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NEUROTROPHIN ROLES IN RETINAL GANGLION CELL SURVIVAL: LESSONS FROM RAT GLAUCOMA MODELS

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Abstract

The neurotrophin (NT) hypothesis proposes that the obstruction of retrograde transport at the optic nerve head results in the deprivation of neurotrophic support to retinal ganglion cells (RGC) leading to apoptotic cell death in glaucoma. An important corollary to this concept is the implication that appropriate enhancement of neurotrophic support will prolong the survival of injured RGC indefinitely. This hypothesis is, perhaps, the most widely recognized theory to explain RGC loss resulting from exposure of the eye to elevated intraocular pressure (IOP). Recent studies of NT signaling using rat glaucoma models, have examined the endogenous responses of the retina to pressure exposure as well as studies designed to augment NT signaling in order to rescue RGC from apoptosis following pressure-induced injury. The examination of these studies in this review reveals a number of consistent observations and provides direction for further investigations of this hypothesis.

Keywords

glaucoma; intraocular pressure; neurotrophic factors; TRK receptors; p75 neurotrophin receptor

1. Introduction

The retinal pathology of glaucoma is characterized by selective RGC loss that is attributed to cell death by apoptosis (Quigley et al. 1995; Kerrigan et al. 1997; Quigley 1999). The specific downstream signals and effector molecules involved in RGC apoptosis have been the subject of intense investigation and scientific review (Nickells 1999; Farkas & Grosskreutz 2001; Huang et al. 2005; Libby et al. 2005; Nickells 2007), and will not be considered in detail here. However, the key upstream signals that trigger the apoptotic cascade in RGC in glaucoma are less clear and deciphering them is the main subject of this review.

Elevated IOP is the most recognized risk factor for primary open-angle glaucoma. Studies in primates demonstrate that experimentally elevated IOP results in axonal transport obstruction at the optic nerve head (Minckler et al. 1977; Minckler et al. 1978). When IOP is acutely elevated in rats, the retrograde transport of radiolabeled brain-derived neurotrophic factor (BDNF), a potent trophic factor for RGC, is obstructed (Quigley et al. 2000). Further,

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immunolocalization studies suggest that the BDNF receptor, TRKB, accumulates in the optic nerve head (Pease et al. 2000). These observations support the suggestion that transport obstruction inhibits the retrograde delivery of NT-TRK receptor complexes from the brain to the RGC soma, resulting in the deprivation of neurotrophic support that triggers apoptosis (Quigley 1995; Pease et al. 2000; Quigley et al. 2000; Vrabec & Levin 2007). An important implication of this concept is the supposition that the appropriate therapeutic manipulation of the NT signaling pathways will indefinitely prolong the survival of injured RGC. These closely related concepts are referred to as the NT hypothesis and will be examined in this review.

2. NT and RGC Survival

This hypothesis is attractive because NT are known to promote neuronal survival and regeneration in many experimental paradigms. When deprived of retrograde neurotrophic support from target neurons, developing sensory and sympathetic neurons die by apoptosis (Purves 1988; Oppenheim 1991). The application of exogenous BDNF to the superior colliculus reduces developmental cell RGC death (Raff et al. 1993; Frade et al. 1997; Ma et al. 1998). NT have important survival properties for adult neurons as well. The application of exogenous NT, especially BDNF, to isolated RGC prolongs their survival in culture (Johnson et al. 1986; Rodriguez-Tebar et al. 1989; Cohen-Cory & Fraser 1994; Meyer-Franke et al. 1995; Rohrer et al. 2001). Importantly, multiple studies indicate that the intravitreal injection of BDNF prolongs injured, adult RGC survival *in vivo* (Mey & Thanos 1993; Mansour-Robaey et al. 1994; Peinado-Ramon et al. 1996; Di Polo et al. 1998; Chen & Weber 2001).

