Neurodegeneration in Glaucoma: Progression and Calcium-Dependent Intracellular Mechanisms

Samuel D. Crish1 and David J. Calkins2
1Department of Pharmaceutical Sciences, Northeastern Ohio Universities Colleges of Medicine and Pharmacy, Rootstown, OH 44272
2Vanderbilt Eye Institute and Vanderbilt Brain Institute, Vanderbilt University Medical Center, Nashville, TN 37232

Abstract

Glaucoma is an age-related optic neuropathy involving sensitivity to ocular pressure. The disease is now seen increasingly as one of the central nervous system, as powerful new approaches highlight an increasing number of similarities with other age-related neurodegenerations such as Alzheimer’s and Parkinson’s. While the etiologies of these diseases are diverse, they involve many important common elements including compartmentalized programs of degeneration targeting axons, dendrites and finally cell bodies. Most age-related degenerations display early functional deficits that precede actual loss of neuronal substrate. These are linked to several specific neurochemical cascades that can be linked back to dysregulation of Ca$^{2+}$-dependent processes. We are now in the midst of identifying similar cascades in glaucoma. Here we review recent evidence on the pathological progression of neurodegeneration in glaucoma and some of the Ca$^{2+}$-dependent mechanisms that could underlie these changes. These mechanisms present clear implications for efforts to develop interventions targeting neuronal loss directly and make glaucoma an attractive model for both interrogating and informing other neurodegenerative diseases.

Glaucosa is a Neurodegenerative Disease

Disease Etiology: Age and Ocular Pressure

Optic neuropathies are the most common source of age-related loss of sensory activity in the central nervous system (CNS). Of these, glaucoma is by far the worst. This disease is the leading cause of irreversible (i.e., neurodegenerative) blindness worldwide. Age is the greatest risk factor: the likelihood of developing glaucoma increases nearly 7-fold after 55 years. Thus, with the aging population, nearly 80 million people worldwide will be afflicted by 2020 (Quigley and Broman, 2006). The disease is also the number one cause of blindness (both irreversible and reversible) in African-Americans, occurring on average 10 years earlier than in the Caucasian population and at an incidence that is 4 to 5-fold higher (Tielsch et al., 1991).
Traditionally, glaucoma has been considered an “eye disease”. This is because elevated intraocular pressure (IOP) is the leading modifiable risk factor for the disease (Sommer, 1989; Gordon et al., 2002). Thus, animal models of the disease generally incorporate induced elevations or exploit naturally-occurring elevations (Pang et al., 2007; Sappington et al., 2010). However, glaucomatous pathology can also be induced by eliciting an autoimmune response (Wax et al., 2008). Lowering IOP is the most common treatment by far, generally through topical application of pharmaceuticals and/or surgery (Heijl et al., 2002).

The relationship between elevated IOP and vision loss is not straightforward as normal pressure glaucoma, or glaucomatous pathology without elevated IOP, represents about 50% of glaucoma diagnoses (Shields, 2008). Ocular hypertension, or chronically elevated IOP without nerve damage, is more common than glaucoma itself (reviewed in Heijl et al., 2002). Even more enigmatic from a neurobiological standpoint, results from the Early Manifest Glaucoma Trial indicate that lowering IOP for those with nominally normal pressure can be an effective preventive treatment for reducing the risk of the age-related neuropathy associated with glaucoma (Heijl et al., 2002), though it is not clear what fraction of large number of patients with normal IOP benefit from IOP-lowering (Anderson et al., 2003). Thus, IOP represents the primary modifiable risk factor whatever its magnitude. The Baltimore Eye Study concluded that the distinction between “low-tension” and “high-tension” glaucoma is artificial (Sommer et al., 1991). Even so, some estimates indicate that optic nerve degeneration may continue in as many as half of glaucoma patients treated with an IOP-lowering regimen (Leske et al., 2003). These observations suggest that we should consider glaucoma not as a disease involving elevations in pressure, but as a disease in which neurological sensitivity to pressure itself, independent of magnitude, is an additional insult against a back-drop of other age-related stressors in the system (Sappington et al., 2009).

The Neurobiological Roots of Vision Loss

Continued progression of visual loss in glaucoma even with lowered IOP has compelled the search for interventions that treat neurodegeneration directly (McKinnon et al., 2008). Through mechanisms that are not well understood, glaucoma selectively targets the 1.5 million retinal ganglion cell (RGC) neurons whose axons comprise the optic nerve. Because RGCs are the output neurons of the eye, their selective loss in glaucoma has reinforced the “eye disease” viewpoint. However, this perception has in fact created lost opportunities. Most of the retinal ganglion cell lies outside of the eye, and it is here that recent advances in understanding the progression of glaucomatous pathology have focused.

