specific ligands (Calissano et al. 2010). During conditions of NT deprivation Trk signaling leads to cell death. The same effect can be caused by an over-production of TrkA which may induce a switch from an ERK→CREB pro-survival pathway to a MEK3/6→p38MAP pro-apoptotic cascade (Figure 5). It has been demonstrated that during NTs deprivation, the pro-apoptotic protein Bad is associated with Bcl-2/Bcl-xl at the mitochondrial membrane and inhibits Bcl-2/Bcl-xl, promoting cell death (Lodish et al., 2000).

A well established function of the p75 receptor is to promote cell death. When p75 receptor does not interact with Trk it promotes pro-apoptotic signal cascades. Signaling mediated through receptor p75 and its partner leucine rich repeat and Ig domain containing 1 (LINGO-1) is coupled to the mitochondrial apoptotic pathway (Nykjaer, 2005). Recently two binding partner proteins namely, ankyrin repeat – rich membrane spanning protein (ARMS) and Fas apoptosis inhibitory molecule (FAIM) were found to interact with p75 as well as Trk and modulate their signaling pathways thereby promoting survival (Chang et al, 2004, Sole et al, 2004). Moreover, when p75 interacts with pro-neutrophins bound to Vsp10p-domain receptor Sortilin, forming a ternary complex, it causes a pro-apoptotic response through TNF-α (Chikar et al 2008 and Nykjaer et al, 2004). p75 receptor can also positively regulate Trk receptor mediated pro-survival events by forming complex with Trk receptor facilitating its binding to NTs (Huang and Reichardt, 2003). p75 is able to promote neuronal survival *via* nuclear factor kappa B (NFκB) signaling (Hamanoue et al, 1999). The simplified signaling pathways regulating neuronal pro-survival or death depending on receptor, receptor-ligand or receptor-receptor-ligand interaction are presented in Figure 5.

2.3 Theory of neurotrophin deprivation and its relevance to glaucoma

During embryonic development, RGCs elongate neurites towards their targets within the brain. These targets actively secrete NTs in particular BDNF. When there is an insufficient supply of NTs to developing RGCs they undergo apoptosis (Meyer-Franke et all, 1995). In adult retinas, similar processes could take place. When a stable complex of NT and its Trk receptor is formed it is taken up by endocytosis and transported retrogradely up the axon to cell body (Ibáñez, 2007). Retrograde and orthograde axonal transport can be blocked by elevated intraocular pressure (elevated IOP) (Johansson, 1988). During conditions of elevated IOP, retrograde transport of NTs and Trk complexes is blocked and their accumulation occurs at the optic nerve head. This phenomenon is believe to be a main cause of neurotrophic deprivation in RGCs (Quigley et al., 2000, Pease et al., 2000) and may lead to neurodegenerative changes seen in glaucoma. Johnson et al. (2000) showed that a gradual depletion of BDNF and NT-4/5 occurs in the proximal optic nerve and in the superior region of the retina, as a possible response to elevated IOP. It is known that the survival of adult RGCs is maintained by transported as well as locally produced NTs (Raju et al., 1994). The blockage of retrograde transport of NTs causes gradual depletion of NTs in the retina. It seems that NTs trafficked from the brain can help in RGC survival in cases of injury, where the efficacy of local neurotrophins is not sufficient.. During glaucoma, in the situation of injury to RGCs, decreased local production of NTs in the retina as well as additional obstruction to retrograde transport could have significant implications in disease progression. The obstruction of retrograde transport precipitated by posterior displacement of the lamina cribrosa due to elevated IOP is the main hypothesis underlying glaucomatous

optic neuropathy (Quigley et al., 2000, Pease et al., 2000). Figure 6 shows the mechanisms involved in the blockage of retrograde transport of growth factors due to ischemia or increased IOP.
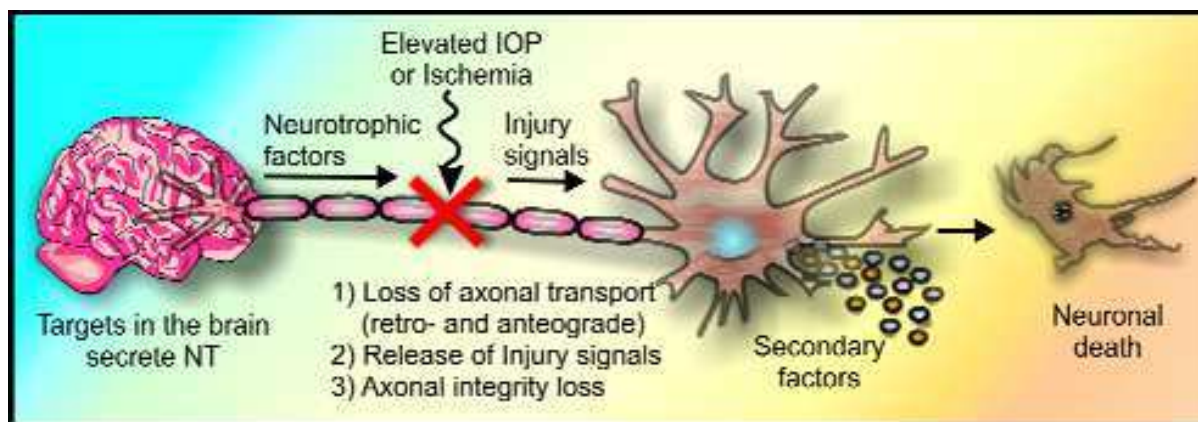

Fig. 6. Schematic representation of blockade of transport of neutrophic factors

While there is acceptance that neurotrophin deprivation leads to glaucoma, several studies found transiently elevated levels of NTs (BDNF and NT3) in the retina after injury. In acute optic nerve injury like optic nerve crush, there was an initial short term increase in retinal BDNF and TrkB levels (Hirsch et al., 2000) followed by a a decrease in TrkB receptors below normal level which had a correlation to apoptosis of RGCs (Chen and Weber, 2004). One plausible explanation for the short term increase in retinal BDNF and TrkB levels might be endogenous synthesis of NTs in response to the cessation of axonal transport. There was a loss of BDNF and NT-4/5 from the superior retina which coincided with evidence of axonal degeneration during elevated IOP in rats (Johnson et al., 2000). However, neurotrophin deprivation by itself could not be the only explanation for RGC death during glaucoma. As discussed earlier, NTs are locally produced in the retina (Ugolini, 1995, Seki, 2005). It is possible that in case of retrograde transport blockade, NTs expressed in situ should serve as proper compensation to the brain derived NTs. However, there is no clear agreement about the relative contribution of brain and locally derived NTs towards neuronal survival, as well as various effects of NTs on somal and axonal compartments (Quigley et al., 2000, Kimpinski et al., 1997 and Kuruvilla et al., 2000).

2.4 Promoting RGC survival in experimental models of neurotrophin deprivation

Exogenous NTs administration can decrease RGC loss during axonal injury; however, so far this strategy resulted only in temporary effects (Clarke et al., 1998, Di Polo et al., 1998, Isenmann et al., 1998, Bahr, 2000). This could be due to a deficit in Trk receptors in injured RGCs (Chen and Weber, 2004). Coassin et al. (2008) demonstrated that during elevated IOP retinal NGF expression was upregulated and it correlated with RGC death, mainly as the ratio of TrkA to p75 was shifted toward pro-apoptotic p75. Rudzinski et al. (2004) suggested that RGC apoptosis during elevated IOP was not only due to neurotrophic factor deprivation, but also dysfunction of NT receptors and their signaling pathways. It is clear that mere administration of NTs would not be efficacious as a neuroprotective strategy, suggesting that a concurrent regulation of receptor expression would be necessary for optimal neuroprotection.

2.4.1 Pure protein delivery

Sawai et al (1996) investigated the effect of brain- derived neurotrophic factor (BDNF), neurotrophin NT-4/5, or NT-3 on retinal ganglion cell (RGC) axons regeneration in the retinas of rats after optic nerve transection. The authors found that intravitreal injections of BDNF as well as NT4/5 were able to increase the branch median lengths by eightfold which could have implications for RGCs regrowth into their CNS targets in future therapies.

In many experimental studies, axotomy or severe nerve crush has been used as a nerve injury model. Administration of BDNF after optic nerve injury in a cat model resulted in a 55% increase in ganglion cell survival 1 week after nerve crush and 79% by the second week. Combined introduction of BDNF to the eye as well as the visual cortex increased these values further by 17%. Based on the results it was concluded that combined treatments of the eye and brain targets presented more effective approaches against nerve injury and RGC survival (Weber et al., 2010). Administration of 200 µg/mL NGF eye drops has been shown to be neuroprotective for RGC in rat eyes by inhibiting apoptosis of RGCs in animals with glaucoma. More significantly, the authors tested the same eye drops on three human glaucoma patients for three months. Patients were evaluated for changes in pattern electroretinography, visual field and visual evoked potential assays. The NGF topical administration caused improvements in inner retinal function, however no placebo controls were included in this study (Lambiase et al, 2009). In another study Colafrancesco et al. (2011) produced elevated IOP by hypertonic saline intravitreal injections into rat eyes to investigate the role of NGF on damaged RGCs and axons. Pressure elevation transiently caused an increase in NGF in the retina, followed by a drastic drop below normal levels leading to RGC apoptosis. This study showed that non-invasive, topic delivery of NGF as eye drops protected RGCs from degeneration and death. It is however unclear how NGF could penetrate the eye and reach higher order brain structures to produce therapeutic effects.

2.4.2 Gene therapy

Gene therapy has emerged as a promising avenue for the treatment of ocular disorders after the successful phase I clinical trial for Leber's Congenital Amaurosis using AAV-mediated gene delivery of the RPE65 gene (Bainbridge et al. 2008). Gene therapy as a delivery method to the retina using viral vectors has also been successful in several animal models of eye diseases including retinal degeneration, inherited retina degeneration, retinitis pigmentosa or canine childhood blindness (Martin et al., 2003). Using an experimental rat glaucoma model, it was shown that AAV mediated administration of BDNF to the RGCs significantly increased RGC survival (up to 52%) after 4 weeks of IOP elevation (Martin et al, 2003). In another study, Cheng et al. (2002) overexpressed TrkB receptor using AAV vector and combined this therapy with exogenous intraocular injections of pure BDNF, and found an increase in RGC survival. Their results indicated that TrkB-induced RGC rescue was caused by activation of the MEK →MAPK but not the PI-3K→AKT pathway. In another approach to protect RGCs, Di Polo et al. (1998) targeted BDNF to the Muller cells and found a 4.5-fold increase in surviving RGCs, 16 days post-injury in an optic nerve axotomy model in rats.

2.4.3 Intravitreal transplantation

Intraocular transplantation of progenitor or stem cells is a rapidly growing research field. Despite the fact that transplantation is a very attractive approach, many barriers to clinical efficacy remain such as an acute immune response and rejection of the transplants. To

overcome these difficulties subsequent multiple immunosuppressive therapies either alone or in combination with exogenous erythropoietin or chondroitinase ABC treatment have been used in parallel. There is a question of ethical issues connected to stem cell therapy. Transplantation of progenitor or stem cells is an emerging approach for treating neurodegenerative conditions of the CNS, and in particular the retina. It has been suggested that the neuroprotective effects of transplanted cells occur *via* the production and secretion of NTFs (Bull et al., 2008). So far, many different cell types including bone marrow mesenchymal stem cells (Inoue et al., 2007), embryonic stem (ES) cells (Banin et al., 2006) and neuronal stem cells (Grozdanic et al.,2006) have been used for the treatment of retinal degeneration and were found to integrate into the retina and differentiate into mature cell types. Transplantation of photoreceptor precursors to rhodopsin-deficient mice retinas resulted in incorporation and differentiation of progenitors to fully functional photoreceptors (MacLaren et al., 2006 and Bartsch et al., 2008). Similar strategies could be used for RGCs, but the mechanism could be more complicated. In the rat model of raised IOP by laser treatment, oligodendrocyte precursor cells (OPCs) were intravitreally transplanted to the retina. Transplanted OPCs survived up to 12 weeks post surgery and were found to localize in proximity to the RGCs. The transplanted cells increased survival rate of injured RGCs by up to 60% (Bull et al., 2009). Use of MSCs derived from bone marrow of patients is therapeutically very attractive because it allows autologous transplantation and has less ethical issues. This strategy was successfully used in an elevated IOP rat model by Yu et al. (2006) which resulted in significant RGC neuroprotection.

2.4.4 Neuroprotection *via* NT receptors and/or their downstream signaling modifications

Increasing NT concentrations was not always sufficient for neuronal survival and/or regeneration and the effects of administration of NTs were usually only transient. In some cases intraocular injections of NTs promoted neuronal survival; however, they also produced axonal dystrophy (Pernet et al., 2006). As previously discussed, there needs to be a balance between NTs and their receptors (Trk and p75), which depending on the cellular context can trigger contrary biological effects (anti- or pro- survival). Since there were difficulties with long-term stable administration of NTs in animal models of glaucoma and retinopathy, the selective agonists of Trk receptors or antagonists of p75 or their downstream pathway components have been studied as anti-glaucoma treatments. Combinations of growth factors and antagonist of protein forming complexes with Trk receptors can promote a sustained and efficacious rescue of ganglion cells. For instance, in the rat experimental model of laser derived ocular hypertension, an antagonist of LINGO-1, which was known to form a complex with TrkB receptor, produced its inhibition and long term protection of RGCs. This study showed that combined treatment of RGC by intravitreal injections of BDNF and LINGO-1-Fc provided long lasting neuroprotection after elevated IOP. This treatment was responsible for stable activation of the TrkB receptor (Fu et al., 2009).

Therapeutic use of NGF has been able to only partially rescue RGCs from apoptosis and failed because the action depends on the receptor (TrkA or p75). This was supported by the finding that the TrkA receptor agonist (peptidomimetic D3) was effective in the treatment of experimental glaucoma caused by elevated IOP and decreased RGCs loss by 25% (She et al., 2007). However the best results were obtained by a combination of a TrkA receptor agonist together with a pressure lowering drug (betaxolol) in which 90% reduction of RGCs loss was observed (Shi et al., 2007). The synergistic effect of a combination of NGF and

peptidomimetic TrkA agonist, along with a peptidomimetic p75 antagonist prevented RGC cells loss following optic nerve transection (Lebrun-Julien et al., 2009). p75 increased production of neurotoxic proteins TNF-α and α(2)-macroglobulin. p75 was found to be expressed by the majority of glia and Muller cells and TrkA was found mainly in RGCs (Lebrun-Julien et al., 2009). In another study, wild type NGF failed to protect RGCs in tested models of glaucoma and nerve axotomy. NGF-C, a NGF selective agonist of TrkA, which does not bind to p75, was found to increase neuronal survival by 17% two weeks following axotomy and by 13% in a ocular hypertension glaucoma model. Other approaches used an anti-NGF monoclonal antibody (mAb NGF30) blocking NGF binding to p75 without changes in NGF-TrkA interaction. This treatment caused a doubling of RGC survival post-axotomy and a 15% increase in RGC survival in an ocular hypotension model. Moreover, authors tested p75 receptor antagonists (THX-B and LM-24) in ON axotomy and ocular hypotension and found 37% and 21% RGC survival in the two experimental models. Antagonists of p75 inhibit TNF-α and α(2)-macroglobulin expression and were found to be neuroprotective in ON axotomy and ocular hypertension (Bai et al, 2010). Other approaches have employed Trk B agonists to produce neuroprotective effects. Unfortunately, many TrkB agonists were not able to fully mimic neuroprotective functions of BDNF on TrkB receptors. To discover small molecules having similar potency as BDNF, the authors developed a cell-based apoptotic assay and identified a potent TrkB agonist, deoxygedunin. Deoxygeduin was able to activate only TrkB, in a BDNF-independent manner evoking antiapoptotic cell pathways (ERK1/2 and AKT activation). When intravenously injected into mice (5mg/kg), deoxygeduin penetrated the blood brain barrier and strongly mimicked BDNF, in terms of its neuroprotective ability (Jang et al., 2010).

There are many complications in developing and testing new active ligands with greater specificity for pro-survival NTF receptors. Due to those difficulties, there were strategies developed to avoid receptor *per se* and activate downstream pro-survival pathways. There is still an incomplete understanding of the complexity of NT receptors signaling pathways, in particular to differentiate between target-mediated and cell body-mediated neurotrophin signaling. Zhou et al. (2005) hypothesized that stable activation of pro-survival ERK1/2 kinase, which is a downstream effector of Trk receptors, inhibits RGC death. Since MEK1 is a direct upstream activator of ERK1/2, increase in MEK1 should activate ERK1/2 expression. The authors found significant RGC protection in the AAV-MEK1 mice, with up to 60% greater survival compared to control mice after the 5th week of treatment (Zhou et al., 2005). Another study evaluated the neuroprotective effect of modification of downstream NTs effectors. A NK-4 cyanine dye produced neurotrophic effects and promoted neurite-outgrowth and cell proliferation of PC12 cells. Action of NK-4 was dependent on activation of PI3K-AKT but occurred independently of Trk. NK-4 additionally induced phosphorylation of AKT kinase. The authors showed increased cell count indicative of enhanced survival in the treated group versus control (Ohta et al., 2011).

Another key target for neuroprotection of RGCs is Rho kinase which is a member of the serine-threonine family of protein kinases activated by the small G protein Rho. Rho kinase inhibition has been shown to be beneficial for IOP regulation in patients and also enhances RGC survival and axon regeneration (Rao and Epstein, 2007). An attempt to modify downstream signaling of NT receptors was to stably inactivate Rho GTPase, which was shown previously to be a downstream effector of p75 /LINGO-1/Nogo-R complex which formation leads to cell death (Nykjaer et al., 2005). It is also known that neurotrophin (NGF, BDNF or NT-3) binding abolished RhoA activation. Inactivation of Rho proteins mimicked

the effect of neurotrophins by increasing the rate of neurite elongation. (Yamashita et al., 2005). Bertrand et al. (2007) used multiple intraocular injections of membrane permeable C3-like Rho antagonists and found that sustained Rho inactivation acts as a pro-survival mechanism following optic nerve injury showing a 1.5 fold increase in RGCs survival. Co-receptor of p75 - NogoR can activate Rho kinase in a p75 dependent manner. In neurons, Rho has been shown to play a role in the regulation of apoptosis. The Rho antagonist C3–05 (to suppress Rho activation) was tested in the rodent model of acute nerve injury. In mice and rats treated with Rho-C3–05 the extent of cell death was significantly reduced by ~50% post- injury. The correlation between Rho kinase and p75 receptor was based on co-localization of active Rho with p75 after injury. Rho inactivation after injury inhibited apoptosis by preventing the synthesis of pro-apoptotic p75 receptor (Wang et al., 2002). The Rho antagonist C3-07 was successfully used in another study resulting in survival of RGCs by 1.5 fold after optic nerve lesion (Bertrand et al., 2007).