BDNF is a member of the nerve growth factor family of NT, which also includes nerve growth factor (NGF), NT3, and NT4/5. These NT exert their effects by binding to either specific tyrosine kinase (TRK) receptors or the P75NT receptor (P75NTR), forming what are generally understood to be survival and death signaling complexes, respectively. The specific TRK/NT partners are: TRKA/NGF, TRKB/BDNF, TRKB/NT4/5 and TRKC/NT3. TRK receptor activation of downstream survival signaling pathways include both extracellular signal-regulated kinases (ERK) and AKT. All four NT bind to P75NTR and, while P75NTR signaling is complex, it generally includes the downstream activation of JUN N-terminal kinases (JNK) and is coupled to the mitochondrial apoptotic pathway (Dhanasekaran & Reddy 2008).

Importantly, NT are locally produced in the retina (Ugolini et al. 1995; Cui et al. 2002; Vecino et al. 2002; Spalding et al. 2004; Seki et al. 2005). Therefore, while increased IOP may prevent the retrograde transport of NT-receptor complexes, endogenous retinal sources could provide adequate compensation. The activity and physiological roles of NT from various sources for the RGC is still lacking and, additionally, NT can have different effects on somal and axonal compartments (Kimpinski et al. 1997; Toma et al. 1997; Kuruvilla et al. 2000).

While the pro-survival role of TRK receptor-mediated NT signaling is well-established and P75NTR can signal cell apoptosis (Miller & Kaplan 2001), studies have continued to reveal diverse, sometimes opposing, roles for both ligands and receptors, as well as to identify new signaling partners (Kalb 2005). Maximal activation and substrate specificity for NGF is achieved when TRKA is co-expressed with P75NTR; suggesting that a high specificity receptor complex of TRK, P75NTR and NT is formed (He & Garcia 2004; Nykjaer et al. 2005; Wehrman et al. 2007) or degradation of the activated receptor is inhibited (Makkerh et al. 2005). Paradoxically, P75NTR can promote neuronal survival via nuclear factor kappa B signaling (NFκB) (Hamanoue et al. 1999; Mamidipudi et al. 2002) and TRK receptor activation has been found to induce neuronal death under certain circumstances (Kalb 2005). Another layer of complexity is added by the discovery that proNT, the precursors of mature NT, can be secreted and serve as death signals in complex with P75NTR and sortilin (Lee et al. 2001; Teng et al. 2005).

While studies show that NT administration can slow the rate of RGC loss following injury, so far NT augmentation strategy has resulted in temporary, rather than permanent, cell survival (Clarke et al. 1998; Di Polo et al. 1998; Isenmann et al. 1998; Bahr 2000; Leaver et al. 2006), perhaps due to a reduced availability of NT receptors in injured RGC (Cheng et al. 2002; Chen & Weber 2004). Sustained RGC survival remains the goal of neuroprotective strategies based on the NT hypothesis. In addition, as appealing as the NT hypothesis may be, it does fail to take into account some potentially important observations. First, BDNF knock-out mice have been shown to have normal axon counts, illustrating that while exogenous BDNF may suppress developmental neuronal loss, an endogenous supply is not essential for RGC survival (Cellerino et al. 1997). Additionally, survival and axon extension of RGC *in vitro* has been shown to require multiple growth factors as well as the elevation of intracellular cyclic AMP and electrical activity (Meyer-Franke et al. 1995; Goldberg et al. 2002), implying that complex signaling relationships are necessary for cell survival and function in vivo. Finally, while the administration of NT can result prolonged RGC survival, there is also evidence that exogenous NT also can downregulate TRK receptor expression levels in the neural cells and tissues, including the retina (Frank et al. 1997; Knusel et al. 1997; Sommerfeld et al. 2000; Chen & Weber 2004; Gibbons & Bailey 2005).

Figure 1 offers a simplified summary of the NT signaling pathways that are mentioned in this review.