Since the retina and optic projection are part of the CNS, it is not surprising that glaucoma shares epidemiological and mechanistic similarities with other CNS neurodegenerations, including Alzheimer’s, Parkinson’s, amyotrophic lateral sclerosis (ALS), and Huntington’s disease. While the etiologies of these diseases are diverse, their progression involves many important common elements that are themselves potential targets for therapeutic interventions. Our recent work and that of other groups demonstrates that neurodegeneration in glaucoma shares these common components and that they hold promise as therapeutic targets (McKinnon, 2003; Steele et al., 2006; Buckingham et al., 2008; Kong et al., 2009; Crish et al., 2010). Thus, animal models of glaucoma are seen increasingly as emergent tools with which to inform studies of other age-related CNS neurodegenerations (Whitmore et al., 2005). In particular, models are useful for helping us understand (1) how aging biases the system towards irreversible loss of function, and (2) how deprivation of this sensory input affects a major portion of the aging brain.
Progression of Neurodegeneration

The Endgame First: Apoptosis in the Retina

Whatever the early events involved in RGC degeneration in glaucoma are, they ultimately result in down-stream caspase-dependent, mitochondrial-mediated apoptosis (Kerrigan et al., 1997; Quigley, 1999; Tatton et al., 2001; McKinnon et al., 2002; Tahzib et al., 2004; Waldmeier and Tatton, 2004; Huang et al., 2005a,b; Qu et al., 2010). Ample evidence supports the typical characterization of glaucoma as a disease inducing the eventual apoptotic loss of RGC somas in the retina. For example, histological examination of the RGC layer of the retina clearly demonstrates thinning of the cell body population; this is so for both human tissue and animal models (Kerrigan-Baumrind et al., 2000; John et al., 1998). Thus, as in other CNS conditions (see Rohn, 2010), investigations exploring the progression of glaucomatous neurodegeneration have focused on apoptotic elimination of RGCs (Quigley et al., 1995; Kerrigan et al., 1997; Huang et al., 2005a,b). Similarly, one of the most common techniques used historically to assay RGC somatic survival is straightforward tract tracing; applying a neuronal tracer such as fuoro-gold to the lateral geniculate body, superior colliculus or other central projection site and examining its localization in the retina some time later (Mittag et al., 2000; Vidal-Sanz et al., 2001; Danias et al., 2003; Filippopolous et al., 2006). However, these assays reflect better the state of retrograde axonal transport and axon survival. If axons are challenged early, and this seems to be case, then lack of retrograde-labeled RGCs does not necessarily indicate they are absent. For example, in the DBA2J mouse model of pigmentary glaucoma, RGC somas labeled by neuronal markers are seen to persist long after retrograde transport is depleted (Buckingham et al., 2008).

However, there is a growing movement away from viewing apoptosis as the cause of clinical presentation of disease (Gould et al., 2006; Jelinger, 2006; Brady and Morfini, 2010). Empirical support for compartmentalized degeneration of neuronal processes, including deficits in axonal transport and physiological dysfunction, is changing the way we view neurodegenerative disorders. This is so for glaucoma as well (Figure 1). For example, deletion of the pro-apoptotic gene BAX in the DBA/2J mouse model of glaucoma has a protective effect on the cell body but does not prevent RGC axon loss (Libby et al., 2005). Evidence supporting the idea that neuronal processes are affected separately from cell bodies and actually precede cell body loss has been steadily accumulating (Whitmore et al., 2005; Libby et al., 2005; Jakobs et al. 2005; Schlamp et al., 2006; Jakobs et al., 2006; Stevens et al., 2007; Howell et al., 2007; Buckingham et al., 2008; Soto et al., 2008, Fu et al., 2009; Crish et al., 2010; Baltan et al., 2010). Furthermore, structure in the optic projection persists after axonal transport is depleted (Buckingham et al., 2008; Crish et al., 2010). These and similar findings raise the question of if not cell death, what is blinding in glaucoma?