2.4.4.1 Other neuroprotective approaches

Shi et al. (2008) studied α-2-macroglobulin (α2-M) inhibitors for their neuroprotective abilities. It was found that α-2M act as a sink for NTs and therefore inhibits their bioavailability during glaucoma. The authors elevated IOP in rats by cauterization and by the Morrison's model to test their hypothesis that neutrophic deficit is due to neutralization of neutrophins by α-2M. They proved that blocking α-2M, using specific inhibiting antibodies against α-2M was protective for RGCs even without lowering IOP (29% protection). Together with normalization of IOP the neutralization effect were even more pronounced (35% protection) (Shi et al., 2008).

Erythropoietin (Epo) is a glycoprotein hormone, synthesized in the kidney secreted by interstitial cells of the adrenal cortex in response to tissue hypoxia. Epo is a neurotrophic factor that could be developed as a new drug for neurological disorders. Epo posses a specific Epo/Epo-receptor system in the CNS and cerebrospinal fluid (Buemi et al., 2002). Zhong et al. (2007) studied the neuroprotective effects of intraperitoneal administration of erythropoietin(Epo) in the DBA/2J mouse model of glaucoma. Treatment with Epo at doses of 3000, 6000, and 12,000 U/kg body weight per week promoted RGC survival in DBA/2J glaucomatous mice without affecting IOP. These results suggested that Epo may be used as a potential therapeutic neuroprotectant in glaucoma. Since Epo does not cross the blood-brain barrier (BBB) there is a tendency to recombine its structure that way that it will became permeable to BBB. Boado et al. (2010) developed new drug (HIRMAb(IgG)-EPO fusion protein) enabling Epo to cross the BBB.

2.4.5 Potential methods for NTs delivery

Topical eye drops, intraocular injections and systemically-administered compounds have been the conventional systems for delivering drugs to glaucomatous eye. However, these methods have many limitations and disadvantages. First, there is a factor of patient compliance, efficacy, side effects and absorption of the chemical. Several methods were discovered recently to improve long-term delivery of the drug, control dosage and reduce side effects. The goal might be achieved by different strategies such a slow-release biodegradable microspheres devices commonly made of poly-DL-lactic-co-glycolic acid (PLGA). PLGA spheres can be filled with chosen NT or receptor agonist which when intraocularly injected will result in slow, sustained drug release (Ward et al., 2007). Devices can be filled with not only NTs or receptor agonist/antagonist. It could be also fortified with

chemical compounds which have the ability to activate pro-survival or inhibit pro-apoptotic pathways, downstream to NTs. It is also possible to deliver viral constructs or siRNAs to the retina: however, genetic manipulation of human cells always represents a potential biohazard issue. The other new method for sustained delivery of glaucoma medication involves incorporation of the drug into a punctal plug. This method is already used for patients suffering from dry eye syndrome. Another approach is usage of solid lipid nanoparticles loaded with neuroprotective drugs. Researchers have reported promising results from experiments using laboratory-created nanoparticles to deliver a glaucoma medication (Li et al., 2011). Investigations into this approach for treating glaucoma were in very early stages. This approach can also be used for NTs delivery. Animal experiments using nanoparticles demonstrated that they can actively migrate through the vitreous and neurosensory retinal layers, reaching the retinal pigment epithelium and choroid. This capability has proved to be useful particularly in treatment of retinal degeneration diseases. Nanotechnology can be used in ophthalmology to treat retinal degenerative disease with gene therapy, prosthetics, and regenerative nanomedicine (Zarbin et al., 2010). Another emerging technology for drug delivery to the eye is encapsulated cell technology (ECT). ECT has also been developed to treat diseases of the central nervous system and the eye (Tao et al., 2002). ECT could be applied to neurodegeneration treatment in glaucomatous eyes. This device can serve as a source of neurotrophic factors produced by encapsulated cells in the eye. Such an approach was already successfully used to delivery CNTF in a dog model of retinitis pigmentosa.

3. Calcium channel blockers

Calcium channel blockers (CCBs) have a therapeutic potential in treating glaucomatous optic neuropathy. However, their therapeutic potential as neuroprotectants has not yet been fully realized to treat glaucoma or other chronic neurodegenerative diseases. This section will discuss the importance of neuronal calcium signaling under normal physiological conditions. Secondly this section will then discuss the proposed hypothesis of calcium dysregulation in playing a key role in apoptosis, and the proposed mechanism of ischemia-reperfusion that has been demonstrated to occur in some cases of glaucoma. Lastly, this section will then discuss CCBs as neuroprotective pharmaceutical agents, retinal vessel dilators, and IOP lowering agents.

Under physiological conditions, calcium is an important neuronal intracellular signaling molecule found at very low cytoplasmic concentrations (100 nM) under normal non-excited states (1-2 mM extracellularly). This low intracellular calcium concentration allows a strong inward electrochemical gradient, and facilitates the ability for calcium to be used as a quick communicator to activate and suppress a number of different genes and signaling pathways that are critical for neuronal survival, long term potentiation, and synaptic plasticity. Interestingly, it seems that not all calcium influx is considered equal. Different subset of neuronal signaling pathways can be activated depending on which channel is used to mediate calcium conduction inside neurons.

RGCs are the output neurons of the retina that conveys all the visual information obtained from the retina to the brain. Different synaptic inputs are received on the dendrites of RGCs from bipolar cells and amacrine cells. The normal flow of electrical potentials through the retina is initiated at the back of the retina starting from the photoreceptor cells. The electrical information then travels from photoreceptor cells to bipolar cells, and finally to RGCs.

Glutamate is the main excitatory neurotransmitter that is released from photoreceptor cells and biopolar cells. Typically horizontal cells and amacrine cells modulate the electrical signal at the outer and inner plexiform layers respectively. Figure 7 shows various layers of the retina(A) and a representative retina flat mount (B).
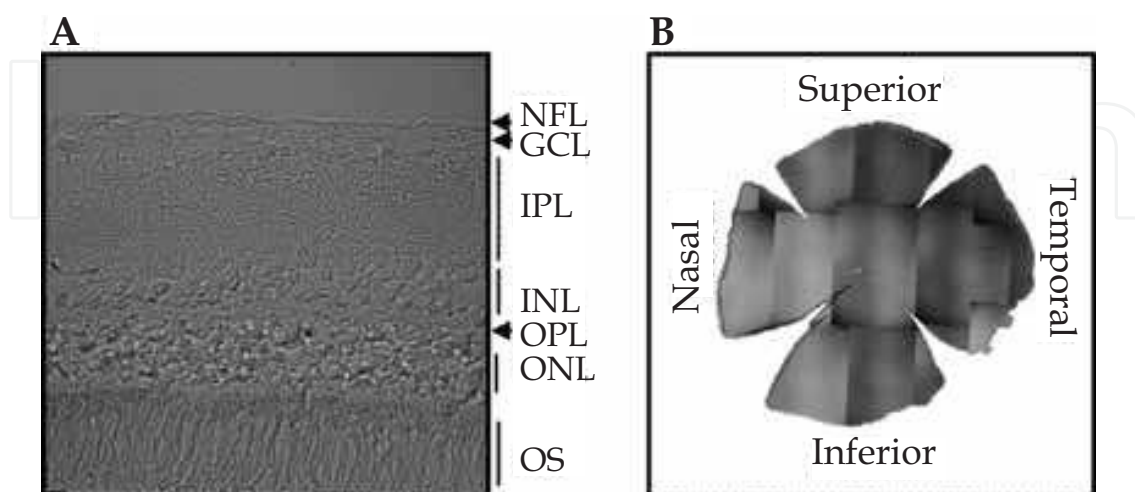

Fig. 7. A. Organization of the retinal layers. Confocal microscopic image of a rat retina section. The different layers of the retina are indicated in the picture: Nerve fiber layer (NFL), Ganglion cell layer (GCL), Inner plexiform layer (IPL), Inner nuclear layer (INL), Outer plexiform layer (OPL), Outer nuclear layer (ONL) and Outer Segment (OS). B. Representative flat mount showing different areas of the retina including superior, inferior, nasal and temporal

Once glutamate is released from the axonal terminals of bipolar cells, it will migrate through the synaptic cleft to interact with glutamate receptors found on the dendrites of RGCs. Glutamate receptors are divided into two main classes: ionotropic and metabotropic. The ionotropic glutamate receptors are further divided into NMDA and non-NMDA subtypes. The non-NMDA receptors are also known as AMPAr and Kainate receptors. NMDA receptors are known for their ability to be highly permeable to Ca^{2+} ion influx once they are activated. Typically NMDA receptors need both glutamate and glycine to be activated. However, NMDA receptors are subject to a Mg^{2+} blockade preventing calcium ion influx when the membrane potential is at rest (-90 to -70 mV). This Mg^{2+} can only be removed from the NMDA receptor complex once the membrane potential of the dendrites reaches between -20 and -30 mV. (Mayer et al., 1984; Ozawa et al., 1998) The Mg^{2+} blockade is typically relieved by raising the membrane potential through the activation of non-NMDA receptors. The stimulation of these ionotropic receptors causes excitatory postsynaptic currents that then go to further activate voltage gated calcium channels (VGCCs). VGCCs are divided into two major classes of Ca^{2+} channels based upon the membrane potential that opens them. There are low-voltage activated (LVA) and high-voltage activated (HVA) channels. These channels can be further divided into T-type which is the LVA and L-, N-, P/Q, and R-type which are all HVA. Of the VGCCs, L-type is the one that is associated with calcium-mediated neuronal injury because of the prolong calcium influx that occurs when this channel is activated (Mark et al., 2001). Additionally, prolonged stimulation of VGCCs and NMDA channels has been shown in cortical neurons to suppress mitochondrial movement in the dendrites (Li et al., 2004).

Under normal physiological conditions, calcium signaling inside neurons mediates a number of second messenger pathways. For example, when calcium influx occurs through neuronal L-type VGCCs that are encoded by the CaV1.2 and CaV1.3 pore-forming subunits, the channel has the ability to activate CREB, MEF, and NFAT that can lead to the expression of genes like *c-fos* and *bdnf* (Graef et al., 1999; Mao et al., 1999; Sheng et al., 1990; Morgan and Curran, 1986; Murphy et al., 1991; Zafra et al., 1990). Similarly, NMDA receptors expressing NR2A-containing glutamate receptors have demonstrated to promote long term potentiation through Ras-GRF2 and ERK MAP kinase activation (Flammer et al., 1994). It is evident that calcium signaling through ionotropic glutamate receptors and VGCCs are essential and needed for neuronal survival and functionality. However, one caveat in all of these studies is that the data has not been replicated in primary RGCs. So though, it can be assumed that RGCs are an excitatory neuron and probably do behave in a similar fashion to cortical excitatory neurons, there is not much information to determine if cell survival and long term potentiation pathways are also activated in RGCs when NMDA and L-type VGCCs are activated.

In chronic neurological disease processes, it is plausible that the beneficial effects that occur from neuronal calcium signaling are suppressed, and an overload of calcium causes activation of the apoptotic cascade. For example, normal stimulation of intrasynaptic NMDA receptors under physiological conditions causes activation of many pro-survival pathways which lead to phosphorylation of cAMP response element-binding protein (CREB) through activation of calmodulin-dependent protein (CaM) kinase pathway and Ras-extracellular signal-regulated kinase 1/2 (ERK1/2) pathway (Hardingham et al., 2001; Wu et al., 2001; and Hardingham et al., 2001b). However, extrasynaptic NMDA stimulation suppresses CREB activation through the inactivation of Ras-ERK1/2 pathway (Hardingham et al., 2002; Hardingham et al., 2002b; and Ivanov et al. 2006). These opposing pathways are activated through similar receptors in different neuronal locations and demonstrate the importance of compartmentalization of calcium signaling in the neuron. When the same ionotropic calcium channel is stimulated, the location of that channel dictates the secondary pro-survival/pro-apoptotic pathways that are stimulated. It is clear from all these studies that basal calcium signaling is beneficial for the neuron, but overstimulation of these extrasynaptic NMDA receptors is detrimental for neuronal survival causing stimulation of apoptotic enzymes including calcineurin and calpain.

Calcineurin is a calmodulin-dependent serine-threonine phosphatase that activates Bad through dephosphorylation. This dephosphorylation causes BAD to dissociated from the inhibiting protein 14-3-3 and cause it to move into the mitochondrial outer membrane. Once Bad is on the outer mitochondrial members, it will bind to anti-apoptotic Bcl-2 or Bcl-XL and promote release of cytochrome c. (Wang et al., 1999; Heckman et al., 2006) Calcineurin activation and cytochrome c release has been linked with RGC death after optic nerve crush. (Huang et al., 2005) Lastly, RGC death has been shown to be attenuated in experimental models of optic nerve crush after treatment with a calcineurin inhibitor, FK506 (Huang et al., 2005).

Calpain is a Ca^{2+}-dependent cysteine protease that is implicated in many neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease (Nixon, 2003; Goll et al., 2003) Calpain activates calcineurin and transforms it into its constitutively activated form. Calpain activation has been detected in rat experimental glaucoma, (Huang et al., 2010) while its inactivation has demonstrated protection against excitotoxic neuronal cell death *in vitro* and *in vivo* (Wu et al., 2004).
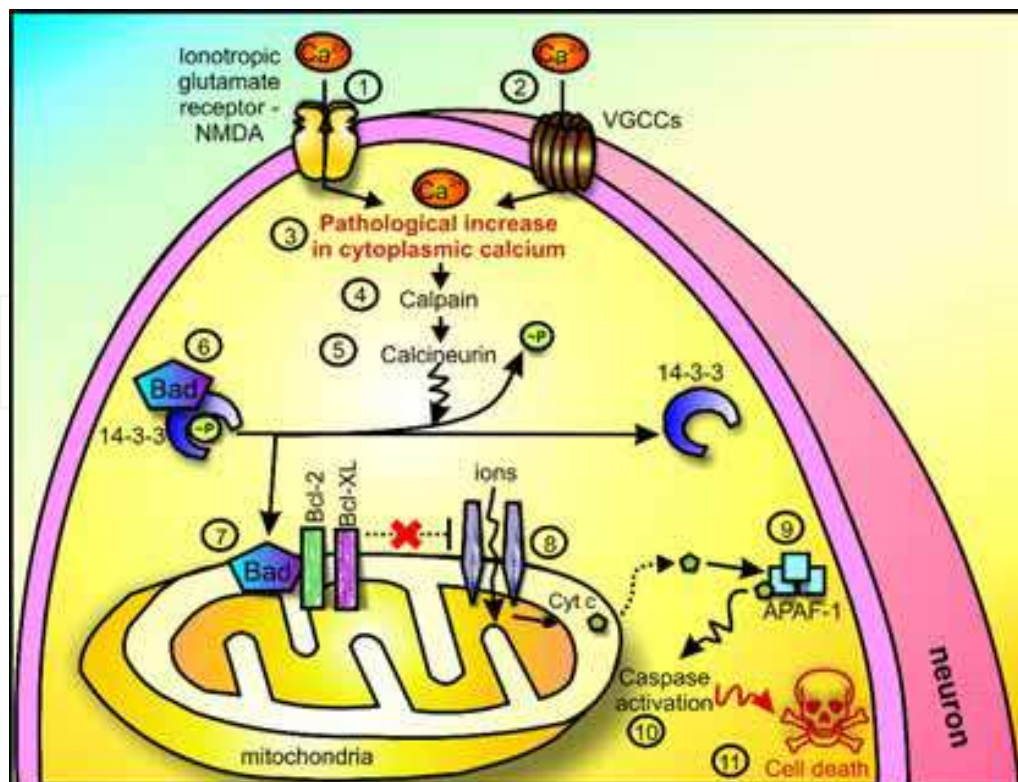
Fig. 8. Calcium-induced apoptotic changes in neurons. Intracellular calcium in increased through the sequential actions of NMDA and voltage-dependent calcium channels. Elevated Ca²⁺ activates a number of factors including calpain and calcineurin which have downstream effects on substrates. Calcineurin activation promotes dephosphorylation of Bad which in turn initiates mitochondrial dysfunction by its interaction with Bcl2 or Bcl-XL. The disruption of mitochondria releases cytochrome c which activates the apoptotic cascade

Calcium channel blockers (CCBs) are pharmaceutical agents geared at halting calcium influx across cellular membranes and decreasing intracellular calcium levels. These agents are widely used clinically to control patients suffering from benign essential hypertension, angina pectoris, cardiac arrhythmias (like atrial fibrillation and paroxysmal supraventricular tachycardia), and pulmonary hypertension. CCBs have been studied to treat many of the chronic neurological diseases afflicting individuals worldwide. CCBs could in theory act as neuroprotectants to help preserve neuronal viability, especially by halting the upstream activation of calcium sensitive apoptotic proteins (calcineurin and calpain) and can prevent permanent damage from the late stage activation of apoptosis (Loetscher et al., 2001). Many of the theories implicated in RGC death in glaucoma involve some form of calcium dysregulation. So if an appropriate CCB can be identified to treat RGCs vulnerable to apoptosis, then this pharmaceutical agent could halt the activation of many apoptotic pathways early on in the activation of the cell-death pathway. Additionally, CCBs have therapeutic potential in decreasing IOP and increasing retinal blood flow. Therefore, it is possible that a CCB treatment could act as a "silver bullet glaucoma therapy" by decreasing IOP, increasing retinal blood, and protecting RGCs by maintaining calcium homeostasis by acting as a neuroprotectant. The rest of this section will highlight CCBs as a pharmaceutical treatment to treat glaucoma as a neuroprotectant, retinal vessel vasodilator, and IOP reducing molecule.

The use of CCBs to treat glaucoma as a neuroprotectant is based upon the assumption that two fundamental molecular hypothesizes implicated in RGC death in glaucoma are correct. These two hypothesizes are that glutamate excitotoxicity exist, and that calcium dysregulation occurs and activates apoptotic enzymes in glaucoma. The debate over glutamate excitotoxicity as a pathological correlate to RGC death in glaucoma is addressed in the glutamate excitotoxicity portion of this chapter. There does appear to be evidence, as mentioned above, that calcium dysregulation does occur prior to RGC death. Drugs that are geared at blocking calcium ion influx mainly target two classes of ion channels, NMDA receptors and L-type VGCCs. The section on excitotoxicity will touch upon treatments that have shown neuroprotective potential through the blockade of NMDA receptors.