3. Modeling Glaucoma in Laboratory Rats

With these concepts in mind, experimental glaucoma models in rats have been used to explore aspects of the NT hypothesis. These models all rely on the production of elevated IOP and their characteristics and suitability have been reviewed (Danias et al. 2002; Levkovitch-Verbin 2004; Morrison et al. 2005; Pang & Clark 2007; Morrison et al. 2008a). While many variations in how the models are modified and implemented exist, there are basically three techniques commonly used to elevate IOP. These are referred to as the laser, hypertonic saline and cautery models, designations that will be used throughout this review.

In the laser model, the limbus and underlying trabecular meshwork are damaged by multiple laser burns (Ueda et al. 1998; WoldeMussie et al. 2001; Levkovitch-Verbin et al. 2002). In the hypertonic saline model, a method originated in our laboratory, the trabecular meshwork and Schlemm's canal are sclerosed by the retroinjection of hypertonic saline into the isolated limbal plexus via a tiny episcleral vein (Morrison et al. 1997). In both models, damage is to the aqueous outflow pathways and results in outflow obstruction (Morrison et al. 1997; Levkovitch-Verbin et al. 2002; Nissirios et al. 2008). This is important because aqueous outflow obstruction is a principle characteristic of human primary open angle glaucoma (Tamm & Fuchshofer 2007).

In addition, both methods result in IOP elevation up to approximately twice normal levels, producing a roughly equivalent range of optic nerve axon and RGC loss (up to 90%) over a period of weeks (Morrison et al. 2005). In the hypertonic saline model, pressure elevation is generally delayed until several days following the injection and multiple injections may be necessary to produce stable IOP elevation. We find that the pattern and magnitude of pressure elevation varies among animals. In the laser model, pressure is rapidly elevated following treatment but tends to normalize after a period of a few weeks, requiring a second laser treatment if sustained elevation is desired.

In the other commonly used method, a number of larger veins located just posterior to the rectus muscle insertions are cauterized, apparently reducing venous outflow from the globe and producing ocular venous congestion (Grozdanic et al. 2003; Morrison et al. 2005). These veins have been identified as either episcleral or vortex veins (Shareef et al. 1995; Grozdanic et al. 2003). The method results in a similar degree of IOP elevation as the others. However, in the

cautery model, the injury progresses at a slower, linear rate (Garcia-Valenzuela et al. 1995; Sawada & Neufeld 1999; Ko et al. 2001), reaching a maximum of about 40% in 10 to 28 weeks. Axonal degeneration in this model has not been well characterized, but also appears to be significantly less than in the laser or hypertonic saline models (Shareef et al. 1999; Grozdanic et al. 2003; Wang et al. 2004) and aqueous outflow may not be significantly obstructed (Nissirios et al. 2008).

In all methods, careful documentation of IOP is necessary to allow pressure dose comparisons between experimental groups and correlation of responses to IOP history. The equipment and techniques available for IOP measurement in rodents have been recently reviewed (Morrison et al. 2005; Pang et al. 2005). Two important factors that impact IOP are frequently ignored when pressure data is collected. The use of common anesthetics produces an unpredictable lowering of IOP in rats (Jia et al. 2000b). Additionally, there is a 10 mm Hg circadian range of IOP in rats, peaking during the dark phase. In glaucoma model eyes with normal light phase IOP measurements, this dark phase peak may be doubled and result in axonal degeneration (Jia et al. 2000a). IOP measurements in conventionally housed rats should be obtained during both light and dark phases, or alternatively, housing in constant, low-level light will stabilize the circadian variations without producing retinal injury (Jia et al. 2000a; Morrison et al. 2005). Ignoring the effects of circadian rhythms and anesthesia is likely to lead to a significant underestimation of the actual IOP experienced by the eye.

4. Endogenous NT Function In Rat Glaucoma Models

4.1 Endogenous NT And Receptor Levels

Using the hypertonic saline model and immunohistochemistry, we examined the sequence of changes in retinal and optic nerve head protein distribution that accompany exposure to elevated IOP (Johnson et al. 2000). At IOP levels approximately twice normal values for one week, immunostaining for BDNF and NT4/5 was reduced in the optic nerve head and the inner layers of the retina, changes that were accompanied by evidence of optic nerve axon degeneration. After longer exposures, retinas contained scattered individual RGC layer somas that were strongly labeled by NT antibodies, suggesting upregulation of NT expression in some injured RGC. RGC layer apoptosis was increased at all time points, even in retinas with no immunohistochemical evidence of altered NT distribution. Still, the relatively early loss of retinal NT immunostaining offers some support for the hypothesis that NT deprivation contributes to apoptotic RGC loss.