Early Deficits in Axonal transport

Functional deficits occur early in the progression of glaucoma, an intriguing finding that may change not only how glaucoma is viewed, but eventually how it is treated. The idea of deficits in axonal transport in glaucoma is not new, first appearing in the 1970’s (Anderson and Hendrickson, 1974; Mincker et al., 1976; 1977). Since then, focus has been on the optic nerve head (ONH) as the site of impaired transport through obstruction of normal axoplasmic flow due to effectors such as mechanical blockade (Quigley and Addicks, 1980; Quigley et al., 1981; Hollander et al., 1995; Burgoyne et al., 2005). ONH blockade has figured prominently in a common view of glaucoma by which retrograde transport of pro-survival factors (most notably BDNF) from the RGC synaptic terminal in the brain to the cell body is challenged, thereby triggering apoptosis (Pease et al., 2000; Quigley et al.,...
This view is appealing, since RGC axons are naturally most vulnerable as they pass unmyelinated through the lamina cribrosa plates on their way to the optic nerve (reviewed in Whitmore et al., 2005). The nerve head is an early site for dramatic changes in glial reactivity (Son et al., 2010). Reactive glia in the nerve head are thought to induce compositional changes in the extracellular matrix of the lamina (Fuchshofer et al., 2005; Guo et al., 2005). This process may involve the release of inflammatory cytokines from astrocytes and microglia (Hernandez, 2000; Yuan and Neufeld, 2000; Tezel et al., 2001) and the death of oligodendrocytes (Nakazawa et al., 2006).

Vision loss in glaucoma develops in a well-defined manner, progressing from a paramacular lesion in the temporal field along the arcuate pattern formed by RGC axons and the retinal vasculature (Levin, 2001; Goldblum and Mittag, 2002). It has been argued that this general pattern correlates with anatomical features of the ONH, suggesting that glaucomatous field defects are primarily axogenic in origin, moving retrograde from the nerve head to the retina (Quigley, 1999). Several lines of evidence suggest that a component of glaucoma involves ischemic reperfusion injury at the ONH due to compromised vascular circulation (reviewed in Osborne et al., 2004). It is widely held that these processes represent the primary initiators of RGC death and are among the earliest events associated with RGC loss (Quigley, 1999; Schlamp et al., 2006; Mabuchi et al., 2004; see discussion in Pang et al., 2005).

In other neurodegenerative diseases, however, deficits in axonal transport also precede, and most likely cause, eventual axonal and somatic degeneration (Stokin et al., 2005; Coleman et al., 2005; Morfini et al., 2009). In these disorders active intra-axonal mechanisms downstream from the stressors are promoted as causative agents in defective transport. These include metabolic abnormalities or changes to the molecular motors and cytoskeletal structures that underlie axonal transport (Morfini et al., 2009) resulting in deficits occurring at locations far removed from the site of the stressor (Conforti et al., 2007; Cuchillo-Ibanez et al., 2008). Similar changes occur in the glaucomatous nerve head (Kashiwagi et al., 2003; Barron et al., 2004; Martin et al., 2006; Band et al., 2009) raising the possibility that these mechanisms can produce transport deficits outside of this region.

In animal models of glaucoma, impaired axonal transport first appears in the distal portion of the RGC projection in the brain, well removed from the ONH (Crish et al., 2010). In addition, anterograde axonal transport (cell body to synaptic terminal) appears to be affected earlier than retrograde transport (Danias et al., 2003; Filippopoulous et al., 2006; Buckingham et al., 2008; Crish et al., 2010). If transport blockade was due to mechanical factors at the nerve head, one would expect both anterograde and retrograde transport to be affected similarly. Instead, intra-axonal, functional and cytoskeletal defects affect anterograde transport disproportionally (Wang et al., 1995; Ross et al., 2006). Differences in the specifics of transport blockade, including location, are not trivial but result in very different patterns and mechanisms of degeneration, with deficits in anterograde transport producing axonopathy distal to the defective site (Coleman et al., 2005). This is not to say the ONH is not a critical site or even the critical site for pathogenesis, but rather that whatever ONH processes challenge axonal transport, the insult may be transduced within the axons themselves. We discuss various possibilities for this cascade below.