Of the drugs that have been developed and used to treat human pathological conditions, there are three classes of drugs geared at blocking L-type VGCCs. These three main classes are the phenylalkylamine (PAA), or verapamil-like CCBs, the benzothiazepine (BTZ), or diltiazem-like CCBs, and the dihydropyridine (DHP), or nifedipine-like CCBs (Araie and Mayana, 2010). In animal studies, intraperitoneal injections of nifedipine showed neuroprotection and improved b-wave amplitude when rats received retinal ischemic damage (Crosson et al., 1990) Lomerizine is another L-type and T-type CCB that has demonstrated to have neuroprotective effects on ischemic retinal neurons in both *in vitro* and *in vivo* (Toriu et al., 2000). In primary RGC cultures exposed to hypoxic conditions (5% normal partical pressure), iganidipine, nimodipine, and lomerizine demonstrated robust RGC protection (Yamada et al., 2006; Chen et al., 2007).

Another pharmaceutical agent that has demonstrated neuroprotection to RGCs by blocking calcium currents is betaxolol, a β1-adrenoceptor antagonists (Araie and Mayana, 2010). This molecule has demonstrated to have binding affinity to L-type VGCCs and NMDA, and has been shown to decrease calcium influx through the cellular plasma membrane. (Melena et al., 1999; Bessho et al., 1991; Hester et al., 1994; Hoste and Sis, 1998; Setoguchi et al., 1995; Dong et al., 2006; Nagata et al., 2008) Considering the potential that blocking L-type VGCCs has demonstrated as being a neuroprotectant as described above, their may be therapeutic potential for treating patients afflicted with glaucoma with betaxolol as well. Betaxolol may act as an IOP lowering agent and as a neuroprotectant by blocking pathological influxes of calcium through L-type VGCCs. (Hirooka et al., 2000) Topical application of betaxolol has also been reported to induce expression of BDNF in the rat retina. (Wood et al., 2001). In vivo experiments also demonstrated protection through the attenuation of retinal damage to rabbits and rats subjected to ischemic insult (Wood et al., 2001; Wood et al., 2003; Osborne et al., 1999; Osborne et al., 2004; Cheon et al., 2002) or to rat eyes injected with NMDA or kainic acid (Osborne et al., 1999; Cheon et al., 2006). However given betaxolol's L-type VGCC blocking effect, when this drug was tested next to timolol in a large comparative human clinical trial, there was no difference in disease progression between those patients taking either timolol or betaxolol (Watson et al., 2001; Araie et al., 2003). Lastly, in another human clinical trial, the conversion rate from ocular hypertension to glaucoma between betaxolol and placebo was not attenuated between the two treatment groups (Kamal et al., 2003).

The predominant theory of what precipitates the cascade of RGC death in glaucoma is the mechanical compression theory highlighted above. However, another theory of retinal ischemia reperfusion has been put forth to account for RGC loss as a consequence of insufficient retinal blood flow (Flammer, 1994). The retina receives its blood supply from the central retinal artery which is a branch from the ophthalmic artery (Olver, 1998). Insufficient blood flow to the retina is able to produce glaucomatous-like visual field defects, and

researchers have speculated that patients suffering from normal tension glaucoma could be developing glaucomatous optic neuropathy because of a vasospastic syndrome (Flammer et al., 1987; Broadway and Drance 1998). Research studies have actually demonstrated that patients suffering from glaucoma are more prone to suffer from vasospastic episodes (Gasser and Flammer, 1991; Rojanapongpun and Drance, 1993; O'Brien 1998). In fact, in some glaucoma patients, improvements are seen in retinal circulation and visual field defects when patients are treated with CCBs (Flammer and Guthauser, 1987; Guthauser et al., 1988).

As mentioned above, CCBs have the ability to block intracellular calcium influx, which can lead to relaxation of vascular smooth muscle and enable enhanced blood flow to perfuse certain organs. There are numerous studies that have been conducted in looking at the effects of nifedipine, nicardipine, verapamil, nimodipine, nilvadipine, and lomerizine on ocular blood flow and their abilities to counteract retinal vasospasm. In a prospective clinical trial, Kitazawa et al. (1989) treated 25 patients suffering from normal tension glaucoma with oral nifedipine for 6 months and was able to demonstrate improved visual fields. However, another study performed by Harris et al. (1997) showed that 6-month treatment with oral nifedipine in 21 patients failed to demonstrated significant difference in visual field and spatal contrast sensitivity. Another prospective study performed on patients with open angle glaucoma showed that a 3 month treatment with nifedipine had no affect on improving visual fields compared with the control group (Rainer et al., 2001). In contrast, nimodipine has demonstrated in multiple prospective clinical trials to improve visual function in normal tension glaucoma patients (Bose et al., 1995; Piltz et al., 1998; Boehm et al., 2003; Luksch et al., 2005) How some of these CCBs demonstrated improvement in visual function in patients suffering from glaucoma is unknown, but perhaps it could have been through both a vasodilator affect, and a direct neuroprotective effect on the RGCs.

A final therapeutic target for CCBs is their ability to lower IOP when given topically. The mechanism of action of CCBs in lowering IOP is still controversial. L-type VGCCs have been shown to exist on both human and bovine trabecular meshwork (TM) cells (Steinhausen et al., 2000; Wiederholt et al., 2000; Thieme et al., 2005). Ciliary epithelial cells also have DHP-sensitive, VGCCs (Farahbakhsh et al., 1994). CCBs act by decreasing in-flow through its effects on ciliary epithelial cells, or improve outflow through its effects on the TM cells. Out of all the therapies used to treat glaucoma, CCBs have the capability to lower IOP while also increasing retinal blood flow and protecting RGCs from pathological influxes of cytoplasmic calcium.

Though there is a lot of evidence correlating pathological influx of cytoplasmic calcium with RGC death, the fact of the matter is that there are probably many pathological pathways acting in concert with one another to elicit RGC apoptosis (Qu et al., 2010). For example, astrocytes and retinal glial cells can fail to take up glutamate from the excess glutamate that is being released from damaged RGCs in glaucoma (Adachi et al., 1998; Danbolt, 2001) Therefore, excitoxicity then can cause pathological influx of calcium ions through overactivation of NMDA receptors and VGCCs. This elevation of calcium ions can not only cause activation of calcium specific apoptotic enzymes, but also generate large amounts of free radicals which can in-turn cause oxidative stress (Tezel, 2006). So therefore, it is not only important to investigate potential therapeutic targets that are geared at protecting RGCs from cell death caused by glaucomatous optic neuropathy insults, but it is just as important to understand basic cellular and molecular mechanisms underlying neuronal cell death in glaucoma.

4. Glutamate excitotoxicity

Excitotoxicity is a process by which excitatory amino acids such as glutamic acid (glutamate) and aspartic acid produce overstimulation of their receptors leading to neurotoxicity and/or neurodegeneration. Glutamate is a major excitatory neurotransmitter both in the brain and in the retina. The excitatory neurotransmitters in the brain include glutamate and aspartate, while the inhibitory neurotransmitters include GABA, glycine and taurine. In the retina, glutamate is the primary neurotransmitter that is involved in the visual transduction pathway. Glutamate is an α-amino acid containing a second carboxylic acid (-COOH) group attached to the γ-carbon atom. In the CNS, glutamate does not cross the blood brain barrier, hence in neurons, glutamate is made from α-ketoglutarate, an intermediate of the TCA cycle. Another source of glutamate is the conversion of glutamine obtained from glial cells to glutamate within neurons. This cycle is described in Figure 9.
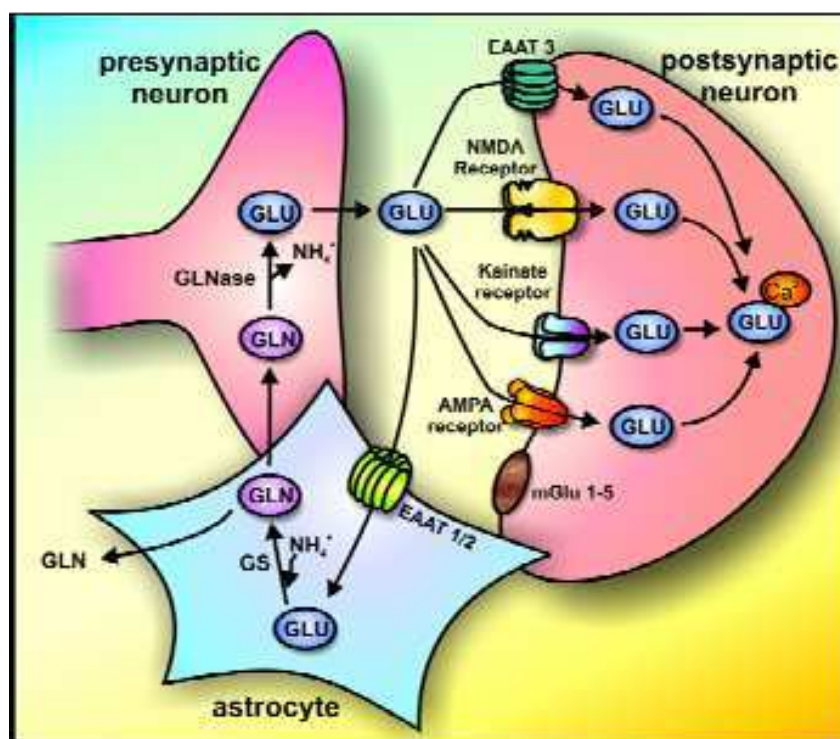

Fig. 9. The Glutamate-Glutamine cycling between neurons and glia. Glutamate (GLU) is taken up by glial cells and converted to glutamine (GLN) by the enzyme glutamine synthase (GS). Glutamine is transported by the action of excitatory amino acid transporters (EAATs) back to the neurons where it is converted back to glutamate by the enzyme glutaminase (GLNase)

Glutamate is generated by the enzyme, glutaminase, in the endoplasmic reticulum, packaged in the golgi apparatus into membrane-bound vesicles and transported down the axon by anterograde axonal transport on microtubules to the synaptic junctions. The membranes of the vesicles merge with the synaptic membrane and glutamate is released by exocytosis into the synaptic space. Upon release into the synapse, glutamate binds to glutamate receptors in the post-synaptic membranes. There are two principal classes of glutamate receptors: ionotropic and metabotropic receptors. Ionotropic receptors are ligand-gated ion channels which produce an influx of calcium and sodium ions upon binding to

glutamate and similar agonists. Metabotropic receptors are G-protein coupled receptors which couple ligand binding to intracellular signal transduction mechanisms mediated by a variety of second messengers including cAMP and intracellular calcium. Excitotoxicity is mediated by ionotropic receptors which can be characterized into various subtypes according to the type of the selective agonist including, N-methyl D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate. These agonists are not naturally occurring, but have a structural resemblance to glutamate. Glutamate binds to and activates all three classes of receptors namely, NMDA, AMPA and kainate receptors. NMDA receptor activation is important for synaptic plasticity which plays a key role in memory and learning. However, overstimulation of NMDA receptors contributes to elevation of intracellular calcium leading to neuronal injury.

The NMDA receptor has a tetrameric structures composed of two subunits of NR1 and NR2 subunits (Figure 10). There are four subtypes of NR2 subunits called NR2A, NR2B, NR2C and NR2D. Other related subunits including NR3 and NR4 have inhibitory effect on receptor activity. Activation of the NMDA receptor requires binding of two molecules of glutamate and two molecules of glycine which acts as a co-agonist. The AMPA receptor (AMPAr) also functions as a tetramer comprised of four different types of subunits including GluR1 to GluR4. Most AMPA receptors have a structure comprising of a combination of a dimer of GluR2 with a dimer of either GluR1, GluR3 or GluR4 (Figure 10). Each subunit of the AMPA receptor has a binding site for glutamate. Binding of glutamate to AMPA receptors causes an opening of the ion channel leading to an influx of sodium ions. AMPA and kainate receptors play an important role in facilitating rapid excitatory neurotransmission by rapid conduction of Na^+ ions. AMPA receptors in the retina are also permeable to Ca^{2+} ions. Electrical stimulation of a presynaptic neuron causes glutamate release which activate AMPA receptors in the post-synaptic neuron. Activation of AMPAr causes depolarization of the post-synaptic neuron. However glutamate binding to NMDA receptors in weakly depolarized postsynaptic neurons is not sufficient to trigger activation of the receptors. NMDA receptors possess a high conductance Ca^{2+} channels which in normal resting state have a magnesium block that prevents activation of the channel. However, high frequency activation of the AMPAr (due to arrival of a train of nerve impulses) causes a release of the magnesium block of the NMDA receptors producing an influx of calcium ions. AMPArs have a dual role in neuroprotection depending upon their spatiotemporal pattern of expression. Increased expression of GluR1 in neurons has been demonstrated to facilitate dendrite outgrowth and synaptogenesis, while increased expression of GluR2 preserves existing dendritic arbors (Prithiviraj et al., 2008). This dynamic receptor moves into and out of the plasma membrane, and when the AMPAr complex is comprised of both the GluR1 and GluR2 subunits, it is correlated with synaptogenesis and long term potentiation (LTP) (Malinow and Malenka, 2002).

Under pathological conditions, when neurons are under stress, AMPAr is also implicated in cell death by excitotoxicity through the increase permeability of AMPAr to calcium ions by the endocytosis of GluR2 (Beattie et al., 2010). During elevation of IOP in a rodent model, GluR2 expression in the dying RGCs demonstrated a decreased level of expression, implying that this decrease level of GluR2 expression made the RGCs more susceptible to calcium overload through the increased permeability of calcium through the AMPArs. Typically AMPArs are impervious to calcium ion influx when GluR2 is associated with the AMPAr complex on the plasma membrane. So how can increased AMPAr expression be implicated both as a neuroprotective, neurotrophic physiological cellular process, and also

associated with increased neuronal excitotoxicity when neurons are under pathological stress? The answer appears to be in the ability to maintain GluR2 expression in the AMPAr complex in order to impede calcium ion influx through AMPAr on the plasma membrane while maintaining the beneficial role that AMPAr (GluR1/GluR2) plays in promoting dendritic outgrowth and promoting LTP (Gainey et al., 2009). Kainate receptors also have a tetrameric structure comprising of a combination of GluR5, GluR6 and GluR7 subunits. The role of kainite receptors is not as well understood as the NMDA and AMPA receptors (Figure 10).
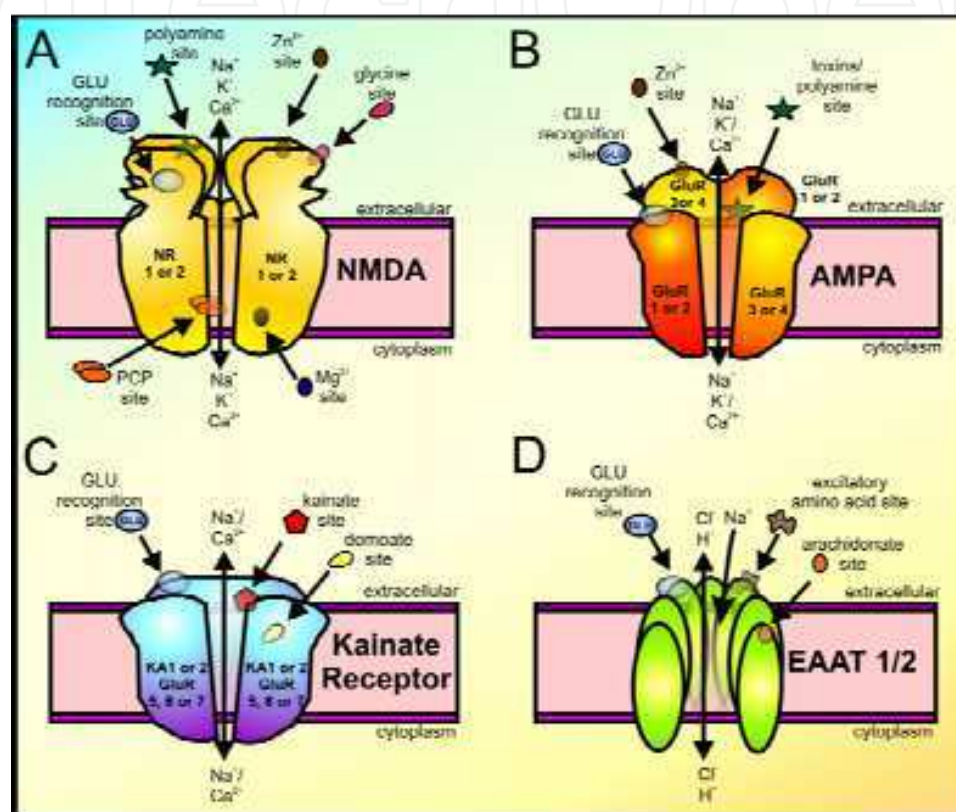

Fig. 10. Classes of glutamate receptors and transporters. Glutamate acts by binding to three main classes of receptors including NMDA (A), AMPA (B) and Kainate receptors (C) which are ligand-gated ion channels. All the glutamate receptors are permeable to sodium, and calcium ions. NMDA receptor is a tetramer comprising of two subunits of NR1 and NR2. AMPA receptor contains four different types of subunits called GluR 1 to GluR4. Kainate receptors are also tetrameric in structure and their subunits are named KA1 and KA2 and GluR5 to GluR7. EAAT is a excitatory glutamate transporter which is a high affinity sodium dependent membrane-bound carrier protein having a ion channel-like structure

While glutamate is the primary neurotransmitter in the vertical pathway of the retina (from photoreceptor to bipolar cells to ganglion cells), abnormal increase in glutamate has been thought contribute to retinal injury. One of the earliest observations describing glutamate excitotoxicity was made by Hayashi in 1954 who observed seizure activities in brain after administration of glutamate to neonatal mice. Subsequently, Lucas and Newhouse (1957) reported that postnatal mice fed with monosodium glutamate show loss of inner retinal neurons. These observations were the basis of "glutamate excitotoxicity" hypothesis in glaucoma. Excitotoxicity has also been shown to occur in clinical conditions including

traumatic brain injury, stoke, epilepsy, and also in various neurodegenerative diseases including Huntington disease, amyotrophic lateral sclerosis, AIDS dementia. Elevation of intracellular calcium is thought to be the primary mechanism leading to excitotoxicity by glutamate. The intracellular calcium levels are maintained at low levels (typically 100 nM in most cell types), while extracellular calcium concentrations are much higher (1 to 2 mM). NMDA receptors activation cause calcium influx into cells which produces membrane depolarization and this activates voltage-dependent calcium channels. Opening of these channels amplifies the calcium response, thereby exacerbating calcium overload in the cells. Calcium levels homeostasis maintained by a variety of calcium pumps and antiporters in the plasma membrane, endoplasmic reticulum and mitochondria. Ca^{2+}-ATPases use energy inherent in ATP hydrolysis to pump calcium ions across the plasma membrane by active transport. These pumps have high affinity but low capacity, pumping out one Ca^{2+} ion for each molecule of ATP hydrolyzed. Sodium calcium exchangers act as antiporters use the sodium gradient across the plasma membrane to exchange calcium ions for sodium ions. However under conditions of ischemia, due to poor energy (ATP) production, the sodium gradients across cell membranes are decreased thereby affect the ability antiporters to extrude Ca^{2+} ions (Figure 11). All these factors contribute to elevation of cytosolic calcium. Increase in cytosolic calcium activates a variety of signaling molecules that influence the fate of cell survival. For example calcium binds to calmodulin and activate CaM kinase II which phosphorylates a variety of substrates, various enzymes and transcription factors. Calcium activates calpains which degrades essential cellular proteins. Calcium also activates endonucleases and promotes cleavage of genomic DNA.