Rudzinski and colleagues, using the vein cautery model, evaluated the effect of elevated IOP on endogenous retinal NT and NT receptors at time points up to 28 days post surgery (Rudzinski et al. 2004). Retinal BDNF mRNA levels remained relatively unchanged until day 28, when they approximately doubled, NT4/5 was unchanged, NT3 declined slightly and NGF peaked at about two-fold at 14 days, then declined to control levels at 28 days. Receptor message level analysis indicated that TRKB remained constant during the experimental period, while TRKC mRNA levels were significantly increased to approximately 300%. This TRKC increase was due to the upregulation of truncated, inactive TRKC isoforms, while levels of the active, full-length form remained unchanged. TRKA levels increased slowly, reaching significance at about 2.3 fold at day 28 while increases in P75NTR levels were not significant. By western analysis, BDNF, NGF, NT3 and TRKA protein levels paralleled message levels, while levels of TRKB and TRKC were not determined. Fluorescent co-localization studies indicated that increases in TRKA occurred in RGC, while the increased, inactive TRKC was localized to RGC layer glia. From this study, the authors concluded that elevated IOP induces time-dependent changes in retinal NT and receptor levels that are related to the stress and consequent death of RGC. Their study also confirms the linear rate of RGC loss in this model and also suggests that the observed fluctuations have negligible impact on the rate of apoptosis. Further,

levels of NT and their receptors are generally increased, providing little evidence that elevated IOP results in RGC NT deprivation. In contrast, these observations illustrate the potential importance of NT-receptor signaling other than that of BDNF-TRKB.

Also using the cautery model, Kim et al (Kim et al. 2007) found that elevated IOP resulted in elevated retinal BDNF message levels that peaked over four times control levels at 1 week, then declined to approximately twice control levels at 4 and 8 weeks. While the pattern of BDNF response was different from that found by Rudzinski et al., this study also found an overall increase in BDNF mRNA levels in glaucoma model retinas.

In a recent study, Coassin et al (Coassin et al. 2008), used the hypertonic saline model to produce eyes with at least 20% IOP elevation. They found a gradual increase in retinal NGF protein by ELISA, reaching significance at 35 days post-injection, coinciding with a significant increase in the number of apoptotic RGC. They also detected a gradual increase in NGF mRNA expression and, at 35 days, decreased ratios for TRKA relative to P75NTR. From these observations, they concluded that NGF is overexpressed in experimental glaucoma, but not to an extent sufficient to support RGC survival, perhaps due to the relatively greater upregulation of P75NTR. Again, this study illustrates the potential importance of multiple NT signaling pathways to RGC survival.

Over the last few years, we have also investigated *in vivo* alterations in retinal NT and receptor mRNA and protein levels using our hypertonic saline glaucoma model, with preliminary findings published in abstract form (Jia et al. 2004; Cepurna et al. 2007). In contrast to some previous findings, our completed studies [manuscript currently under review at EER] indicate that while retinal BDNF mRNA and mature protein levels are not significantly altered, there are moderate alterations in receptor expression. Message levels for TRKB decrease and P75NTR increase linearly, reaching values in the most injured retinas that are 67% ($p<0.01$) and 210% ($p<0.001$) of controls, respectively. However, regardless of these message changes, the activated, pro-survival form of TRKB protein actually demonstrates a slight, but significant, linear increase with increasing nerve injury ($p<0.03$), while P75NTR protein levels are not significantly altered.

In summary, these studies offer no more than scant support for the NT hypothesis. Rather, they suggest that multiple NT signaling pathways are activated in pressure-induced RGC injury, implying complex cellular responses.