**Neurophysiology**

Though RGC cell bodies in the retina and axonal structures in the projection may persist until late in disease progression (Buckingham et al., 2008; Crish et al., 2010), physiological deficits can produce clinical symptoms that mirror degenerative processes. Since the goal of studying mechanisms of degeneration must be to identify targets for ameliorating loss of vision, this subtle aspect cannot be overlooked. The pattern electroretinogram (PERG) is a potential tool to detect early glaucomatous changes, though its use as a diagnostic measure is
actively debated (Johnson et al., 1989; Bach and Hoffman, 2008). PERG has produced intriguing results in animal models, showing dysfunctional RGC activity that precedes outright degeneration (Saleh et al., 2007); this has recently been supported in human glaucoma (North et al., 2010). Interestingly the association between elevated IOP and PERG outcome remains ambiguous. There is evidence of a positive relationship in that lowering IOP improves PERG amplitude (Nagaraju et al., 2007; Porciatti and Nagaraju, 2010), but other studies indicate no relationship between PERG amplitude and IOP elevation (Johnson et al., 1989; Sehi et al., 2010).

While these conflicting results may be due to species or experimental differences, it is quite possible that physiological changes in glaucoma are first reflected extra-retinally, e.g., in the RGC axon or axonal terminals. This view is supported by some direct measurements of nerve function (King et al., 2006; Baltan et al., 2010). Two salient points emerge in viewing these studies as a whole. First, the results support the idea that glaucoma is about sensitivity to IOP itself (as the clinical data suggest), rather than just elevated IOP, and second that physiological changes may not report actual degeneration or but rather earlier dysfunction. If these changes are functional in nature, elements of the neural substrate of the retinal projection still exist – and therefore do not need to be regenerated, at least at the scale of replacing cell bodies or long, convoluted axon segments. In this view, functional measures may serve as sensitive indicators of underlying pathology and as valid targets for intervention. It remains to be seen how attempts to reverse loss of function improve eventual outcome.

**Distal Axonopathy**

Compartmentalized degeneration in which neuronal processes and cell bodies are differentially affected is taking a major role as a mechanism of progression in CNS disorders (Fischer et al., 2004; Stokin et al., 2005; Coleman et al., 2005). Long or extensive processes present unique metabolic and logistical challenges to neurons, making compartments such as axons more susceptible to stressor-induced damage.

Axon degeneration traditionally has been described as occurring in one of two programs: Wallerian degeneration or dying back (see Coleman, 2005). Although current evidence blurs the distinction between these two programs, a convenient definition frames Wallerian degeneration as a synchronous event along the entire affected axon, while dying back occurs as a progressive distal-to-proximal cascade that begins at the synaptic terminals. Wallerian degeneration typically results from axon trauma. While transection is most often used to study Wallerian degeneration, it can arise from more subtle localized insults - thus its relevance for glaucoma. Insult at the nerve head could certainly result in axon degeneration that precedes somatic loss, causing Wallerian degeneration to occur along the entire post-ONH axon (Salinas-Navarro et al., 2010). However, typical morphological indications of Wallerian degeneration along the entire length such as axonal beading are not present prominently in glaucomatous human nerves or in animal models (Nickells, 2007; Crish et al., 2010). Progressive degenerative diseases exhibit distal axonopathy early followed by dying back (Fischer et al., 2004; Stokin et al., 2005; Coleman et al., 2005). RGC axons in the optic nerve and brain demonstrate distal axonopathy indicative of underlying dying back, even as more proximal axonal processes appear normal (Nickells, 2007; Crish et al., 2010).

**Synaptic and Dendritic Remodeling**

Of all the neuronal compartments, synapses are the most sensitive to modification – these mechanisms playing the major role in normal plastic changes in the nervous system. Therefore, one could expect synapses to be affected in disease, and this is certainly the case in other degenerative disorders (Spires and Hyman, 2004; Zaja-Milatovic et al., 2005; Zang...
et al., 2005; Day et al., 2006; Knobloch and Mansuy, 2008). Glaucoma is no different, with RGC synapse elimination in the retina implicated early in disease progression (Stevens et al., 2007; Fu et al., 2009). Even so, MRI studies indicate that activity in the synaptic layer of the inner retina persists presumably well after synaptic pruning has started (Calkins et al., 2008). In the distal projection, little has been done examining RGC synaptic terminals in the brain. However, recent studies have indicated that RGC terminals are affected later in disease progression, with changes in NMDA physiology and synaptic loss occurring well after other functional pathologies emerge in glaucoma (Georgiou et al., 2010; Crish et al., 2010). It is not known how the dynamics of RGC terminal loss relate to those governing dendritic pruning for RGC-recipient neurons in central targets (Gupta et al., 2007). Another open question is how changes in synaptic terminals and their postsynaptic retinal targets relate to dendritic and synaptic changes in the retina.