One of the key targets of elevated intracellular calcium is the mitochondria, which undergoes changes that could lead to opening of the permeability transition pore (Nickells, 2004). The mitochondrial calcium antiporter is a low affinity high capacity calcium transporter that pumps calcium ions into the mitochondria upon elevation of cytoplasmic calcium. Ca^{2+} build up in the mitochondria produces mitochondrial swelling by dissipating the voltage gradient between the outer and inner mitochondrial membrane. The inner mitochondrial membrane is flexible and thrown into multiple folds called cristae, and is therefore able to withstand the increase in volume. However the outer mitochondria membrane is unable to accommodate the increase in volume and ruptures, leading to release of cytochrome c from the mitochondria. The released cytochrome c forms a complex with apoptotic protein activating factor-1 (APAF-1) and caspase 9 to form the apoptosome which in turns activates the caspases which cleave a variety of structural and regulatory protein thereby leading to apoptotic death of cells.

Excitatory amino acid transporters (EAATs): After glutamate is released into the synaptic cleft, it is not degraded since there are no extracellular enzymes for its degradation. Glutamatergic transmission is terminated by rapid uptake of glutamate by a variety of specific glutamate transporters which are called excitatory amino acid transporters (EAATs) (Beretta et al., 2003). There are several classes of EAATs including EAAT1 (GLAST), EAAT2 (GLT), EAAT3 (EAAC), EAAT4 and EAAT5 (expressed mainly in the retina) each of which is encoded by distinct genes (Beretta et al., 2003). EAAT1 expression has been observed mainly in astrocytes throughout the CNS. EAAT1 is a plasma membrane bound protein and rarely detected in the cytoplasm. EAAT2 is also a plasma membrane protein expressed in astrocytes in various cerebral areas. EAAT3 is a major excitatory amino acid transporter in the neurons and highly expressed in hippocampus, cerebellum, and basal ganglia. EAAT4 expressed has been observed both in the cytoplasm and plasma membrane. EAAT4 is
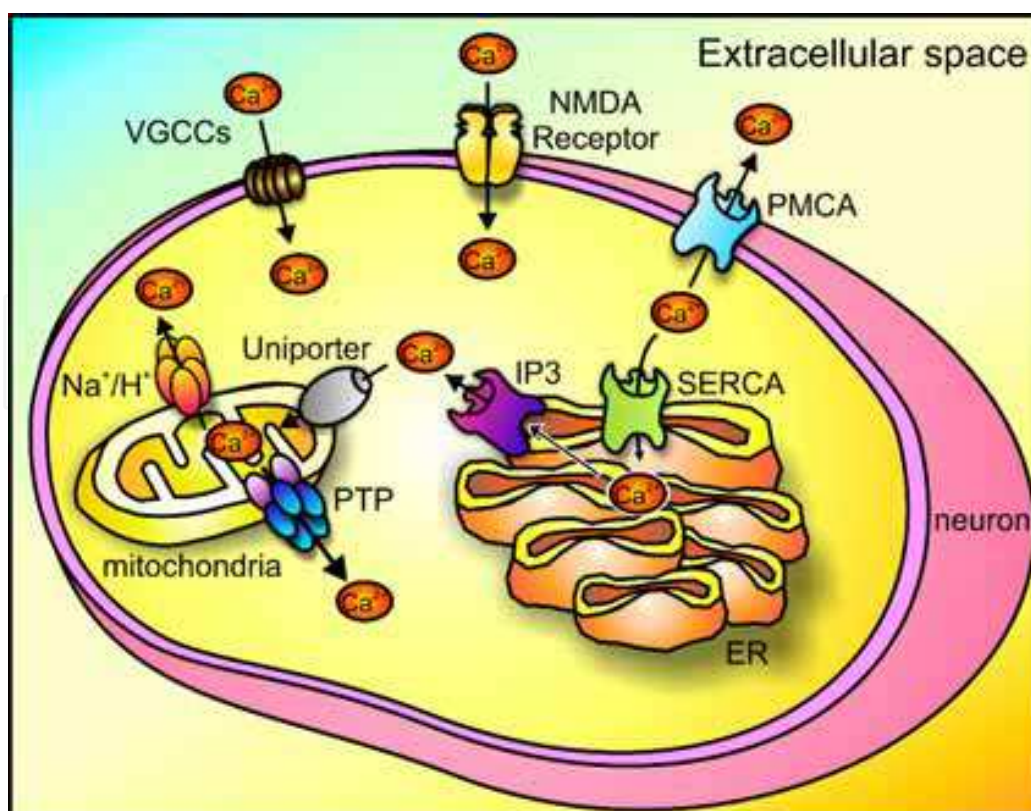
Fig. 11. Calcium homeostasis in cells. Intracellular calcium is elevated mainly by ionotropic receptors (for example, glutamate receptors), which in turn could activate voltage-sensitive calcium channels and store-operated channels. Elevated intracellular Ca^{2+} can produce a variety of cellular changes by binding to calmodulin and activating various signaling pathways or can be sequestered into the endoplasmic reticulum or mitochondria. Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps promote uptake of Ca^{2+} into the endoplasmic reticulum. On the other hand, activation of inositol-1,4,5-trisphosphate (IP_3) receptors in the endoplasmic reticulum or sarcoplasmic reticulum promote release of Ca^{2+} from these intracellular stores . The resting calcium is maintained at 100 nM by both uptake into the ER and Ca^{2+} release from cells by the action of plasma-membrane Ca^{2+}-ATPase (PMCA). The mitochondria has a uniporter that takes up Ca^{2+} from the cytosol, however it can release it by reversal of the uniporter. Other mechanisms contributing to release of Ca^{2+} from the mitochondria include Na^+/H^+-dependent Ca^{2+} exchange, or opening of the permeability transition pore (PTP). Ca^{2+} efflux from cells occurs mainly by the PMCA, which binds calmodulin and has a high affinity for Ca^{2+}

localized in the plasma membrane mainly in Purkinje cells. Expression of EAATs is decreased by conditions of transient global ischemia. Treatment with different cytokines including tumor necrosis factor-α (TNF-α), interferon-γ and interleukin-1β have been shown to inhibit glutamate uptake. TNF-α appears mediate this effect by decreasing GLAST and GLT expression in astrocytes. TNF-α levels have been shown to be elevated in glaucoma primarily in glial cells (Nakagawa et al., 2006; Tezel, 2008) and this could be one factor that contributing to glutamate elevation during glaucomatous insults. These observations are significant in the light of recent studies which suggest that glial activation is an early and key event in glaucoma disease progression (Nickells, 2007).
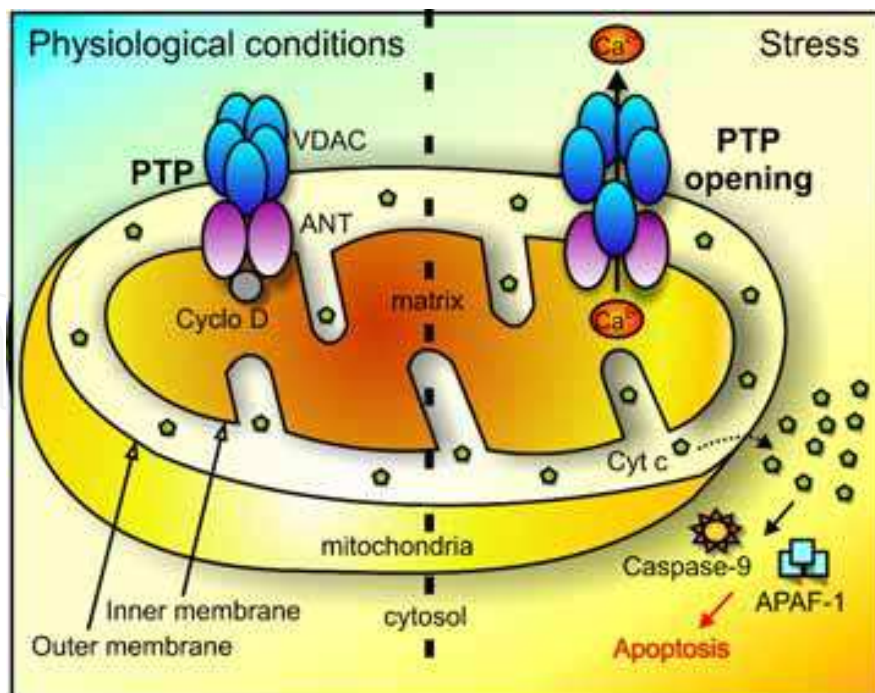
Fig. 12. Ca²⁺ and Mitochondrial Permeability Transition Pore. One of the consequences of elevation of intracellular calcium is the opening of permeability transition pore, which is a conductance pore comprising of a complex of adenine nucleotide translocator (ANT) (in the inner mitochondrial membrane) and voltage-dependent anion channel (in the outer membrane). Under conditions of cellular stress, the complex forms an open pore at the junction of the outer and inner mitochondrial membrane. Opening of the opens dissipates the ionic gradient across the mitochondrial membrane, produces mitochondrial swelling and release of Ca²⁺ from the mitochondria. Cytochrome c (Cyt c) is another factor that is released from the mitochondria due to opening of the PTP and rupture of the outer mitochondrial membrane. The released cytochrome c forms a complex with procaspase 9 and apoptotic protein activating factor-1 (APAF-1) to activate the caspase cascade and promote apoptosis

4.1 Glutamate elevation in glaucoma

Excitotoxicity due to elevation of glutamate concentrations is an attractive hypothesis to account for retinal ganglion cell death in glaucoma. However, there is no consistent evidence for increased glutamate in animal models of glaucoma. Dreyer et al (1996) found a 2-fold increase in glutamate levels in human glaucoma patients compared to control subjects. The study also found 6-8 fold higher levels of vitreal glutamate in monkeys with experimentally-induced ocular hypertension. Another study found 5-fold higher levels of glutamate in dogs with primary glaucoma, compared with normal dogs. However two subsequent studies carried out in primates found no significant increase in vitreal glutamate in IOP elevated monkey eyes, compared to those in control eyes (Carter-Dawson et al., 2002; Wamsley et al., 2005). It is possible that the confounding factors such as increased activity of EAATs in glial cells that could be masking the increased glutamate concentrations that may occur in a narrow time window following ocular hypertension. Another significant omission in many of these studies is assessment of glutamate levels in the retina, since glial cells in the retina including Muller cells and astrocytes could be

paracrine sources of glutamate that contribute to excitotoxiciy. A more detailed analysis of various retinal sources of glutamate is necessary for a comprehensive assessment of glutamate elevation in glaucoma.

4.2 Excitotoxic cell death of retinal ganglion cells

Excitotoxicity is a recurring theme in the pathogenesis of retinal ganglion cell death, however the detailed cellular and biochemical mechanisms have eluded understanding for the past five decades. There are several unanswered questions about the relevance of excitotoxicity in glaucoma. The basic premise of elevation of glutamate needs further evaluation in both animal models of ocular hypertension and in human patients. If an increase in glutamate concentrations does occur in glaucoma, it is unclear if it is secondary to ischemia/hypoxia at the optic nerve head. Treatment with MK801 an NMDA glutamate receptor antagonist prevented apoptotic cell death of RGCs in ischemic rat retina after 12 h after insult (Ju et al., 2008). If an increase in glutamate occurs independent of ischemia, the underlying mechanisms for glutamate efflux from glial cells are not yet clear. The major line of evidence for excitotoxic cell death of retinal ganglion cells was from animal studies in which injection of nanomolar amounts of NMDA produce a loss of inner retinal neurons, particularly the retinal ganglion cells and inner plexiform layers. For instance, studies (Silliprandi et al., 1992) have shown that a single intravitreal injection of 20 nmole of NMDA resulted in 70% loss of cells with a soma diameter of greater than 8 microns in the ganglion cell layer, which were presumed to be retinal ganglion cells. Similar observations were made by Manabe and Lipton (2002) who demonstrated 80% retinal ganglion cell death after intravitreal injection of 20 nmole of NMDA in rats. Treatment with MK801, an uncompetitive NMDA antagonist was shown to block NMDA-mediated loss of retinal ganglion cells. A more detailed analysis of retinal ganglion cell death by excitotoxicity emerged from in vitro studies using primary cultures of retinal ganglion cells. Using chick retinal cells, Ferreira et al. demonstrated that under sodium-free conditions (to block activation of VGCC), calcium entry through NMDA receptors was sufficient to produce cell death. Hahn et al. (1988) showed that the extracellular Ca^{2+} concentration, plays a key role in glutamate-induced cell death in retinal ganglion cells. Under these conditions, both Mg^{2+} and the amino acid antagonist MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo-(alpha,gamma)-cyclohepten-5, 10-imine maleate], blockers of N-methyl-D-aspartate receptor-coupled ion channels, completely blocked cell death induced by glutamate. The study suggests that Ca^{2+} entry through N-methyl-D-aspartate-activated channels is responsible for retinal ganglion cell death. Another NMDA antagonist, eliprodil (1 µM) was also found be very effective in protecting cultured retinal ganglion cells from NMDA-mediated cell death (Pang et al., 1999). Activation of p38 MAP kinase appears to mediate some of the neurotoxic effects of NMDA since treatment with SB203580 (a specific inhibitor of p38 MAP kinase) is effective in protecting against NMDA-mediated cell death (Manabe and Lipton, 2003). Most of the studies that demonstrate an acute effect of NMDA in producing neurodegeneration in the inner retina, may have limited relevance to primary open angle glaucoma which is a chronic neurodegenerative disease, in which clinical manifestation occurs over a period of several years. Perhaps, it would be worthwhile to study the involvement of metabotropic glutamate receptors in neurodegeneration in glaucoma. One study reported changes in several metabotropic glutamate receptors, particularly mGluR2 which was increased nearly 10-fold after ocular hypertension in DBA/2J mice (Dyka et al., 2004). Additional studies are needed

to analyze the significance of changes in metabotropic glutamate receptors in glaucoma and their role in neurodegeneration.

Despite numerous observations about excitotoxic cell death of retinal ganglion cells and loss of inner plexiform layer, one study reported no effect of NMDA on retinal ganglion cells (Ullian et al., 2004). The study found that NMDA treatment killed mainly the amacrine cells, while retinal ganglion cells were unaffected. This study was unable to reproduce the NMDA-mediated cell death of cultured primary retinal ganglion cells that was observed in many of the previous studies. There are many possibilities for this apparent discrepancy between various research findings about excitotoxicity in retinal ganglion cells. RGCs are highly dependent on trophic factor-mediated signaling for their cell survival. Many of the earlier studies employed NMDA treatment in cell culture conditions that did not have the optimum levels of neurotrophins and trophic factors which could account for differences in the findings. Depending upon the concentration of neurotrophins in the culture medium of retinal ganglion cells, there could be variation in viability of the cells, which could account for variations in the data for excitotoxicity. For instance BDNF has been shown to inhibit the activation of Bim, a pro-apoptotic factor that is important for promoting apoptosis of trophic-factor deprived RGCs. Thus the experimental conditions of primary culture could greatly influence the outcome of experiments when excitotoxicity of NMDA is assessed in primary cultures of retinal ganglion cells. The localization of NMDA receptors is another factor contributing to susceptibility to excitotoxicity. Chen and Diamond (2002) report that NMDA receptors are localized predominantly in the extrasynaptic region suggesting that NMDA receptors are less likely to be overactivated by synaptic events. It is unclear how this distribution of receptors would contribute to excitotoxicity of retinal ganglion cells.

Cell culture experiments with glutamate are often problematic due to poor solubility of L-glutamic acid and high concentrations of glutamate tend to generate artifacts due to acidification of the cell culture medium. Some investigators use monosodium glutamate to circumvent this problem, but it is unclear if this has different binding properties to glutamate receptors. It would be interesting to analyze glutamate levels in a chronic animal model of ocular hypertension and determine if NMDA antagonist would be effective as neuroprotective agents in these experimental models of glaucoma.

Since NMDA receptor activity, is important for synaptic plasticity and normal neuron physiology, many NMDA antagonist that block all NMDA activity have undesirable side effects. In contrast, studies by Stuart Lipton's group demonstrated that memantine, an adamantane derivative, preferentially blocks excessive NMDA receptor activity without disrupting normal activity (Lipton, 2004). Memantine acts as an open-channel blocker by preferential binding when there is overstimulation of the receptor and does not appreciably bind to the receptor channel and interfere with normal channel activity and neurotransmission. Memantine was found to be well tolerated in patients and has been approved by FDA for treatment of dementia in Alzheimer's disease patients. Studies with the uncompetitive NMDA antagonist, memantine, met with a degree of success in ocular hypertensive rats. However phase III clinical trials with memantine were largely unsuccessful and patients showed no improvement in visual field. This study raises several issues about the contribution of NMDA receptor activation in neurodegeneration in glaucoma patients. The involvement of glutamate excitotoxicity in retinal ganglion cell death needs further investigation and further studies are necessary to understand its relevance to neurodegeneration in glaucoma.