4.2 Alterations In Endogenous NT Survival And Apoptosis Signaling Pathway Intermediates

Neuronal injury is linked to induction of the JUN transcription factor and its prolonged phosphorylation by JNK is linked to apoptosis (Oppenheim et al. 1990; Koistinaho et al. 1993; Estus et al. 1994; Tournier et al. 2000; Lei et al. 2002). Therefore, using the laser model, Levkovitch-Verbin and colleagues examined the time course of retinal immunostaining for JUN and activated pJUN following IOP elevation (Levkovitch-Verbin et al. 2005). They found immunolabeling for both peaked at 1 week following IOP elevation and simultaneously retinal levels of pJUN protein were increased by approximately 60%. While JUN activation is associated with RGC death by apoptosis, it also is implicated in regenerative and survival responses (Hull & Bahr 1994; Robinson 1994; Schaden et al. 1994; Lu et al. 2003), supporting multiple roles for the transcription factor following retinal injury.

Using the laser model, Kwong and Caprioli also examined the correlation between RGC layer apoptosis labeling and activated JNK labeling of RGC in eyes with IOP levels at about 150% (Kwong & Caprioli 2006). By immunolabeling, they found that the number of activated JNK-labeled RGC layer cells doubled at 5-weeks in glaucoma model retinas. Retrograde fluorescent labeling identified some of these cells as RGC and some apoptotic cells were positive for

activated JNK. These observations support the association of JNK activation with the process of RGC apoptosis.

Kim and Park used the cautery model to examine activation of NT survival pathway signaling at time points up to 6 weeks (Kim & Park 2005). They also confirmed the gradual, steady loss of RGC in this model, with a 7% loss at two weeks and 30% at 6 weeks. Using immunohistochemistry and western analysis, they examined TRK-activated signaling via AKT that results in the phosphorylation of BAD (pBAD), inhibiting its pro-apoptotic activity. They found that levels of activated AKT and pBAD peaked at 1 week post-surgery, before falling to levels below control values by 6 weeks, suggesting that this pro-survival pathway response is short lived.

They also found evidence of activation of the extrinsic (caspase 8) signaling pathway, involving FAS ligand and FAS Associated Death Domain (FADD). Generally, this pathway is associated with immune and cell mediated toxicity, but it also may be activated via the intrinsic (mitochondrial) caspase pathway (Weishaupt et al. 2003). Kim and Park found elevated levels of pro-apoptotic FAS ligand, FADD, and activated caspase 8 at all time points following cautery. By fluorescent retrograde labeling and immunohistochemistry, they showed co-localization of these proteins to RGC. In a follow-up study, they found that FAS ligand colocalized to microglia and FADD to both Muller cells and RGC, suggesting potential glial participation in the extrinsic caspase activation process (Ju et al. 2006). Because pro-survival events tended to peak earlier than some of the pro-apoptotic ones, Kim and Park concluded that the former delayed RGC death, although they did find apoptotic RGC even during peak pro-survival signaling.

Levkovitch-Verbin et al followed up their previous study by an examination of both apoptotic and survival pathway activation in the laser model (Levkovitch-Verbin et al. 2007). Prior to peak levels of apoptotic caspase 3 activation at day 15, they found peak expression of activated pro-apoptotic p38 as well as pro-survival ERK1 and AKT. While IOP levels returned to normal values at about 3 weeks, active caspase 3 remained at very high levels up to 30 days and JNK levels peaked at the same time, suggesting prolonged retinal pro-apoptotic responses following IOP normalization.

The upregulation of caspase pathways in experimental glaucoma was further studied by Huang et al using the hypertonic saline model (Huang et al. 2005). In eyes with 10 days of elevated IOP, they used laser capture microdissection to select retrograde-labeled RGC and quantitative RT-PCR to determine message levels. They demonstrated upregulated levels of both caspase 8 and 9 mRNA, initiators of the extrinsic and intrinsic (mitochondrial) caspase cascades, respectively. Simultaneously, the number of retrograde labeled RGC had fallen by 34%. Western analysis showed increased levels of activated caspases 8 and 9 proteins in glaucoma model retinas. The activation of caspase 8 adds to the evidence that death signaling pathways other than those regulated by P75NTR play a role in RGC loss in glaucoma.