While typically not as long as axons, dendritic processes are often very extensive, placing similar sorts of metabolic demands on a cell. Consequentially, early dendritic changes may be expected in glaucoma — and there is evidence to support this case in animal models. Jakobs et al. (2006) demonstrated dendritic field remodeling preceding cell body loss in the DBA/2J mouse model consisting of both loss of arborization and abnormal morphologies in existing processes. These changes appear to parallel the degeneration in the distal axon in the same model (Crish et al., 2010), but a direct comparison has not yet been made. Importantly, dendritic pruning (like axonal degeneration) can be mitigated at least in part by treatment with trophic factors (Weber et al., 2008; Weber et al., 2010).

The Calcium Hypothesis
Ca\(^{2+}\) and Neurodegeneration

In healthy neurons, Ca\(^{2+}\)-dependent cascades influence a variety of cellular functions, including exocytosis, gene transcription, membrane trafficking and intracellular respiration (Berridge et al., 2000). Normally, the concentration of cytosolic Ca\(^{2+}\) is roughly 10,000 times lower than that in the extracellular space (Hernandez-Fonseca and Massieu, 2005). An important, common component of axonopathy across neurodegenerative disorders is increased influx of extracellular Ca\(^{2+}\), which triggers cytoskeletal degradation through enzymatic activity (Coleman, 2005). Excessive levels of neuronal Ca\(^{2+}\) lead to breakdown of Ca\(^{2+}\) homeostasis and to a series of cytoplasmic processes that promote caspase-dependent neuronal cell death (Bredesen et al., 2006). Importantly, while intra-axonal Ca\(^{2+}\) increases prior to degeneration, the source of the influx can be either directly through the axon or through the neuronal cell body and dendrites (Coleman and Perry, 2002). There are many mechanisms that could contribute to increased influx, which are likely to depend on neuronal cell type and the nature of the initiating insult. For example, in Alzheimer’s disease, neurons bearing neurofibrillary tangles demonstrate higher levels of free and bound Ca\(^{2+}\). activated Ca\(^{2+}\)-dependent proteases, and the Ca\(^{2+}\)-activated enzyme transglutaminase (reviewed in Mattson and Chan, 2003). Elevated intracellular Ca\(^{2+}\) could arise through direct conductance through amyloid β protein channels (Kawahara and Kuroda, 2000).

Axonal degeneration in glaucoma bears several striking similarities to that in other neurological diseases (Tatton et al., 2003; Whitmore et al., 2005). These include abnormal processing of APP (McKinnon, 2003), dependence on target-derived trophic support (Quigley et al., 2000), and involvement of various Ca\(^{2+}\)-mediated cascades (Whitmore et al., 2005). For example, cleavage of calcineurin, a Ca\(^{2+}\)-dependent protein phosphatase, occurs in response to elevated IOP, and inhibiting calcineurin systemically inhibits pressure-induced RGC axon loss in the optic nerve (Huang et al., 2005). Relevant to transport deficits and axonopathy is Ca\(^{2+}\) activation of calpains. Calpains are a class of Ca\(^{2+}\)-dependent proteases that have been implicated in a number of neurodegenerative conditions (Vosler et
al., 2008), including glaucoma (Huang et al., 2008). While \( \text{Ca}^{2+} \) dysregulation-induced calpain activation can elicit a number of pathological changes in the cell, most relevant here are its activities on the cytoskeleton. Calpains act directly on synaptic components (Lu et al., 2000; Jourdi et al., 2005) and breaks down the structural proteins alpha-II-spectrin and heavy chain neurofilaments (Siman et al., 1984; Chan and Mattson, 1999). Calpains also activate kinases such as cyclin dependent kinase 5 (Lee et al., 2000), extracellular signal regulated kinases (Veeraana et al., 2004), and stress activated protein kinases (Goni-Oliver et al., 2007). The latter serve to phosphorylate neurofilaments and the microtubule associated protein Tau, slowing or blocking axonal transport (Figure 2; Shea et al., 2004, Shea and Chan, 2008). Recent evidence demonstrates that in experimental glaucoma, calpains are activated with cleavage of associated substrates in RGCs (Huang et al., 2010).