5. Alpha-2-adrenergic agonists

Alpha-2 adrenergic agonists including clonidine and brimonidine have been known to reduce intraocular pressure, however clonidine produces a number of undesirable cardiovascular side effects. Brimonidine has a structure similar to clonidine, and is effective loweing IOP both in normotensive and ocular hypertensive primates (Burke and Potter, 1986). The mechanism underlying its ocular hypotensive effects are possibly related to reducing aqueous humor formation, since alpha-2-adrenergic receptors have been shown to be expressed in the iris and ciliary body. In recent years, studies have shown that in addition to lowering IOP, brimonidine has neuroprotective effects in animal models of glaucoma (Wheeler et al., 2003; Ahmed et al., 2001). For instance, in a rat model of elevated IOP, brimonidine administration initiated 10 days after IOP elevation prevented any further loss of ganglion cells. In vehicle- or timolol-treated rats, ganglion cell loss continued to occur (Woldmussie et al., 2001). In another glaucoma model, seven days after inducing transient ischaemia, there was loss of approximately half of the RGC population. Topical pre-treatment with 0.1% or 0.5% brimonidine attenuated ischaemia-induced RGC death. Brimonidine appers to preserve RGC survival and protect against a variety of glaucomatous insults. For instance, Lee et al. (2010) showed that brimonidine increased survival of rat RGCs in the presence of glutamate neurotoxicity, oxidative stress, and hypoxia.

6. Other emerging targets for neuroprotection

6.1 Endothelins

Endothelin-1 (ET-1) is a potent vasoactive peptide (Yanagisawa et al., 1988), has gained attention for its neurodegenerative role in glaucoma (Yorio et al., 2002). However the precise mechanisms underlying ET-1 mediated neurodegeneration are not completely understood. ET-1 levels are elevated in the aqueous humor of primary open angle glaucoma patients (Noske et al., 1997; Tezel et al., 1997) and in animal models of glaucoma (Kallberg et al., 2002; Thanos and Naskar 2004; and Prasanna et al., 2005). The clinical relevance of these findings is not completely clear but it appears plausible that ET-1 in aqueous humor could be secreted in response to an increase in IOP. There is considerable evidence to suggest that ET-1 could produce optic nerve damage similar to that seen in glaucoma. For instance, continuous peribulbar administration of ET-1 in primates produces optic nerve damage, similar to that seen in glaucoma (Orgul et al., 1996; Cioffi and Sullivan, 1999). Lau et al. (2005) have shown that a single ET-1 injection into rat eyes promoted loss of retinal ganglion cells, accompanied by increased GFAP labeling in the Muller cells end feet and astrocytic cell layer in retina and optic nerve. Chauhan et al. (2004) demonstrated that chronic administration of low doses ET-1 that do not appreciably alter blood flow in the retrobulbar region of the optic nerve, also results in a progressive loss of retinal ganglion cells and their axons without changes in optic disc morphology.

Since all known actions of ET-1 occur through binding to its receptors, it is important to understand which receptors may be mediating its damaging effects in glaucomatous optic neuropathy. Endothelin-1 acts mainly through two classes of receptors, the ETA and ETB receptors (Rubanyi and Polokoff, 1994). Both ETA and ETB belong to the rhodopsin superfamily of G-protein coupled receptors. Recently studies suggest that the endothelin B (ETB) receptor may be involved in mediating ET-1's neurodegenerative effects. For instance, Wang et al. (2006) showed increased immunohistochemical staining for ETB receptors in

human glaucomatous optic nerves as compared with age-matched controls Rogers et al. (1997) demonstrated an increase in ETB expression in glial cells in the optic nerve, after optic nerve transection. ET-1 acts through its receptors to promote proliferation and activation of optic nerve head astrocytes, which are key steps in the progression of glaucoma (Prasanna et al., 2002). Yang et al. (2007) showed that ETB mRNA expression in the retina increased in a rodent elevated IOP model of glaucoma, from as early as 1 day and persisted up to 8 weeks of IOP elevation. It therefore appears that endothelin receptor activation could be an early event in the etiology of glaucoma. Krishnamoorthy et al. (2008) showed that ETB receptor expression is increased in RGCs after intravitreal injection of ET-1 and this was accompanied by apoptosis of RGCs. ET-1 mediated apoptosis was attenuated in ETB receptor-deficient rats (Krishnamoorthy et al., 2008). Recent studies from Simon John's lab showed that blocking endothelin receptors using bosentan (antagonist for both ETA and ETB receptors) is neuroprotective in a mouse ocular hypertension model of glaucoma (Howell et al., 2011). It is becoming increasingly evident from several corroborative observations that the endothelin system may play a pivotal role in neurodegeneration in glaucoma. Together these studies suggest the possibility of using endothelin antagonists as potential neuroprotective agents for glaucoma treatment.

6.2 Sigma-1 receptor

Sigma-1 receptors have emerged as a promising new neuroprotective candidate to protect RGCs from many of the noxious factors that are associated with the disease process of glaucoma (Dun et al., 2007). Sigma-1 receptors are a 26 kD transmembrane protein found on the endoplasmic reticulum (ER) that translocate from the ER to interact with ionotropic channels located on the plasma membrane (Hayahsi and Su 2007). This molecular signaling pathway enables the ubiquitously expressed sigma-1 receptor to associate and regulate many ligand gated ion channels that are found on the plasma membrane of many different cell types throughout the body (Hayashi and Su 2007; Zhang and Cuevas 2002). Sigma-1 receptors have been shown to be expressed in the retina, with the predominating level of expression in the retinal ganglion cell layer (Liu et al., 2010). There are several factors that contribute to neuroprotective ability of sigma receptors. Sigma-1 receptor's ability to bind and associate with L-type voltage gated calcium channels, regulate neuronal intracellular calcium concentrations when exposed to ischemic conditions, prevent glutamate excitotoxicity of RGCs when exposed to sigma-1 agonist (+)-pentazocine are some of the key mechanisms underlying sigma-1's neuroprotective effects (Tchedre et al., 2008; Katnik at al., 2006; Dun et al., 2007). Other pathways affected by sigma-1 receptor include attenuation of intrinsic death signal of glutamate-exposed retinal cell lines, which is a likely candidate to maintain the viability, and homeostatic intracellular calcium regulation of RGCs when they are exposed to a number of noxious stimuli that cause RGC apoptosis (Tchedre and Yorio 2008). Lastly, sigma-1 receptor stimulation has also demonstrated *in vivo* protection against retinal degeneration in a diabetic retinopathy animal model (Smith et al., 2008). More pre-clinical research needs to be preformed to demonstrate the neuroprotective efficacy of sigma receptors in glaucomatous animal models.

6.3 Tumor necrosis factor-α

Another molecule that has emerged as a key contributor to glaucoma progression is the cytokine, tumor necrosis factor-α (TNF-α) (Tezel, 2008). In the anterior chamber of the eye,

TNF-α could have beneficial effects by promoting outflow through conventional and uveoscleral pathways (Husain et al., 2008). However, TNF-α appears to produce damaging effects in the posterior segment of the eye. Several publications point to the involvement of TNF-α in glaucomatous neurodegeneration. Yang et al. (2007) showed an increase in TNF-α receptor 1A expression in an ocular hypertension model of glaucoma in rodents. Increased labeling for TNF-α and its receptor was observed in glaucomatous eyes compared to controls (Tezel et al., 2001). In the same study, double immunofluorescence suggested TNF-α labeling mainly in glial cells, while TNF-α receptors were found mainly on RGCs. In a mouse model of ocular hypertension, Nakazawa et al. (2008) found increased concentrations of TNF-α in the retina, which was followed by microglial activation and loss of RGCs. The authors found that RGC loss was greatly attenuated in TNF-receptor 2-deficient mice, suggesting that TNF-α acts through TNF receptor-2 to produce neurodegeneration in glaucoma (Nakazawa et al., 2006). TNF-α promotes ET-1 release from ciliary non-pigmented ciliary epithelial cells (Prasanna et al., 1998) as well as retinal pigmented epithelium (Narayan et al., 2003), suggesting that some of TNF-α's effects could be due to the downstream effects of endothelins. The role of TNF-α in glaucomatous degeneration is an evolving area of research with important implications for further research.

6.4 CD44

CD44 is a transmembrane glycoprotein which acts as a receptor for hyaluronic acid. Recent work by several groups suggests that soluble CD44 is significantly increased in aqueous humor of primary open angle glaucoma patients (Choi et al., 2005; Budak et al., 2009; Mokbel et al., 2010). The 32kDa ectodomain fragment of soluble CD44 was found have toxic effects both in the trabecular meshwork and ganglion cells *in vitro*. The cytotoxicity of CD44 was blocked by administration of anti-CD44 antibody in vitro (Choi et al., 2005). CD44 was also found to be increased in other models of neurodegeneration such as RDS mice which is characterized by inherited retinal degeneration (Krishnamoorthy et al., 2000). More work is needed in this developing area of research to fully understand the contribution of CD44 to glaucomatous pathophysiology.

7. Concluding remarks

While the present treatment modalities for glaucoma are effective in terms of lowering intraocular pressure and slowing down neurodegeneration, there are issues with long term application of medications particularly their side effects due to prolonged use. Besides, neurodegenerative effects continue to occur in some patients despite lowering IOP. It is clear that there are numerous targets that could be exploited for developing a neuroprotective agent to reduce axon loss and apoptosis of RGCs. However, it would be imperative to develop stringent measures to assess the efficacy and potency of various test compounds in animal models of glaucoma. After careful analysis using a battery of tests and cellular and molecular assays for neuroprotection, an efficacious candidate drug could make significant progress in clinical trials, so that there would be a good chance for success with neuroprotection in humans. This would involve a concerted effort among basic scientists, clinicians and pharmacologists and should result in the development of the first generation of neuroprotective treatments for glaucoma.

8. Acknowledgements

9. References

Adachi,K.,Kashii,S.,Masai,H.,Ueda,M.,Morizane,C.,Kaneda,K.,Kume,T.,Akaike,A., Honda,Y. (1998).Mechanism of the pathogenesis of glutamate neurotoxicity in retinal ischemia. *Graefes Arch. Clin. Exp. Ophthalmol.* Vol. 236, pp. 766-774.

Allergan Inc, Press Releases on Memantine Trials, Fourth Quarter Operating Results, Jan 30 2008 http://www.allergan.com/newsroom/index.htm

Anderson, M.G., Smith, R.S., Hawes, N.L., Zabaleta, A., Chang, B., Wiggs, J.L. & John SW. (2002). Mutations in genes encoding melanosomal proteins cause pigmentary glaucoma in DBA/2J mice. *Nat Genet.* 30: 81-5.

Araie, M., and Mayama, C. (2010) Use of calcium channel blockers for glaucoma, Progress in Retinal and Eye Research, 2010, 1-18

Araie, M., Azuma, I., Kitazawa, Y., 2003. Influence of topical betaxolol and timolol on visual field in Japanese open-angle glaucoma patients. Jpn. J. Ophthalmol. Vol. 47, pp. 199-207.

Arévalo, J.C., Yano, H., Teng, K.K. & Chao, M.V. (2004). A unique pathway for sustained neurotrophin signaling through an ankyrin-rich membrane-spanning protein. *EMBO J* 23:2358-68.

Badea T.C., and Nathans J. (2004). Quantitative analysis of neuronal morphologies in the mouse retina visualized by using a genetically directed reporter. J Comp Neurol 480:. 331–351

Bähr, M. (2000). Live or let die - retinal ganglion cell death and survival during development and in the lesioned adult CNS. *Trends Neurosci.* 23:483-90.

Bahrami H. (2006) Causal models in primary open angle glaucoma. *Ophthalmic Epidemiol.* 4:291-298.

Bai, Y., Dergham, P., Nedev, H., Xu, J., Galan, A., Rivera, J.C., ZhiHua, S., Mehta, H.M., Woo, S.B., Sarunic, M.V., Neet, K.E. & Saragovi, H.U. (2010). Chronic and acute models of retinal neurodegeneration TrkA activity are neuroprotective whereas p75NTR activity is neurotoxic through a paracrine mechanism. *J Biol Chem.* 285:39392-39400

Bainbridge, J.W., Smith, A.J., Barker, S.S., Robbie, S., Henderson, R., Balaggan, K., Viswanathan, A., Holder, G.E., Stockman, A., Tyler, N., Petersen-Jones, S., Bhattacharya, S.S., Thrasher,A.J., Fitzke, F.W., Carter, B.J., Rubin, G.S., Moore, A.T. & Ali, R.R. (2008) Effect of gene therapy on visual function in Leber's congenital amaurosis. *N Engl JMed* 358:2231–2239.

Banin, E., Obolensky, A., Idelson, M., Hemo, I., Reinhardtz, E., Pikarsky, E., Ben-Hur, T. & Reubinoff, B. (2006). Retinal incorporation and differentiation of neural precursors derived from human embryonic stem cells. *Stem Cells.* 24:246-57.

Barbacid, M. (1995). Neurotrophic factors and their receptors. *Curr Opin Cell Biol.* 7(2):148-55.

Bartsch, U., Oriyakhel, W., Kenna, P.F., Linke, S., Richard, G., Petrowitz, B., Humphries, P., Farrar, G.J. & Ader, M. (2008). Retinal cells integrate into the outer nuclear layer

and differentiate into mature photoreceptors after subretinal transplantation into adult mice. *Exp Eye Res.* 86(4):691-700

Beattie, M.S., Ferguson, A.R. & Bresnahan, J.C. (2010) AMPA-receptor trafficking and injury-induced cell death. *Eur JNeurosci.* 32:290-297.

Bebarta, V., Luyten, D. & Heard K. (2003) Emergency medicine animal research: does use of randomization and blinding affect the results?, *Acad Emerg Med.* 10: 684–687

Benowitz, L.I., Shashoua, V.E. & Yoon, M.G. (1981) Specific changes in rapidly transported protein during regeneration of the goldfish optic nerve. *JNeurosci.* 1: 300-7.

Bensimon, G., Lacomblez, L. & Meininger, V. (1994) A controlled trial of riluzole in amyotrophic lateral sclerosis: ALS/Riluzole Study Group, N Engl J Med 330: 585-591.

Beretta, S., Begni, B. & Ferrarese C (2003) Pharmacological manipulation of glutamate transport *Drug News Perspect.* 16:435-445.

Berkemeier, L.R., Ozçelik, T., Francke, U. &Rosenthal, A. (1992). Human chromosome 19 contains the neurotrophin-5 gene locus and three related genes that may encode novel acidic neurotrophins. *Somat Cell Mol Genet.* 18:233-245.

Bertrand, J., Di Polo, A. & McKerracher, L. (2007). Enhanced survival and regeneration of axotomized retinal neurons by repeated delivery of cell-permeable C3-like Rho antagonists. *Neurobiol Dis.* 25(1):65-72

Bessho, H., Suzuki, J., and Tobe, A., (1991). Vascular effects of betaxolol, a cardioselective b-adrenoceptor antagonist, in isolated rat arteries. Jpn. J. Pharmacol. 55: 351-358.

Boado, RJ., Hui, EK., Lu, JZ., Pardridge, WM. (2010). Drug targeting of erythropoietin across the primate blood-brain barrier with an IgG molecular Trojan horse. *J Pharmacol Exp Ther.* 333:961-969.

Boehm, A.G., Breidenbach, K.A., Pillunat, L.E., Bernd, A.S., Mueller, M.F., Koeller, A.U., (2003). Visual function and perfusion of the optic nerve head after application of centrally acting calcium-channel blockers. *Graefes Arch. Clin. Exp. Ophthalmol.* 241: 34-38.

Bose, S., Piltz, J.R., Breton, M.E., (1995). Nimodipine, a centrally active calcium antagonist, exerts a beneficial effect on contrast sensitivity in patients with normal-tension glaucoma and in control subjects. *Ophthalmology* 102: 1236-1241.

Broadway, D.C., Drance, S.M., 1998. Glaucoma and vasospasm. *Br. J Ophthalmol.* Vol 82 pp. 862–870.

Budak YU, Akdogan M, Huysal K. Aqueous humor level of sCD44 in patients with degenerative myopia and primary open-angle glaucoma. (2009)BMC Res Notes. 2:224.

Buemi, M., Cavallaro, E., Floccari, F., Sturiale, A., Aloisi, C., Trimarchi, M., Grasso, G., Corica, F., Frisina, N. (2002). Erythropoietin and the brain: from neurodevelopment to neuroprotection. *Clin Sci (Lond).* 103:275-82.

Bull N.D., Irvine K.A., Franklin R.J.M, Martin K.R. (2009) Transplanted oligodendrocyte precursor cells reduce neurodegeneration in a model of glaucoma. *Invest Ophthalmol Vis Sci.* 50:4244–4253.

Calissano, P., Amadoro, G., Matrone, C., Ciafrè, S., Marolda, R., Corsetti, V., Ciotti, MT., Mercanti, D., Di Luzio, A., Severini, C., Provenzano, C., Canu, N. (2010). Does the term 'trophic' actually mean anti-amyloidogenic? The case of NGF. *Cell Death Differ.* 17:1126-1133.

Carter-Dawson, L., Crawford, M.L., Harwerth, R.S. & Smith, E.L. 3rd., Feldman, R., Shen, F.F., Mitchell, C.K. & Whitetree, A. (2002) Vitreal glutamate concentrations in monkey with experimental glaucoma. *Invest. Ophthalmol. Vis. Sci.* 43: 2633-2637

Chang, MS., Arevalo, JC., Chao, MV. (2004). Ternary complex with Trk, p75, and an ankyrin-rich membrane spanning protein. *JNeurosci Res.* 78:186-92

Chao, MV. & Hempstead, BL (1995). p75 and Trk: a two-receptor system. *Trends Neurosci.* 18:321-326

Chao, MV. (2003). Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nat Rev Neurosci.* 4:299-309.

Chauhan, B.C., LaVatte, T.L., Jollimore, C.A., Yu, P.K., Reitsamer, H.A., Kelly, M.E.M., Yu, D-Yi., Tremblay, F. & Archibald, MA. (2004) Model of endothelin-1-induced chronic optic neuropathy in rat. *Invest. Ophthalmol. Vis. Sci.* 45: 144-152.

Chen H-SV, Pellegrini JW, Aggarwal SW, Lei SZ, Warach S, Jensen FE and Lipton SA. (1992). Open-channel block of N-methyl-d-aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity. *J Neurosci* 12: 4427-4436.

Chen, H. & Weber, AJ. (2004). Brain-derived neurotrophic factor reduces TrkB protein and mRNA in the normal retina and following optic nerve crush in adult rats. *Brain Res.* 1011:99-106.