5. Neuroprotection Strategies Designed to Rescue Injured RGC

While our knowledge of the early responses of RGC to pressure-induced injury is still very incomplete, glaucoma models have been implemented in a number of studies designed to support RGC survival during and after pressure-induced injury.

5.1 Neuroprotection by Application of Exogenous NT

Using the cautery model, Ko and colleagues examined the effect of multiple intravitreal BDNF injections on RGC survival (Ko et al. 2000). RGC were retrograde labeled and IOP elevated to sustained values of about twice baseline. Without BDNF treatment, RGC loss after 37 days

was 27%. Four sequential injections of BDNF significantly reduced this loss to 19% and augmentation of BDNF with a systemic free-radical scavenger limited the cell loss to 10%. As a follow up to this study, a time course of RGC survival with BDNF treatment alone was determined (Ko et al. 2001). At 33 and 47 days following IOP elevation, vehicle treatment produced 16% and 27% RGC loss, while BDNF significantly decreased this loss to 9% and 17% at the same respective time points.

Martin et al implemented a gene therapy strategy to sustain BDNF availability to injured RGC (Martin et al. 2003). Two weeks prior to IOP elevation by laser treatment, they intravitreally injected a modified viral vector transgene that increased retinal BDNF levels by four fold. Then, at four weeks following IOP elevation, they evaluated RGC survival by counting morphologically intact axons in the optic nerve. They found that axon loss in the BDNF-treated group was 32%, significantly less than the 52% loss found in untreated glaucoma model nerves.

Both these studies lend support to previous observations indicating that application of exogenous BDNF can prolong the survival of injured RGC.

5.2 Neuroprotection by Alteration of NT Receptor Activation

Recently, Shi et al., hypothesized that the pharmacologic activation of TRKA or inhibition of P75NTR might augment the protective effect of therapeutic IOP reduction in glaucoma model eyes (Shi et al. 2007). Using the cautery model, they produced a 1.7 fold IOP elevation over 4 months. RGC loss in untreated cauterized eyes was 16% at 3 weeks and 35% at 6 weeks. Daily betaxolol following two weeks of IOP elevation effectively normalized IOP, similar to previous observations (Morrison et al. 1998; Rudzinski et al. 2004). In betaxolol-treated glaucoma model eyes, RGC loss was 7% at 3 weeks and 20% at 6 weeks, demonstrating that IOP normalization reduced, but did not eliminate, RGC loss.

To determine if residual cell losses could be prevented by TRKA activation, the authors used dual sequential intravitreal injections of a TRKA agonist, with and without betaxolol pressure normalization. With the TRKA agonist alone, the RGC loss was reduced, but with betaxolol and the TRKA agonist, the cell loss was reduced to only 11% at 6 weeks, a value is not significantly different from the loss after initial two weeks of pressure elevation. This suggested that the combination of IOP normalization and TRKA activation successfully prevented the continuing phase of RGC loss following the initial IOP insult. Administration of NGF alone did not provide this protection. Shi and colleagues also used a similar set of experiments to determine if a P75NTR antagonist might be neuroprotective, but no alteration in response was found. Together, these findings suggest that pharmacological activation of TRKA may promote adult RGC survival in eyes with previous pressure-induced injury and provide further evidence for multiple trophic factor signaling pathways in RGC survival.