**\( \text{Ca}^{2+} \) and Oxidative Stress**

With aging, the CNS becomes increasingly susceptible to oxidative damage to DNA and protein (Cakatay et al., 2001). Oxidative stress markers have been implicated in several animal studies involving acutely induced elevations in IOP. These includes higher levels of lipid peroxidation and protein carbonyl content (Moreno et al., 2004; Ko et al., 2005; Tezel et al. 2005; Ferreira et al., 2010). In the DBA2J mouse, ceruloplasmin, an important antioxidant implicated in other neurodegenerative conditions, is strongly upregulated (Steele et al., 2006; Stasi et al., 2007). Ceruloplasmin exerts much of its effect on oxidative stress by converting ferrous (\( \text{Fe}^{2+} \)) iron into the ferric (\( \text{Fe}^{3+} \)) form. This reduces the iron-mediated formation of reactive oxygen species (ROS) and allows iron to interact with transferring or storage proteins such as transferrin and ferritin. All three iron-associated proteins act as antioxidants and are elevated in monkeys with chronically elevated IOP as well as in human glaucoma (Farkas et al., 2004; Stasi et al., 2007). Several anti-oxidative markers are also elevated in the aqueous humor of glaucoma patients, including catalase, glutathione peroxidase, superoxide dismutase, and malondialdehyde (Ferreira et al., 2004; Ghanem et al., 2010). In human glaucomatous retinas and optic nerve heads, glial-related oxidative stress pathways are upregulated (Tezel et. al., 2007).

Oxidative stress can arise from \( \text{Ca}^{2+} \) dysregulation through several mechanisms, including increasing metabolic rate (Zundorf et al., 2009) and activation of ROS-producing enzymes such as nitric oxide synthase and nicotinamide adenine dinucleotide phosphate oxidase (Feissner et al., 2009; Abramov et al., 2007). ROS formation can directly damage proteins, lipids, and nucleic acids. Even worse, oxidative stress creates a positive feedback loop with \( \text{Ca}^{2+} \) dysregulation. ROS impair mitochondrial respiration and depolarize the mitochondrial membrane, thereby decreasing the organelle’s ability to buffer \( \text{Ca}^{2+} \) (Ward et al., 2000; Murchison and Griffith, 2007; Esin, 2007). In addition, ROS promote \( \text{Ca}^{2+} \) release from internal stores due to effects on the ryanodine and inositol triphosphate receptors (Missiaen et al., 1992; Abramson et al., 1995; Feng et al., 2000; Bulynk et al., 2004). Finally, ROS damage plasma membrane proteins, such as \( \text{Ca}^{2+} \) ATPase and the Na-Ca exchanger, responsible for maintaining and restoring the large differential in \( \text{Ca}^{2+} \) concentrations across the membrane (Zaidi and Michaelis, 1999; Huschenbett et al., 1998). Together these mechanisms maintain, and even exacerbate, abnormal \( \text{Ca}^{2+} \) levels in the cell (Figure 2). The neuroprotective drug deprenyl apparently acts by promoting transcription of Bcl2, which over time increases the \( \text{Ca}^{2+} \)-buffering capacity of mitochondria (Rodnitsky, 1999). Bcl2 expression increases 3-fold in RGCs exposed to elevated pressure (Sappington et al., 2006), suggesting that an increase in intracellular \( \text{Ca}^{2+} \) could initiate a similarly protective pathway.
Implications for Neuroprotective Therapies

For years it has been recognized that many drugs used to lower IOP or reduce vasoconstriction also have a secondary, direct action on RGCs themselves by modulating cation influx and accumulation of intracellular Ca$^{2+}$ over time. For example, non-selective β-adrenoceptor antagonists in addition to lowering IOP also blunt both the influx of Na$^+$ and Ca$^{2+}$ into RGCs (Wood et al., 2003; Osborne et al., 2004). In the DBA2J mouse, the β-blocker timolol promotes RGC axonal survival almost as well as the NMDA antagonist memantine (Schuettauf et al., 2002). Iganidipine is a broad Ca$^{2+}$ antagonist that reduces vascular constriction in the retina and nerve head induced by endothelin-1 and so is thought to relieve pressure-induced ischemic insult (Ishii et al., 2003). Studies of pressure-related ischemic/reperfusion injury at the nerve head indicate that influx of extracellular Ca$^{2+}$ into the RGC axoplasm is critical to the pathology associated with ischemic insult (Stys et al., 1992). In addition to reducing ischemic injury through their action as vasodilators, β-blockers also counteract ischemia by reducing Ca$^{2+}$ influx into the RGC (Tomita, 2000). This has been demonstrated directly in spectrometry studies comparing the efficacy of various β-adrenoceptor antagonists in attenuating RGC intracellular Ca$^{2+}$ (Wood et al., 2003). Optical imaging studies have demonstrated that betaxolol, a β1-antagonist, is highly effective at reducing Ca$^{2+}$ influx to RGCs induced by glutamate receptor agonism (Zhang et al., 2003). In axonal injury, an action of Na$^+$ is thought to involve leakage through non-NMDA channels that induces a reversal of the intra-axonal Na$^+$/Ca$^{2+}$ exchanger, leading to a rise in intracellular Ca$^{2+}$ (Stys et al., 1992).