Chen, S. & Diamond, J.S. (2002) Synaptically released glutamate activates extrasynaptic NMDA receptors on cells in the ganglion cell layer of rat retina. J. Neurosci. 22: 2165-2173.

Chen, Y.N., Yamada, H., Mao, W., Uchida, S., Matsuyama, S., Aihara, M., and Araie, M.(2007). Hypoxia-induced retinal ganglion cell death and the neuroprotective effects of beta-adrenergic antagonists. Brain Res. 1148: 28-37.

Cheng, L., Sapieha, P., Kittlerova, P., Hauswirth, WW., Di Polo, A. (2002). TrkB gene transfer protects retinal ganglion cells from axotomy-induced death in vivo. *J Neurosci.* 22:3977-86

Cheon, E.W., Kim, Y.H., Cho, Y.Y., Kim, H.J., Kang, S.S., Cho, G.J., Yoo, J.M., Song, J.K., Choi, W.S., 2002. Betaxolol, a b1-adrenoceptor antagonist, protects a transient ischemic injury of the retina. Exp. Eye Res. 75: 591-601.

Cheon, E.W., Park, C.H., Kim, Y.S., Cho, C.H., Chung, Y.C., Kwon, J.G., Yoo, J.M., Choi, W.S., Cho, G.J., 2006. Protective effects of betaxolol in eyes with kainic acid-induced neuronal death. Brain Res. 1069: 75-85.

Chikar, JA., Colesa, DJ., Swiderski, DL., Di Polo, A., Raphael, Y., Pfingst, BE. (2008). Over-expression of BDNF by adenovirus with concurrent electrical stimulation improves cochlear implant thresholds and survival of auditory neurons. *Hear Res.* 245:24-34.

Choi J, Miller AM, Nolan MJ, Yue BY, Thotz ST, Clark AF, Agarwal N, Knepper PA. (2005) Soluble CD44 is cytotoxic to trabecular meshwork and retinal ganglion cells in vitro. Invest Ophthalmol Vis Sci. 46:214-222.

Cioffi, G.A. & Sullivan, P. (1999) The effect of chronic ischemia on the primate optic nerve. *Eur. J Ophthalmol.* 9: S34-S36

Clark, A.F. & Yorio, T. *Ophthalmic Drug Discovery. Nature Review Drug Discovery.* 2: 448-459

Clarke, PG., Posada, A., Primi, MP., Castagné, V. (1998). Neuronal death in the central nervous system during development. *Biomed Pharmacother.* 52:356-62.

Coassin, M., Lambiase, A., Sposato, V., Micera, A., Bonini, S., Aloe, L. (2008). Retinal p75 and bax overexpression is associated with retinal ganglion cells apoptosis in a rat model of glaucoma. *Graefes Arch Clin Exp Ophthalmol.* 246:1743-1749.

Colafrancesco, V., Parisi, V., Sposato, V., Ross,i S., Russo, MA., Coassin, M., Lambiase, A., Aloe, L. (2011). Ocular application of nerve growth factor protects degenerating retinal ganglion cells in a rat model of glaucoma. *JGlaucoma.* 20:100-108

Coombs J, Van Der List D, Wang G-Y, Chalupa LM. (2006). Morphological properties of mouse retinal ganglion cells. *Neuroscience* 140: 123–136.

Crosson, C.E., Willis, J.A., Potter, D.E. (1990). Effect of the calcium antagonist, nifedipine, on ischemic retinal dysfunction. J. Ocul. Pharmacol. 6: 293-299.

Danbolt, N.C.,2001.Glutamate uptake.*Prog.Neurobiol.* 65: 1-105.

Danesh-Meyer H.V. &Levin L. A. (2009) Neuroprotection: Extrapolating from Neurologic Diseases to the Eye. American Journal of Ophthalmology 148: 186-191.

Danesh-Meyer, H.V. (2011). Neuroprotection in glaucoma: recent and future directions. *Current Opinion in Ophthalmology* 22: 78-86

Di Polo, A., Aigner, LJ., Dunn RJ., Bray, GM., Aguayo, AJ. (1998). Prolonged delivery of brain-derived neurotrophic factor by adenovirus-infected Müller cells temporarily rescues injured retinal ganglion cells. *Proc Natl Acad Sci U S A.* 95:3978-3983.

Diebold, Y. & Calonge, M. (2010)Applications of nanoparticles in ophthalmology. Progress in Retinal and Eye Research: 29: 596-609

Dong, Y., Ishikawa, H., Wu, Y., Shimizu, K., Goseki, T., Yoshitomi, T. (2006). Effect and mechanism of betaxolol and timolol on vascular relaxation in isolated rabbit ciliary artery. Jpn. J. Ophthalmol. 50:504-508.

Dryer, E.B., Zurakowski, D., Schumer, R.A., Podos, S.M, & Lipton, S.A. (1996) Elevated glutamate levels in the vitreous body of humans and monkeys with glaucoma. *Arch. Ophthalmol.* 114: 299-305.

Dun Y, Thangaraju M, Prasad P, Ganapathy V, Smith SB. (2007) Prevention of excitotoxicity in primary retinal ganglion cells by (+)-pentazocine, a sigma receptor-1 specific ligand. *Invest Ophthamol Vis Sci.* Vol. 48:4785-4794.

Dyka, F.M., May, C.A. & Enz, R. (2004) Metabotropic glutamate receptors are differentially regulated under elevated intraocular pressure. *J Neurochem.* 90: 190-202.

Farahbakhsh, N.A., Cilluffo, M.C., Chronis, C., Fain, G.L., (1994). Dihydropyridinesensitive $Ca2þ$ currents in rabbit ciliary body epithelial cells. *Exp. Eye Res.* 58:197-205.

Fatma, N., Kubo, E., Sen, M., Agarwal, N., Thoreson, W.B., Camras, C.B. & Singh, D.P. (2008)Peroxiredoxin 6 delivery attenuates TNF-alpha-and glutamate-induced retinal ganglion cell death by limiting ROS levels and maintaining Ca^{2+} homeostasis. *Brain Res.*1233:63-78.

Ferreira, I.L., Duarte, C.B. & Carvalho, A.P. (1996) Ca^{2+} influx through glutamate receptor-associated channels in retina cells correlates with neuronal cell death. *Eur J Pharmacol.* 302(1-3):153-62.

Flammer J.(1994) The vascular concept of glaucoma. *Surv Ophthalmol.* Vol. 38 (May 1994) Suppl:S3-6. Review.

Flammer, J., Guthauser, U., (1987). Behandlung chorioidaler Vasospasmen mit Kalziumantagonisten. *Klin. Monatsbl. Augenheilkd.* 190:299–300.

Flammer, J., Guthauser, U., Mahler, F., 1987a. Do ocular vasospasms help cause low-tension glaucoma? *Doc. Opthalmol. Proc. Ser.* 49: 397–399.

Frank T., Schlachetzki J.C., Göricke B., Meuer K., Rohde G., Dietz G.P., Bähr M., Schneider A., Weishaupt J.H. (2009) Both systemic and local application of granulocyte-colony stimulating factor (G-CSF) is neuroprotective after retinal ganglion cell axotomy. *BMC Neurosci*. 10: 49-59.

Fraser S., Wormald R. Ophhthalmology (eds yanoff, M. & duker, J. S.). . 1999,12:1.1-12:1.6.

Fu, Q.L., Li, X., Yip, H.K., Shao, Z., Wu, W., Mi, S., So, K.F. (2009). Combined effect of brain-derived neurotrophic factor and LINGO-1 fusion protein on long-term survival of retinal ganglion cells in chronic glaucoma. *Neuroscience*. 162:375-82

Gainey, M.A., Hurvitz-Wolff, J.R., Lambo, M.E. & Turrigiano GG. (2009) Synaptic scaling requires the GluR2 subunit of the AMPA receptor. *Journal of Neuroscience*. 29:6479-6489.

Gasser, P., and Flammer, J. (1991). Blood-cell velocity in the nailfold capillaries of patients with normal-tension and high-tension glaucoma. *Am. J Ophthalmol*. 111:585–588.

Ghate, D. and Edelhauser, H.F. (2008) Barriers to Glaucoma Drug Delivery. *J Glaucoma* 17:147-156

Goll DE, Thompson VF, Li H, Wei W, Cong J. (2003) The calpain system. *Physiol Rev*. Vol. 83: 731-801. Review

Graef IA, Mermelstein PG, Stankunas K, Neilson JR, Deisseroth K, Tsien RW, Crabtree GR. (1999) L-type calcium channels and GSK-3 regulate the activity of NF-ATc4 in hippocampal neurons. *Nature*. 401: 703-708.

Greene, L.A. & Kaplan, D.R. (1995). Early events in neurotrophin signalling via Trk and p75 receptors. *Curr Opin Neurobiol*. 5:579-87.

Grozdanic, S.D., Ast, A.M., Lazic, T., Kwon, Y.H., Kardon, R.H., Sonea, I.M., Sakaguchi, D.S. (2006). Morphological integration and functional assessment of transplanted neural progenitor cells in healthy and acute ischemic rat eyes. *Exp Eye Res*. 82:597-607.

Gupta N., Ang L.C., Noel de T.L., Bidaisee L., Yu¨ cel Y.H.(2006) Human glaucoma and neural degeneration in intracranial optic nerve, lateral geniculate nucleus, and visual cortex. *Br JOphthalmol*. 90: 674–678.

Guthauser, U., Flammer, J., Mahler, F. (1988). The relationship between digital and ocular vasospasm. *Graefes Arch. Clin. Exp.Ophthalmol*. 226: 224–226.

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. *Proc. Natl. Acad. Sci*. 85:6556-60.

Hamanoue, M., Middleton, G., Wyatt, S., Jaffray, E., Hay R.T., Davies, AM. (1999) p75-mediated NF-kappaB activation enhances the survival response of developing sensory neurons to nerve growth factor. *Mol Cell Neurosci*. 14:28-40

Hardingham, G. E. & Bading, H. (2002b) Coupling of extrasynaptic NMDA receptors to a CREB shut-off pathway is developmentally regulated. *Biochim. Biophys. Acta* 1600: 148–153.

Hardingham, G. E., Arnold, F. J. & Bading, H. (2001a) Nuclear calcium signaling controls CREB-mediated gene expression triggered by synaptic activity. *Nature Neurosci*. 4, 261–267.

Hardingham, G. E., Arnold, F. J. & Bading, H. (2001b)A calcium microdomain near NMDA receptors: on switch for ERK-dependent synapse-to-nucleus communication. *Nature Neurosci*. 4: 565–566.

Hardingham, G. E., Fukunaga, Y. & Bading, H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nature Neurosci.* 5, 405–414 (2002a).

Harris, A., Evans, D.W., Cantor, L.B., Martin, B. (1997). Hemodynamic and visual function effects of oral nifedipine in patients with normal-tension glaucoma. *Am. J Ophthalmol.* 124:296-302.

Hayashi T, Su T.P.(2007) Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca^{2+} signaling and cell survival. *Cell.* 131:596-610.

Heacock, A.M. & Agranoff, B.W. (1976) Enhanced labeling of a retinal protein during regeneration of optic nerve in goldfish. *Proc Natl Acad Sci USA.* 73: 828-832.

Hekman, M., Albert, S., Galmiche, A., Rennefahrt, U.E., Fueller, J., Fischer, A., Puehringer, D., Wiese S. & Rapp, U.R. (2006) Reversible membrane interaction of BAD requires two C-terminal lipid binding domains in conjunction with 14-3-3 protein binding, *J Biol. Chem.* 281:17321–17336.

Hempstead, B.L. (2006). Dissecting the diverse actions of pro- and mature neurotrophins. *Curr Alzheimer Res* 3 (1): 19–24.

Hester R.K., Chen Z., Becker E.J., McLaughlin M., DeSantis L. (1994) The direct vascular relaxing action of betaxolol, carteolol and timolol in porcine long posterior ciliary artery. *Surv Ophthalmol.* 38: Suppl:S125-34.

Hirooka, K., Kelly, M.E., Baldridge, W.H., Barnes, S. (2000). Suppressive actions of betaxolol on ionic currents in retinal ganglion cells may explain its neuroprotective effects. Exp. Eye Res. 70: 611-621.

Hirsch, S., Labes, M., Bähr, M. (2000). Changes in BDNF and neurotrophin receptor expression in degenerating and regenerating rat retinal ganglion cells. *Restor Neurol Neurosci.*,17:125-134

Hoste, A.M., Sys, S.U. (1998). Ca^{2+} channel-blocking activity of propranolol and betaxolol in isolated bovine retinal microartery. J. Cardiovasc. Pharmacol. 32: 390-396.

Howell, G.R., Macalinao, D.G., Sousa G.L., Walden, M., Soto, I., Kneeland, S.C., Barbay, J.M., King, B.L., Marchant, J.K., Hibbs, M., Stevens, B., Barres, B.A., Clark, A.F., Libby, R.T. & John, S.W. (2011) Molecular clustering identifies complement and endothelin induction as early events in a mouse model of glaucoma. *J Clin Invest.* doi: 10.1172/JCI44646.
http://www.kegg.com/dbget-bin/www_bget?hsa:4803

Huang W., Fileta J., Rawe I., Qu J., Grosskreutz C.L. (2010) Calpain activation in experimental glaucoma. *Invest Ophthalmol Vis Sci.* 51: 3049-3054.

Huang, W., Fileta, J.B., Dobberfuhl, A., Filippopolous, T., Guo, Y., Kwon G. & Grosskreutz, C.L. (2005) Calcineurin cleavage is triggered by elevated intraocular pressure, and calcineurin inhibition blocks retinal ganglion cell death in experimental glaucoma, *Proc. Natl. Acad. Sci. U.S.A.* 102: 12242–12247.

Huebner, K., Isobe, M., Chao, M., Bothwell, M., Ross, AH., Finan, J., Hoxie, JA., Sehgal, A., Buck, CR., Lanahan, A (1986). The nerve growth factor receptor gene is at human chromosome region 17q12-17q22, distal to the chromosome 17 breakpoint in acute leukemias. *Proc Natl Acad Sci U S A.* 83(5):1403-7.

Husain, S., Yates, P.W., Crosson, C.E. (2008). Latanoprost-induced changes in rat intraocular pressure: direct or indirect? J *Ocul Pharmacol Ther.* 24:367-72.

Ibáñez, C.F. (2007). Message in a bottle: long-range retrograde signaling in the nervous system. *Trends Cell Biol.* 17(11):519-28

Inoue, Y., Iriyama, A., Ueno, S., Takahashi, H., Kondo, M., Tamaki, Y., Araie, M., Yanagi, Y. (2007). Subretinal transplantation of bone marrow mesenchymal stem cells delays retinal degeneration in the RCS rat model of retinal degeneration. *Exp Eye Res*. 85:234-241.

Isenmann, S., Klöcker, N., Gravel, C., Bähr ,M. (1998). Short communication: protection of axotomized retinal ganglion cells by adenovirally delivered BDNF in vivo. *Eur J Neurosci*. 10:2751-6

Ito Y, Shimazawa M, Inokuchi Y, Fukumitsu H, Furukawa S, Araie M, Hara H.(2008) Degenerative alterations in the visual pathway after NMDA-induced retinal damage in mice. *Brain Res* 1212: 89–101.

Ivanov A., Pellegrino C., Rama S., Dumalska I., Salyha Y., Ben-Ari Y., Medina I. (2006) Opposing role of synaptic and extrasynaptic NMDA receptors in regulation of the extracellular signal-regulated kinases (ERK) activity in cultured rat hippocampal neurons. J Physiol. 572(Pt 3):789-98.

Jang, S.W., Liu, X., Chan, C.B., France, S.A., Sayeed, I., Tang, W., Lin, X., Xiao, G., Andero, R., Chang, Q., Ressler, K.J., Ye, K. (2010). Deoxygedunin, a natural product with potent neurotrophic activity in mice. *PLoS One*. 5(7):e11528.

Johansson, J.O. (1988). Inhibition and recovery of retrograde axoplasmic transport in rat optic nerve during and after elevated IOP in vivo. *Exp Eye Res*, 46 (2): 223-7

John, S.W., Smith, R.S., Savinova, O.V., Hawes, N.L,., Chang, B., Turnbull, D., Davisson, M., Roderick, T.H. & Heckenlively, J.R. (1998) Essential iris atrophy, pigment dispersion, and glaucoma in DBA/2J mice. *Invest Ophthalmol Vis Sci*, 39(6):951-962

Johnson T.V., Bull N.D., Hunt D.P., Marina N., Tomarev S.I., Martin K.R. (2010) Neuroprotective effects of intravitreal mesenchymal stem cell transplantation in experimental glaucoma. *Invest Ophthalmol Vis Sci* , 51(4): 2051–2059.

Johnson, E.C., Guo, Y., Cepurna, W.O. and Morrison, J.C. (2009) Neurotrophin roles in retinal ganglion cells survival: lessons from rat glaucoma models. *Exp Eye Res* 88(4):808-815.

Johnson, E.C., Deppmeier, L.M., Wentzien, S.K., Hsu, I., Morrison, J.C. (2000). Chronology of optic nerve head and retinal responses to elevated intraocular pressure. *Invest Ophthalmol Vis Sci*. 41(2):431-42.

Ju W.K., Lindsey J.D., Angert M., Patel A., Weinreb R.N. (2008) Glutamate receptor activation triggers OPA1 release and induces apoptotic cell death in ischemic rat retina. Mol Vis.,14: 2629-2638.

Kallberg, M.E.., Brooks, D.E.., Garcia-Sanchez, G.A.., Komaromy, A.M.., Szabo, N.J., and Tian, L. (2002). Endothelin-1 levels in aqueous humor of dogs with glaucoma. *J Glaucoma* 11(2): 105-109.

Kamal, D., Garway-Heath, D., Ruben, S., O'Sullivan, F., Bunce, C., Viswanathan, A., Franks, W., Hitchings, R., (2003). Results of the betaxolol versus placebo treatment trial in ocular hypertension. Graefes Arch. Clin. Exp. Ophthalmol. 241(3), 196-203.

Katnik C., Guerrero W.R., Pennypacker K.R., Herrera Y., Cuevas J. (2006) Sigma-1 receptor activation prevents intracellular calcium dysregulation in cortical neurons during in vitro ischemia. *J Pharmacol Exp Ther*, 319(3): 1355-1365.