In an alternative approach to inhibiting P75NTR signaling, Fu and colleagues, hypothesized that blocking the function of a P75NTR signaling partner, Lingo1, would be neuroprotective to injured RGC (Fu et al. 2008). Lingo1-P75NTR signaling was originally associated with the inhibition of axonal regeneration (Mi et al. 2004), but it may be important to note that Lingo-1 has other signaling partners in addition to P75NTR (Zhao et al. 2008). Dual laser treatments were used to produce sustained IOP elevation to approximately twice normal levels. Without additional treatment, Lingo1 protein levels increased approximately 1.6 fold and RGC loss was approximately 13% at 2 weeks and 20% at 4 weeks. When Lingo1 function was blocked by intravitreal injection of either soluble Lingo1 or anti-Lingo1 antibody, this loss was successfully eliminated. Further, blocking Lingo1 was found to reduce pro-apoptotic RHOA GTPase, reduce JNK activation and sustain pro-survival AKT signaling in glaucoma model retinas (Dubreuil et al. 2003). Together these findings suggest that Lingo1-related signaling plays a more important role in RGC death than previously appreciated.

5.3 Neuroprotection by Enhancement Of Downstream NT Survival Signaling Pathways

ERK1/2, a common downstream kinase activated by many trophic factors, plays a key role in RGC survival (Cheng et al. 2002). Therefore, Zhou et al hypothesized that stable activation of ERK1/2 would protect RGC in a glaucoma model (Zhou et al. 2005). To test this, they used the hypertonic saline model and gene transfer of the immediate upstream kinase (MEK1) responsible for ERK1/2 activation. Initially, they demonstrated that transfection with constitutively active MEK1 (CA-MEK1) resulted in a significant increase in levels of activated ERK1/2. Then, they labeled RGC with a retrograde tracer and generated CA-MEK1, wild-type MEK1 (WT) and other control groups. Next, they used the hypertonic saline method to produce groups of rats with consistent IOP histories within and between groups. Finally, they examined RGC survival following 5 and 7 weeks of ocular hypertension. At both durations, they found significant RGC protection in the CA-MEK1 group, with approximately 50% greater RGC survival than in the WT group. They also found approximately 50% greater axon survival in the CA-MEK1 group. This data supported their conclusion that prolonged Erk1/2 pathway activation is neuroprotective to injured RGC.

6. Roles for Additional Trophic Factors in RGC Survival

In addition to members of the NGF family, other trophic factors may contribute to RGC survival by signaling via AKT and/or ERK kinases. Using the cautery model to initially double IOP levels, Kanamori and colleagues found a slow decline to baseline IOP at about three months and a 35% RGC loss at six months (Kanamori et al. 2004). They examined retinal immunostaining patterns for total and activated forms of AKT in flat-mounted retinas during that time course. Labeling for activated AKT was absent in the normal retina, but peaked at three days in glaucoma model retinas and was localized, in part, to RGC and their processes. The authors had anticipated a lower level of active AKT due to inhibited retrograde transport of NT-TRK complexes. This unexpected observation led them to hypothesize that signaling via the separate insulin-like growth factor (IGF)/IGF receptor signaling pathway might lead to the activation of the AKT pro-survival pathway in the glaucomatous retinas. By western analysis, they found significantly increased activated forms of both IGF receptor and AKT at three days following IOP elevation, supporting their conclusion that endogenous retinal signaling via IGF may provide an early, although ultimately inadequate, neuroprotective response to RGC injury.

A potential survival enhancing role for glial cell line-derived neurotrophic factor (GDNF) was investigated by Jiang et al recently using the hypertonic saline model (Jiang et al. 2007). GDNF also signals via pathways distinct from TRK or P75NTR. After showing that biodegradable microspheres persist in the vitreous for at least 6 weeks, they injected GDNF and control microspheres at one week after an initial hypertonic saline injection. At eight weeks following a second hypertonic saline injection, retinas and optic nerves were collected and analyzed by immunohistochemistry and histology. In this study, pressure levels rose gradually, stabilizing at 3 weeks at about twice normal values in all experimental groups. Immunolabeling for GDNF and its receptors showed that these proteins were localized, in part, to RGC. In retinas treated with GDNF spheres, the authors reported decreased nerve head cupping, increased nerve fiber layer thickness and significantly increased inner plexiform layer thickness. RGC layer neuronal and axonal survival was significantly increased by about 50%, while glial activation appeared less. These observations led the authors to conclude that sustained GDNF delivery offers significant neuroprotection to injured RGC.