In so-called normal pressure glaucoma, the absence of elevated IOP has compelled several hypotheses to explain the progression of visual field loss and nerve damage, which are nearly identical to those with elevated IOP (Heijl et al., 2002). A significant risk factor is nonuse of Ca$^{2+}$ channel antagonists (Tomita, 2000). A focus has been on ocular blood flow through the optic nerve in the absence of elevated pressure, since most β-adrenoceptor antagonists modulate hemodynamics (Tomita et al., 1999). Other evidence suggests a more likely mechanism may be direct modulation of RGC intracellular Ca$^{2+}$. An earlier investigation compared the effects of nimodipine, a Ca$^{2+}$ channel antagonist, on ocular blood flow using Doppler imaging in normal tension glaucoma patients (Boehm et al., 2003). These investigators found that while the drug did not affect blood flow or IOP, contrast sensitivity was dramatically improved for patients in the treatment group. Their conclusion was similar to that derived from other studies using Ca$^{2+}$ channel antagonists: that the efficacy is a direct result of decreasing Ca$^{2+}$ influx to the RGC or its axon. An open question is whether other types of neuroprotective therapies also modulate Ca$^{2+}$-dependent processes more directly, such as those involving modulation of glial signals (Bosco et al., 2008), trophic factors (Morquette and di Polo, 2008; Saragovi et al., 2009), or mitochondrial dysfunction (Ju et al., 2010).

Perspective

The evidence summarized here points towards a progression of pathology in glaucoma that mirrors recent findings in other major neurodegenerative disorders. Degeneration and functional loss appear to be compartmentalized, affecting the neuronal processes well before the cell body in the retina (Figure 1). This has important ramifications for the search for new neuronal-directed treatments. Addressing loss of function or axonal dystrophy are far more tractable problems than replacing lost cell bodies in the retina or long stretches of their axons in the optic projection. Indeed an exciting avenue of therapeutics seeks to supplant loss of function by supplying exogenous ionic activity (Corredor and Goldberg, 2009). Compartmentalized pathogenesis allows for a considerably more generous therapeutic window between the onset of the disease and irreversible degeneration (Adalbert et al., 2009). The primary message gleaned from completed work is that (1) axonal injury is early
with deficits in active transport among the first events, (2) axon injury can occur independently of cell body loss, and (3) synapse loss and dendritic pruning precede drop-out in the retina. However, much work remains. The temporal relationship between axonal and retinal events remains an important issue to resolve, as does that between synaptic and dendritic pruning.

Glucoma is a multi-faceted syndrome that is etiologically complex. We have focused on describing known relationships between degenerative events in the RGC and possible dysregulation of Ca\textsuperscript{2+}-dependent processes, including oxidative stress mechanisms. Our review of pre-clinical and clinical literature indicates that targeting these processes may have relevance as therapeutic opportunities, especially for those who do not respond to IOP-lowering regimens (Baltmr et al., 2010). They are, however, downstream of key initiating events we do not as yet understand. If the disease involves sensitivity to IOP, what are the transducing elements? Are they extrinsic to the RGC, perhaps mediated exclusively by glial cells of the nerve head and retina? If not, RGCs themselves may sense changes in their own microenvironment independent of glial signaling. RGCs utilize a rich variety of cation channels, including many with a robust Ca\textsuperscript{2+} conductance, for processing excitatory signals from bipolar neurons. Recent results indicate that RGCs also express a number of additional membrane channels that in other neuronal systems respond directly to pressure-related insults. For example, the vanilloid-1 transient receptor potential (TRPV1) channel increases in RGCs with elevated IOP and contributes to increased intracellular Ca\textsuperscript{2+} with exposure to pressure (Sappington et al., 2009). The channel may also mediate secretion of protective cytokines (Sappington and Calkins, 2008). Similarly, pressure-induce release of ATP in the retina has been linked to activation of pannexin hemichannels (Reigada et al., 2008). Whether these or similar channels form a substrate through which RGCs can transduce pressure stimuli and respond directly to glaucomatous insults in vivo remains to be seen. Even so, it seems likely that the best bet for novel neuroprotective opportunities lies in improving our understanding of how RGCs respond to the various stressors associated with glaucoma.