Kimpinski, K., Campenot, R.B., Mearow, K. (1997). Effects of the neurotrophins nerve growth factor, neurotrophin-3, and brain-derived neurotrophic factor (BDNF) on neurite growth from adult sensory neurons in compartmented cultures. *J Neurobiol*. 33(4):395-410

Kitazawa, Y., Shirai, H., Go, F.J., (1989). The effect of Ca2þ-antagonist on visual field in low-tension glaucoma. *Graefes Arch. Clin. Exp. Ophthalmol.* 227(5),408-412.

Kong J-H, Fish D.R., Rockhill R.L., Masland R.H. (2005). Diversity of ganglion cells in the mouse retina: unsupervised morphological classification and its limits. *J Comp Neurol* Vol. 489(3): 293–310.

Krishnamoorthy, R., Agarwal, N., Chaitin, M.H. (2000) Upregulation of CD44 expression in the retina during the rds degeneration. Brain Res Mol Brain Res. 77(1):125-30.

Krishnamoorthy, R.R., Rao, V.R., Dauphin, R., Prasanna, G., Johnson, C., Yorio, T. (2008) Role of the ETB receptor in retinal ganglion cell death in glaucoma. Can J Physiol Pharmacol , 86 (6), 380-393.

Krupin, T, Liebmann, J.M, Greenfield, D.S, Ritch, R., Gardiner, S. Low-Pressure Glaucoma Study Group. (2011) A randomized trial of brimonidine versus timolol in preserving visual function: results from the Low-Pressure Glaucoma Treatment Study. Am J Ophthalmol. 151(4):671-81.

Kuruvilla, R., Ye, H., Ginty, DD. (2000). Spatially and functionally distinct roles of the PI3-K effector pathway during NGF signaling in sympathetic neurons. *Neuron.* 27(3):499-512.

Lacomblez, L., Bensimon, G., Leigh, P.N., Guillet, P., Powe, L., Durrleman, S., Delumeau, J.C. & Meininger, V. (1996) A confirmatory dose-ranging study of riluzole in ALS: ALS/Riluzole Study Group-II, Neurology ,47(6 Suppl 4):S242-50.

Lambert, W., Agarwal, R., Howe, W., Clark, AF., Wordinger, RJ. (2001). Neurotrophin and neurotrophin receptor expression by cells of the human lamina cribrosa. *Invest Ophthalmol Vis Sci.* 42(10):2315-2323.

Lambiase, A., Aloe, L., Centofanti, M., Parisi, V., Mantelli, F., Colafrancesco, V., Manni, GL., Bucci, MG., Bonini, S., Levi-Montalcini, R. (2009). Experimental and clinical evidence of neuroprotection by nerve growth factor eye drops: Implications for glaucoma. *Proc Natl Acad Sci U S A.* 106(32):13469–13474

Lau, J., Dang, M., Hockmann, K. & Ball AK (2005) Effects of acute delivery of endothelin-1 on retinal ganglion cell loss in the rat. *Exp. Eye Res.* 82(1):132-145.

Lebrun-Julien, F., Morquette, B., Douillette, A., Saragovi, HU., Di Polo, A. (2009). Inhibition of p75(NTR) in glia potentiates TrkA-mediated survival of injured retinal ganglion cells. *Mol Cell Neurosci.* 40(4):410-20

Levin, L.A. & Danesh-Meyer, H.V. (2010) Lost in translation: bumps in the road between bench and bedside. *JAMA* 303(15): 1533-1534.

Levkovitch-Verbin, H., Quigley, H.A., Martin, K.R., Valenta, D., Baumrind. L.A. & Pease, M.E. (2002) Translimbal laser photocoagulation to the trabecular meshwork as a model of glaucoma in rats. *Invest Ophthalmol Vis Sci.* 43(2): 402-10.

Lewin, G.R. & Barde, Y.A. (1996). Physiology of the neurotrophins. *Annu Rev Neurosci.* 19:289-317.

Li, R., Jiang, S., Liu, D., Bi, X., Wang, F., Zhang, Q., Xu, Q. (2011). A potential new therapeutic system for glaucoma: solid lipid nanoparticles containing methazolamide. *J Microencapsul.* 28(2):134-41

Li, Z., Okamoto, K., Hayashi, Y., Sheng, M. (2004) The Immportance of Dendritic Mitochondria in the Morphogenesis and Plasticity of Spines and Synapses. *Cell*, 119(6), 873-887.

Lipton, S.A. (2003) Possible role for memantine in protecting retinal ganglion cells from glaucomatous damage. *Surv Ophthalmol.* 48,Suppl 1:S38-46.

Lipton, S.A. (2004) Failures and successes of NMDA receptor antagonists: Molecular basis for the use of open channel blockers like memantine in the treatment of acute and chronic neurologic insults. NeuroRx 1(1): 101-110.

Liu LL, Wang L., Zhong Y. M., Yang X.L. (2010) Expression of sigma receptor 1 mRNA and protein in rat retina. *Neuroscience*. 167(4), 1151-1159.

Liu, X., Wang, D., Liu ,Y., Luo, Y., Ma, W., Xiao, W., Yu, Q. (2010). Neuronal-driven angiogenesis: role of NGF in retinal neovascularization in an oxygen-induced retinopathy model. *Invest Ophthalmol Vis Sci.,*51(7):3749-57

Lodish, H, BERK., A, Zipursky, S. L, Matsudaira, P, Baltimore, D, Darnell, J E. (1999). Molecular Cell Biology. 4th ed. New York: *W H Freeman & Co,*. Section 23.8

Loetscher, H., Niederhauser, O., Kemp, J., Gill, R., (2001). Is caspase-3 inhibition a valid therapeutic strategy in cerebral ischemia? *Drug Discov. Today* 6(13): 671-680.

Luksch, A., Rainer, G., Koyuncu, D., Ehrlich, P., Maca, T., Gschwandtner, M.E., Vass, C., Schmetterer, L., (2005). Effect of nimodipine on ocular blood flow and colour contrast sensitivity in patients with normal tension glaucoma. *Br. J Ophthalmol.* 89(1), 21-25.

MacLaren, R.E., Pearson, R.A., MacNeil, A., Douglas, R.H., Salt, T.E., Akimoto, M., Swaroop, A., Sowden, JC., Ali RR. (2006). Retinal repair by transplantation of photoreceptor precursors. *Nature*. 444(7116):203-207.

MacPhee, I. & Barker, P.A. (1999). Extended ceramide exposure activates the trkA receptor by increasing receptor homodimer formation. *JNeurochem*. 72(4):1423-1430.

Maisonpierre, P.C., Le Beau, M.M., Espinosa, R 3rd., Ip NY., Belluscio, L., de la Monte SM., Squinto, S., Furth, ME., Yancopoulos, GD. (1991). Human and rat brain-derived neurotrophic factor and neurotrophin-3: gene structures, distributions, and chromosomal localizations. *Genomics* 10 (3): 558–568.

Malinow, R. & Malenka, R.C. AMPA receptor trafficking and synaptic plasticity. (2002) *Annu Rev Neuroscience.*, 25:103-126.

Manabe S. & Lipton S.A. (2003) Divergent NMDA signals leading to proapoptotic and antiapoptotic pathways in the rat retina. Invest. Ophthalmol. Vis. Sci. 44(1):385-392.

Mao Z, Bonni A, Xia F, Nadal-Vicens M, Greenberg ME.(1999) Neuronal activity-dependent cell survival mediated by transcription factor MEF2. *Science*. 286(5440), 785-790.

Mark, L.P., Prost, R.W., Ulmer, J.L., Smith, M.M., Daniels, D.L., Strottmann, J.M., Brown, W.D. & Hacein-Bey L (2001) Pictorial review of glutamate excitotoxicity: Fundamental concepts for neuroimaging. *Am. J Neuroradiol*. 22(10): 1813-1824.

Marshall, C. (1995) Specificity of receptor tyrosine kinase signalling: transient versus sustained extracellular signal-regulated kinase activation. *Cell* 80(2): 179–185

Martin, K.R., Quigley, H.A., Zack, D.J., Levkovitch-Verbin, H., Kielczewski, J., Valenta, D., Baumrind, L., Pease, M.E., Klein, R.L., Hauswirth, W.W. (2003). Gene therapy with brain-derived neurotrophic factor as a protection: retinal ganglion cells in a rat glaucoma model. *Invest Ophthalmol Vis Sci*. 44(10):4357-65

Mayer M. L., Westbrook G. L., and Guthrie P. B. (1984) Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature* 309(5965):261-3..

Melena, J., Wood, J.P.M., Osborne, N.N., (1999). Betaxolol, a β1-adrenoceptor antagonist, has an affinity for L-type Ca2þ channels. *Eur. J Pharmacol*. 378(3), 317-322.

Meyer-Franke, A., Kaplan, M.R., Pfrieger, F.W., Barres, B.A. (1995). Characterization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture. *Neuron,* 15(4): 805–819

Mokbel T.H., Ghanem A.A., Kishk H, Arafa L.F., El-Baiomy A.A. (2010) Erythropoietin and soluble CD44 levels in patients with primary open-angle glaucoma. Clin Experiment Ophthalmol. 38(6):560-655.

Morgan J.I, Curran T. (1986). Role of ion flux in the control of c-fos expression. *Nature*. 322(6079): 552-555.

Morrison, J.C., Moore, C.G., Deppmeier, L.M., Gold, B.G., Meshul, C.K. and Johnson, E.C. (1997) A rat model of chronic pressure-induced optic nerve damage. *Exp Eye Res*. 64: 85–96.

Murphy, T.H., Worley, P.F., Baraban, J.M. (1991) L-type voltage-sensitive calcium channels mediate synaptic activation of immediate early genes. *Neuron*. 7 (4), 625-35.

Nagata, T., Ueno, S., Morita, H., Kubota, T., Toyohira, Y., Tsutsui, M., Tawara, A., Yanagihara, N., (2008). Direct inhibition of N-methyl-D-aspartate (NMDA)-receptor function by antiglaucomatous b-antagonists. J. Pharmacol. Sci. 106(3), 423-434.

Nakagawara, A., Liu, XG., Ikegaki, N., White, PS., Yamashiro, DJ., Nycum LM., Biegel JA., Brodeur GM. (1995). Cloning and chromosomal localization of the human TRK-B tyrosine kinase receptor gene (NTRK2). *Genomics*. 25(2):538-46.

Nakazawa T., Nakazawa C., Matsubara A., Noda K., Hisatomi T., She H., Michaud N, Hafezi-Moghadam A, Miller JW, Benowitz LI. (2006) Tumor necrosis factor-alpha mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma. J Neurosci. 26(49), 12633–12641.

Nakazawa, T., Nakazawa, C., Matsubara, A., Noda, K., Hisatomi ,T., She, H., Michaud, N., Hafezi-Moghadam A., Miller JW., Benowitz LI. (2006). Tumor necrosis factor-alpha mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma. *JNeurosci*. 26(49):12633-12641.

Nakazawa, T., Nakazawa, C., Matsubara, A., Noda, K., Hisatomi, T., She, H., Michaud, N., Hafezi-Moghadam, A., Miller, J.W. & Benowitz LI (2006) Tumor necrosis factor-alpha mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma. *J Neurosci*. 26(49): 12633-12641.

Narayan, S., Prasanna, G., Krishnamoorthy, RR., Zhang, X., Yorio, T. (2003). Endothelin-1 synthesis and secretion in human retinal pigment epithelial cells (ARPE-19) :differential regulation by cholinergics and TNF-alpha. *Invest Ophthalmol Vis Sci*.44(11):4885-4894.

Nickells, R.W. (2004) The molecular biology of retinal ganglion cell death: caveats and controversies. *Brain Res. Bull*. 62(6): 439-446.

Nickells, R.W. (2007) From ocular hypertension to ganglion cell death: a theoretical sequence of events leading to glaucoma. *Canadian J Ophthalmol*. 42(2):278-287.

Nixon, R.A. (2003) The calpains in aging and aging-related diseases, *Ageing Res. Rev*. 2(4) , 407–418.

Noske, W., Hensen, J.., and Wiederholt, M. (1997). Endothelin-like immunoreactivity in aqueous humor of primary open angle glaucoma and cataract patients. *Grafes Arch. Clin. Exp. Ophthamol*. 235(9): 551-552.

Nykjaer, A., Lee, R., Teng, KK., Jansen, P., Madsen, P., Nielsen, MS., Jacobsen, C., Kliemannel, M., Schwarz, E., Willnow, TE., Hempstead, BL., Petersen, CM. (2004). Sortilin is essential for proNGF-induced neuronal cell death. *Nature*. 26,427(6977):843-8

Nykjaer, A., Willnow, TE., Petersen, CM. (2005). p75NTR-live or let die. *Curr Opin Neurobiol*. 15(1):49-57

O'Brien, C., (1998) Vasospasm and glaucoma. *Br. J Ophthalmol*. 82, 855–856.

Ohta, H., Arai, S., Akita, K., Ohta, T., Fukuda, S. (2011). Neurotrophic effects of a cyanine dye via the PI3K-Akt pathway: attenuation of motor discoordination and neurodegeneration in an ataxic animal model. *PLoS One*. 6(2):e17137.

Olver, J.M., (1998). Angioarchitecture of the human optic nerve. In: Bisantis, C., Carella, G. (Eds.), Vascular System of the Optic Nerve. Innovation-News-Communication, Rome, pp. 29–44.

Orgul, S., Cioffi, G.A., Bacon, D.R. & Van Buskirk, E.M. (1996) An endothelin-1 induced model of chronic optic nerve ischemia in rhesus monkeys. *J Glaucoma* 5: 135-138.

Osborne, N.N., Casson, R.J., Wood, J.P., Chidlow, G., Graham, M., Melena, J., (2004). Retinal ischemia: mechanisms of damage and potential therapeutic strategies. Prog. Retin. Eye Res. 23, 91-147.

Osborne, N.N., DeSantis, L., Bae, L.H., Ugarte, M., Wood, J.P.M., Nash, M.S., Chidlow, G., (1999). Topically applied betaxolol attenuates NMDA-induced toxicity to ganglion cells and the effects of ischaemia to the retina. Exp. Eye Res. 69, 331-342.

Ozawa S., Kamiya H., and Tsuzuki K. (1998) Glutamate receptors in the mammalian central nervous system. *Prog. Neurobiol.* 54, 581–618.

Pang I.H., Wexler E.M., Nawy S., DeSantis L., Kapin M.A. (1999) Protection by eliprodil against excitotoxicity in cultured rat retinal ganglion cells. Invest. Ophthalmol. Vis. Sci. 40:1170-1176.

Parsons C.G., Gruner R., Rozental J., Millar J. and Lodge D. (1993). Patch clamp studies on the kinetics and selectivity of N-methyl-d-aspartate receptor antagonism by memantine (1-amino-3,5-dimethyladamantane). *Neuropharmacology* 32(12), 1337-1350.

Pease, M.E., McKinnon, S.J., Quigley, H.A., Kerrigan-Baumrind, L.A., Zack, D.J. (2000). Obstructed axonal transport of BDNF and its receptor TrkB in experimental glaucoma. *Invest Ophthalmol Vis Sci*, 41: 764–774

Pernet, V., Di Polo, A. (2006). Synergistic action of brain-derived neurotrophic factor and lens injury promotes retinal ganglion cell survival, but leads to optic nerve dystrophy in vivo. *Brain*. 129(Pt 4):1014-26

Piltz, J.R., Bose, S., Lanchoney, D., (1998). The effect of nimodipine, a centrally active calcium antagonist, on visual function and macular blood flow in patients with normal-tension glaucoma and control subjects. *J Glaucoma* 7, 336-342.

Prasanna, G., Dibas, A., Tao, W., White, K., Yorio, T. (1998). Regulation of endothelin-1 in human non-pigmented ciliary epithelial cells by tumor necrosis factor-alpha. *Exp Eye Res*. 66: 9-18.

Prasanna, G., Hulet, C., Desai, D., Krishnamoorthy, R.R., Narayan, S., Brun, A.M., Suburo, A.M. & Yorio T. (2005) Effect of elevated intraocular pressure on endothelin-1 in a rat model of glaucoma. *Pharmacol Res*. 51: 41-50.

Prasanna, G., Krishnamoorthy, R., Clark, AF., Wordinger, RJ., Yorio, T.(2002) . Human optic nerve head astrocytes as a target for endothelin-1. *Invest Ophthalmol Vis Sci*. 43: 2704-13.

Prasanna, G., Krishnamoorthy, R., Yorio, T. (2010) Endothelin, astrocytes and glaucoma. *Experimental Eye Research*. doi:10.1016/j.exer.2010.09.006

Prithviraj, R., Kelly, K.M., Espinoza-Lewis, R., Hexom, T., Clark, A.B.& Inglis, F.M. (2008) Differential regulation of dendrite complexity by AMPA receptor subunits GluR1 and GluR2 in motor neurons. *Dev Neurobiol*. 1:247-264.

Qu J, Wang D, Grosskreutz C.L. (2010) Mechanisms of retinal ganglion cell injury and defense in glaucoma. *Exp Eye Res*. 91(1), 48-53.

Quigley H.A., Dunkelberger G.R., Green W.R. (1989) Retinal ganglion cell atrophy correlated with automated perimetry in human eyes with glaucoma. *Am J Ophthalmol* 107, 453–464.

Quigley, H.A., Addicks, E.M., Green, W.R, & Maumenee, A.E. (1981) Optic nerve damage in human glaucoma. II. the site of injury and susceptibility to damage. *Arch Ophthalmol*. 99:635-649.

Quigley, H.A., McKinnon, S.J., Zack, D.J., Pease, M.E., Kerrigan-Baumrind, L.A., Kerrigan, D.F., Mitchell, R.S. (2000). Retrograde axonal transport of BDNF in retinal ganglion cells is blocked by acute IOP elevation in rats. *Invest Ophthalmol Vis Sci*. 41(11):3460-6

Rainer, G, Kiss, B., Dallinger, S., Findl, O., Georgopoulos, M., Vass, C., Menapace, R., Polak K., Eichler, H.G., Wolzt, M., Schmetterer, L.(2001) A double masked placebo controlled study on the effect of nifedipine on optic nerve blood flow and visual field function in patients with open angle glaucoma. *Br JClin Pharmacol*. 52:210-212.

Raju, T.R., Rao, M.S., Nagaraja, T.N., Meti, B.L., Schulz, M. (1994). Retinal ganglion cell survival and neurite regeneration in vitro after cell death period are dependent upon target derived trophic factor and retinal glial factor(s). *Brain Res*. 21,664(1-2):247-51.