**Highlights**

- Glaucoma is an age-related neurodegenerative disorder
- The disease involves degeneration of the retinal ganglion cell projection
- Ganglion cell degeneration is compartmentalized, with axonal and retinal programs
- Calcium dysregulation is an emerging theme in glaucomatous neurodegeneration

**Acknowledgments**

The Supported by NIH EY017427 (DJC), the Melza M. and Frank Theodore Barr Foundation through the Glaucoma Research Foundation (DJC), a Departmental Unrestricted Award from Research to Prevent Blindness, Inc. (DJC), an American Health Assistance Foundation National Glaucoma Research Award (DJC), Fight for Sight (SDC), the Vanderbilt Discovery Science program (DJC), and Vanderbilt Vision Research Center (P30EY008126).

**References**


Naracine. Author manuscript; available in PMC 2012 March 10.


Jourdi H, Lu X, Yanagihara T, Lauterborn JC, Bi X, Gall CM, Baudry M. Prolonged positive modulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors induces calpain-mediated PSD-95/Dlg/ZO-1 protein degradation and AMPA receptor down-


McKinnon SJ, Lehmam DM, Kerrigan-Baumrind LA, Merges CA, Pease ME, Kerrigan DF, Ransom NL, Tahzib NG, Reitsamer HA, Levkovitch-Verbin H, Quigley HA, Zack DJ. Caspase activation...


Sehi M, Grewal DS, Feuer WI, Greenfield DS. The impact of intraocular pressure reduction on retinal ganglion cell function measured using pattern electroretinogram in eyes receiving latanoprost 0.005% versus placebo. Vision Res. 2010 2010 Sep 8. [Epub ahead of print].


*Neuroscience*. Author manuscript; available in PMC 2012 March 10.
Figure 1. Degenerative Events Affecting the RGC Projection in Glaucoma

The RGC axon passes unmyelinated through a plexus of astrocytes in the nerve head that becomes neurochemically reactive in glaucoma. This is an established zone of vulnerability to IOP- and age-related stressors. All or nearly all RGCs project contralaterally to the superior colliculus, with small collaterals terminating in more anterior sites. These include the suprachiasmatic nucleus (SCN), lateral geniculate nucleus (LGN), and the pretectal nuclei: olivary pretectal (OPT), nucleus of optic tract (NOT), and posterior pretectal (PPT). A small fraction of RGCs also form ipsilateral projections. Early degenerative events in glaucoma include failure of anterograde transport and axonopathy, both of which progress from distal projection sites towards the optic nerve and retina. RGC axon terminals and their synapses eventually degrade. In the retina, degeneration includes loss of excitatory synapses and dendritic pruning.
Figure 2. Possible Ca\(^{2+}\)-Dependent Mechanisms that Could Contribute to Glaucomatous Neurodegeneration

Multiple stressors relevant in glaucoma could disrupt homeostasis of RGC intracellular Ca\(^{2+}\). Two immediate consequences include oxidative stress and activation of calpains. Oxidative stress exacerbates Ca\(^{2+}\) dysregulation by reducing the capacity for mitochondria to buffer Ca\(^{2+}\), increasing release of Ca\(^{2+}\) from internal stores through interactions with the ryanodine (Ry) and inositol-triphosphate (IP3) receptors, and reducing clearance of intracellular Ca\(^{2+}\) by damaging membrane-bound maintenance proteins such as Ca\(^{2+}\) ATPase and the Na\(^{+}/Ca^{2+}\) exchanger. Activated calpains increase the activity of cyclin-dependent kinase 5 (Cdk5), glycogen synthase kinase 2 (GSK3), and stress-activated protein kinases (SAPK) which phosphorylate cytoskeletal elements, slowing or stopping their axonal transport and promoting their aggregation. Calpains also directly degrade both cytoskeletal scaffolding and synapses. Cytoskeletal degradation and reduced mitochondrial function further compromise axonal transport and maintenance of dendrites, thus likely promoting synapse elimination. These degenerative components trigger the eventual apoptotic elimination of the cell body. Components in grey boxes represent likely targets not directly established for glaucoma.