Rao, V.P., Epstein, D.L. (2007) Rho GTPase/Rho kinase inhibition as a novel target for the treatment of glaucoma. *BioDrugs*. 21:167-77.

Reichardt, L.F (2006). Neurotrophin-regulated signalling pathways. *Philos. Trans. R. Soc. Lond., B, Biol. Sci*. 361 (1473): 1545–64.

Reisberg, B., Doody, R., Stoffler, A., Schmitt, F., Ferris, S. & Mobius, H.J. (2003) Memantine in moderate-to-severe Alzheimer's disease, *N Engl JMed* 348:1333-1341

Rogers, S.D., Demaster E., Catton, M., Ghilardi, J.R., Levin, L.A., Maggio, J.E. & Mantyh, P.W. (1997) Expression of endothelin-B receptors by glia in vivo is increased after CNS injury in rats, rabbits and humans. *Exp. Neurol*. 145: 180-195.

Rojanapongpun, P., Drance, S.M. (1993) The response of blood flow velocity in the ophthalmic artery and blood flow of the finger to warm and cold stimuli in glaucomatous patients. *Graefes Arch. Clin. Exp. Ophthalmol*. 231:375–377.

Rubanyi, G.M. & Polokoff, M.A. (1994) Endothelins: molecular biology, biochemistry, pharmacology and pathophysiology. *Pharmacol. Rev*. 46: 325-415.

Rudzinski, M., Wong, T.P., Saragovi, H.U. (2004). Changes in retinal expression of neurotrophins and neurotrophin receptors induced by ocular hypertension. *J Neurobiol*. 15,58(3):341-54.

Sappington, R.M., Carlson, B.J., Crish, S.D. & Calkins, D.J. (2010). The microbead occlusion model: a paradigm for induced ocular hypertension in rats and mice. *Invest Ophthalmol Vis Sci*. 51:207-216

Sariola, H., Sainio, K., Arumäe, U., Saarma, (1994). M. Neurotrophins and ciliary neurotrophic factor: their biology and pathology. Ann Med. 26(5):355-63.

Sawai, H., Clarke, D.B., Kittlerova, P., Bray, G.M., Aguayo, A.J. (1996). Brain-derived neurotrophic factor and neurotrophin-4/5 stimulate growth of axonal branches from regenerating retinal ganglion cells. *JNeurosci*. 15,16(12):3887-94.

Schober M.S., Chidlow G, Wood J.P, Casson R.J. (2008) Bioenergetic-based neuroprotection and glaucoma. *Clin Experimental Ophthalmol* 36:377–385.
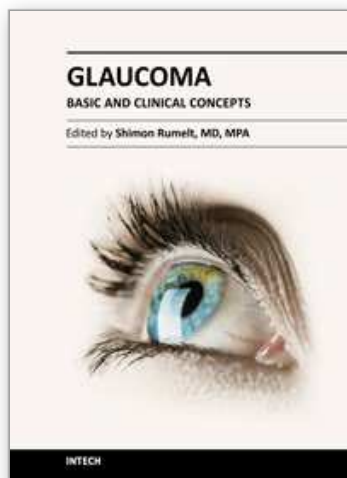
Schwartz M. (2004) Optic nerve crush: protection and regeneration. Brain Res Bull. 15,62(6):467-71.

Seki, M., Fukuchi, T., Tanaka, T., Nawa, H., Takei, N., Abe, H. (2004) Quantitative analyses of mRNA and protein levels of neurotrophin-3 in the rat retina during postnatal development and aging. *Jpn J Ophthalmol.*,48(5):460-4.

Seki, M., Tanaka, T., Sakai, Y., Fukuchi, T., Abe, H., Nawa, H., Takei ,N. (2005). Müller Cells as a source of brain-derived neurotrophic factor in the retina: noradrenaline upregulates brain-derived neurotrophic factor levels in cultured rat Müller cells. *Neurochem Res.* 30(9):1163-70.

Setoguchi, M., Ohya, Y., Abe, I., Fujishima, M. (1995) Inhibitory action of betaxolol, a b1-selective adrenoceptor antagonist, on voltage-dependent calcium channels in guinea-pig artery and vein. Br. J. Pharmacol. 115:198-202.

Sheng, M., McFadden, G., Greenberg, M.E.(1990) Membrane depolarization and calcium induce c-fos transcription via phosphorylation of transcription factor CREB. *Neuron.* 4. 571-582

Shi, Z., Birman, E., Saragovi, HU. (2007). Neurotrophic rationale in glaucoma: a TrkA agonist, but not NGF or a p75 antagonist, protects retinal ganglion cells in vivo. *Dev Neurobiol.* 67(7):884-94. Erratum in: *Dev Neurobiol.* 2007 Sep 15,67(11):1547-8.

Shi, Z., Rudzinski, M., Meerovitch, K., Lebrun-Julien, F., Birman, E., Di Polo, A., Saragovi, HU. (2008). Alpha2-macroglobulin is a mediator of retinal ganglion cell death in glaucoma. *J Biol Chem.* 24,283(43):29156-65

Shimazawa, M., Inokuchi, Y., Ito, Y., Murata, H., Aihara, M., Miura, M., Araie, M., Hara, H. (2007) Involvement of ER stress in retinal cell death. *Mol Vis.* Vol. 13:578–587.

Siliprandi R., Canella R., Carmignoto G., Schiavo N., Zanellato A., Zanoni R.,Vantini G. (1992) N-methyl-D-aspartate-induced neurotoxicity in the adult rat retina. Vis. Neurosci. 8(6):567-573.

Smith SB, Duplantier J, Dun Y, Mysona B, Roon P, Marin PM, Ganapathy V. (2008). In vivo protection against retinal neurodegeneration by sigma receptor 1 ligand (+) pentazocine. *Invest Ophthamol Vis Sci.* 49:4154-4161.

Sole, C., Dolcet, X., Segura, M.F., Gutierrez, H., Diaz-Meco, M.T., Gozzelino, R., Sanchis, D., Bayascas, JR., Gallego, C., Moscat, J., Davies, AM., Comella, JX. (2004). The death receptor antagonist FAIM promotes neurite outgrowth by a mechanism that depends on ERK and NF-kapp B signaling. *J Cell Biol.* 8,167(3):479-92

Sommer, A., Tielsch, J.M., Katz, J., Quigley, H.A., Gottsch, J.D., Javitt J. and Singh K. (1991) Relationship between intraocular pressure and primary open angle glaucoma among white and black Americans. The Baltimore Eye Survey. Arch Ophthalmol 109:1090-5

Spalding, K.L., Rush, R.A., Harvey, A.R. (2004). Target-derived and locally derived neurotrophins support retinal ganglion cell survival in the neonatal rat retina. *J Neurobiol.* 5,60(3):319-27.

Steinhausen, K., Stumpff, F., Strauss, O., Thieme, H., Wiederholt, M., (2000). Influence of muscarinic agonists and tyrosine kinase inhibitors on L-type Ca(2þ) channels in human and bovine trabecular meshwork cells. *Exp. Eye Res.* 70:285-293.

Suemori S, Shimazawa M, Kawase K, Satoh M, Nagase H, Yamamoto T, Hara H.(2006) Metallothionein, an endogenous antioxidant, protects against retinal neuron damage in mice. *Invest Ophthalmol Vis Sci.* 47 (3975–3982)

Sun W, Li X, He S. (2002). Large-scale morphological survey of mouse retinal ganglion cells. *JComp Neurol* 451:(115–126)

Tao, W., Wen, R., Goddard, MD., Sherman, SD., O'Rourke, PJ., Stabila, PF., Bell, WJ., Dean, BJ., Kauper, KA., Budz, VA., Tsiaras, WG., Acland, GM ., Pearce-Kelling, S., Laties, AM., Aguirre GD (2002). Encapsulated cell-based delivery of CNTF reduces photoreceptor degeneration in animal models of retinitis pigmentosa. Invest Ophthalmol Vis Sci. 43:3292-3298

Tchedre, K.T., Huang, R.Q., Dibas, A., Krishnamoorthy, R.R., Dillon, G.H., Yorio, T. (2008) Sigma-1 receptor regulation of voltage gated calcium channels involves a direct interaction. *Invest Ophthamol Vis Sci*. 49:4993-5002.

Tchedre, K.T., Yorio, T.(2008) Sigma-1 receptors protect RGC-5 cells from apoptosis by regulating intracellular calcium, bax levels, and caspase-3 activation. *Invest Ophthamol Vis Sci*. 49:2577-2588

Tezel, G. & Wax, M.B. (2000) Increased production of tumor necrosis factor-alpha by glial cells exposed to simulated ischemia or elevated hydrostatic pressure induces apoptosis in cocultured retinal ganglion cells. *JNeurosci*. 20:8693–8700.

Tezel, G. & Wax, M.B. (2004) The immune system and glaucoma. *Curr Opin Ophthalmol*. 15:80–84.

Tezel, G.(2008) TNF-alpha signaling in glaucomatous neurodegeneration. *Prog Brain Res*. 173:409-21.

Tezel, G., Li, L.Y., Patil, R.V., Wax, M.B.(2001). TNF-alpha and TNF-alpha receptor-1 in the retina of normal and glaucomatous eyes. *Invest Ophthalmol Vis Sci*.42:1787-94.

Tezel,G. (2006) Oxidative stress in glaucomatous neurodegeneration: mechanisms and consequences.Prog.Retin.EyeRes. 25:490-513.

Tezel., G.., Kass, M.A.., Kolker, A.E.., Becker B, Wax MB. (1997). Plasma and aqueous endothelin levels in primary open angle glaucoma. *J Glaucoma* 6: 83-89.

Thanos, S. & Naskar, R. (2004). Correlation between retinal ganglion cell death and chronically developing inherited glaucoma in a new rat mutant. *Exp. Eye Res*. 79:119-29

Thieme, H., Steinhausen, K., Ottlecz, A., Lambrou, G.N., Strauss, O., Wiederholt, M., Rosenthal, R. (2005) Effects of unoprostone and endothelin 1 on L-type channel currents in human trabecular meshwork cells. *Ophthalmic Res*. 37:293-300.

Toriu, N., Akaike, A., Yasuyoshi, H., Zhang, S., Kashii, S., Honda, Y., Shimazawa, M., Hara, H.(2000) Lomerizine, a Ca^{2+} channel blocker, reduces glutamate-induced neurotoxicity and ischemia/reperfusion damage in rat retina. *Exp Eye Res*. 70:475-84.

Ugolini, G., Cremisi, F., Maffei, L. (1995). TrkA, TrkB and p75 mRNA expression is developmentally regulated in the rat retina. *Brain Res*. 15,704(1):121-4.

Ullian, E.M., Barkis, W.B., Chen, S., Diamond, J.S. &, Barres, B.A. (2004) Invulnerability of retinal ganglion cells to NMDA excitotoxicity. *Mol Cell Neurosci*. 26:544-57.

Valent, A., Danglot, G., Bernheim, A. (1997). Mapping of the tyrosine kinase receptors trkA (NTRK1), trkB (NTRK2) and trkC(NTRK3) to human chromosomes 1q22, 9q22 and 15q25 by fluorescence in situ hybridization. *Eur JHum Genet*. 5(2):102-4.

Vecino, E., García-Crespo, D., García, M., Martinez-Millán, L., Sharma, SC., Carrascal, E. (2002). Rat retinal ganglion cells co-express brain derived neurotrophic factor (BDNF)and its receptor TrkB. *Vision Res*. 42(2):151-7. Erratum in: *Vision Res*. 2010 21,50(9):926.

Völgyi, B., Chheda, S., Bloomfield, S.A.,(2009) Tracer coupling patterns of the ganglion cell subtypes in the mouse retina. *JComp Neurol*. 512:664-87.

Wamsley, S., Gabelt, B.T., Dahl, D.B., Case, G,L., Sherwood, R.W., May, C.A., Hernandez, M.R. and Kaufman PL.(2005) Vitreous glutamate concentration and axon loss in monkeys with experimental glaucoma. *Arch Ophthalmol*. 123:64-70.

Wang, H.G., Pathan, I.M., Ethell, S., Krajewski, Y., Yamaguchi, F., Shibasaki, F., McKeon, F., Bobo, T., Franke, T.F. & Reed J.C. (1999) Ca^{2+}-induced apoptosis through calcineurin dephosphorylation of BAD, *Science* 284:339–343.

Wang, K.C., Kim, J.A., Sivasankaran, R., Segal, R., He Z. (2002). P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature*. 7,420(6911):74-8.

Wang, L., Fortune, B., Cull, G., Dong, J & Cioffi GA (2006) Endothelin B receptor in human glaucoma and experimentally induced optic nerve damage. *Arch. Ophthalmol*. 124:717-24

Ward, M.S., Khoobehi, A., Lavik, E.B., Langer, R., Young, M.J. (2007). Neuroprotection of retinal ganglion cells in DBA/2J mice with GDNF-loaded biodegradable microspheres. *JPharm Sci*. 96(3):558-68.

Watson, P.G., Barnett, M.F., Parker, V., Haybittle, J., (2001) A 7 year prospective comparative study of three topical beta blockers in the management of primary open angle glaucoma. Br. J. Ophthalmol. 85:962-968.

Weber, A.J., Viswanáthan, S., Ramanathan, C., Harman, C.D. (2010). Combined application of BDNF to the eye and brain enhances ganglion cell survival and function in the cat after optic nerve injury. *Invest Ophthalmol Vis Sci*. 51(1):327–334

Weier, H.U., Rhein, A.P., Shadravan, F., Collins, C., Polikoff, D. (1995). Rapid physical mapping of the human trk protooncogene (NTRK1) to human chromosome 1q21-q22 by P1 clone selection, fluorescence in situ hybridization (FISH), and computer-assisted microscopy. *Genomics*. 20,26(2):390-3.

Wiederholt, M., Thieme, H., Stumpff, F., (2000). The regulation of trabecular meshwork and ciliary muscle contractility. *Prog. Retin. Eye Res*. 19:271-295.

Wilhelm, B, Lüdtke, H, Wilhelm, H, BRAION Study Group. Efficacy and tolerability of 0.2% brimonidine tartrate for the treatment of acute non-arteritic anterior ischemic optic neuropathy (NAION): a 3-month, double-masked, randomised, placebo-controlled trial. Graefes Arch Clin Exp Ophthalmol. 2006

Wood, J.P.M., Desantis, L., Chao, H.-M., Osborne, N.N. (2001) Topically applied betaxolol attenuates ischaemia-induced effects to the rat retina and stimulates BDNF mRNA. Exp. Eye Res. 72:79-86.

Wood, J.P.M., Schmidt, K.-G., Melena, J., Chidlow, G., Allmeier, H., Osborne, N.N. (2003) The b-adrenoceptor antagonists metipranolol and timolol are retinal neuroprotectants: comparison with betaxolol. Exp. Eye Res. 76:505-516.

Wu, H.Y., Tomizawa, K., Oda, Y., Wei, F.Y., Lu, Y.F., Matsushita, M., Li, S.T., Moriwaki, A., Matsui, H. (2004) Critical role of calpain-mediated cleavage of calcineurin in excitotoxic neurodegeneration. *JBiol Chem*. 279:4929-40.

Wu, G. Y., Deisseroth, K. & Tsien, R. W. (2001) Activitydependent CREB phosphorylation: convergence of a fast, sensitive calmodulin kinase pathway and a slow, less sensitive mitogen-activated protein kinase pathway. *Proc. Natl Acad. Sci. USA* 98:2808–2813.

Yamada, H., Chen, Y.N., Aihara, M., Araie, M. (2006) Neuroprotective effect of calcium channel blocker against retinal ganglion cell damage under hypoxia. Brain Res. 1071:75-80.

Yamagishi, S., Fujitani, M., Hata, K., Kitajo, K., Mimura, F., Abe, H., Yamashita, T. (2005). Wallerian degeneration involves Rho/Rho-kinase signaling. *J Biol Chem.* 27,280(21):20384-8.

Yanagisawa, M, Kurihara, H, Kimura, S, Tomobe, Y, Kobayashi, M, Mitsui, Y, Yazaki, Y, Goto, K, Masaki, T. (1988) A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature. 332(6163):411-5.

Yang, Z., Quigley, H.A., Pease, M.E., Yang, Y., Qian J., Valenta, D. & Zack, D.J. (2007) Changes in gene expression in experimental glaucoma and optic nerve transection: the equilibrium between protective and detrimental mechanisms. *Invest. Ophthalmol. Vis. Sci.* 48:5539-5548.

Yorio, T., Krishnamoorthy, R. & Prasanna, G. (2002) Endothelin: Is it a contributor to glaucoma pathophysiology? *J Glaucoma.* 11:259-70.

Yu, S., Tanabe, T., Dezawa, M., Ishikawa, H., Yoshimura, N. (2006). Effects of bone marrow stromal cell injection in an experimental glaucoma model. *Biochem Biophys Res Commun.* 16,344(4):1071-9.

Zafra, F., Hengerer, B., Leibrock, J., Thoenen, H., Lindholm, D.(1990) Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non NMDA glutamate receptors. *EMBO J* 9:3545-50.

Zarbin, M.A., Montemagno, C., Leary, J.F., Ritch, R. (2010). Nanotechnology in ophthalmology. *Can J Ophthalmol.* 45(5):457-76

Zhang, H, Cuevas, J.(2002) Sigma receptors inhibit high-voltage-activated calcium channels in rat sympathetic and parasympathetic neurons. *J Neurophysiol.* 87:2867-2879.

Zhong, L., Bradley, J., Schubert, W., Ahmed, E., Adamis, AP., Shima, DT., Robinson, GS., Ng YS. (2007). Erythropoietin promotes survival of retinal ganglion cells in DBA/2J glaucoma mice. *Invest Ophthalmol Vis Sci.* 48(3):1212-8.

Zhou, Y., Pernet, V., Hauswirth, WW., Di Polo, A. (2005). Activation of the extracellular signal-regulated kinase 1/2 pathway by AAV gene transfer protects retinal ganglion cells in glaucoma. *Mol Ther.* 12(3):402-12.

Glaucoma - Basic and Clinical Concepts

Edited by Dr Shimon Rumelt

This book addresses the basic and clinical science of glaucomas, a group of diseases that affect the optic nerve and visual fields and is usually accompanied by increased intraocular pressure. The book incorporates the latest development as well as future perspectives in glaucoma, since it has expedited publication. It is aimed for specialists in glaucoma, researchers, general ophthalmologists and trainees to increase knowledge and encourage further progress in understanding and managing these complicated diseases.

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