In studies using the laser model, Ji and colleagues examined neuroprotection following intravitreal ciliary neurotrophic factor (CNTF) injection (Ji et al. 2004). In control glaucoma model eyes, RGC loss was 13% at two weeks and 22% at four weeks. In these eyes, immunostaining for CNTF remained constant in the RGC layer, but increased in the inner

plexiform layer. Activation of the JAK/STAT signaling pathway was indicated by an increase in immunolabeled pSTAT3 in both layers during the first days of IOP elevation. RGC loss was significantly less in CNTF treated eyes, -7% at 2 weeks and 5% at 4 weeks. Western analysis demonstrated that CNTF injections prolonged the retinal activation of pSTAT, although the localization of this increase was not reported. The authors concluded that the pSTAT increase was correlated with the protective effect of CNTF on injured RGC.

7. Conclusions

The NT hypothesis is, perhaps, the most widely recognized theory proposed to explain RGC loss due to elevated IOP and, importantly, it incorporates axonal injury at the optic nerve head as a key component. The currently available rat glaucoma models offer the opportunity to examine this hypothesis in a paradigm in which only the risk factor of IOP is altered. As summarized in this review, recent studies of NT signaling using these *in vivo* models have encompassed both the endogenous responses of the retina to pressure exposure as well as experimental strategies to rescue RGC from apoptosis following pressure-induced injury.

The use of multiple models, variations in their application, small sample sizes, different qualitative and quantitative analysis techniques, as well as the limited scope and duration of many studies undoubtedly impact the conclusions that can be drawn and, perhaps, result in apparent contradictions. Another important limitation is that, in most studies, RGC responses were not isolated from those of other potentially affected retinal cells. However, there are some general observations that have a degree of consistency. First, experimental support for the assumption that RGC are deprived of neurotrophic support before they become committed to apoptosis is quite limited (Johnson et al. 2000; Quigley et al. 2000), particularly considering the ample evidence that NT and other trophic factors are produced endogenously in the retina (Ugolini et al. 1995; Cui et al. 2002; Vecino et al. 2002; Rudzinski et al. 2004; Spalding et al. 2004; Seki et al. 2005; Coassin et al. 2008). Secondly, while multiple NT and other trophic factor signaling pathways contribute to RGC well being, elevated IOP exposure appears to trigger multiple pro-apoptotic signaling pathways as well (Kim & Park 2005; Levkovitch-Verbin et al. 2007; Fu et al. 2008). Thirdly, pro-survival and pro-apoptotic NT signaling events demonstrate dynamic inter-relationships (Rudzinski et al. 2004; Kim & Park 2005; Levkovitch-Verbin et al. 2007; Coassin et al. 2008). While our knowledge and understanding of the process initiated by elevated IOP exposure is fragmented and incomplete, efforts to inhibit pro-apoptotic signaling have been limited and deserve further attention. It is important to note that the only effective long-term survival strategy for RGC soma in an experimental glaucoma model so far is the knockout of the pro-apoptotic BAX gene in DBA/2J mice (Libby et al. 2005). Additionally, multiple studies suggest that while initial pro-survival signals are short-lived and inadequate, prolonging and intensifying these signals can delay RGC loss (Ko et al. 2000; Cheng et al. 2002; Kanamori et al. 2004; Jiang et al. 2007).

Finally, although experimental support for the NT hypothesis is limited, the theory still provides the best explanation of how axonal injury at the optic nerve head results in RGC death in glaucoma. Therefore, further examination of the theory and its implications is essential, particularly investigations utilizing experimental animal glaucoma models and evolving microanalysis techniques, such as laser capture microdissection and microproteomics, to more precisely determine *in vivo* RGC responses to glaucomatous insults (Huang et al. 2004; Huang et al. 2006; Zhang et al. 2006; Guo et al. 2008; Gutstein et al. 2008; Morrison et al. 2008b).

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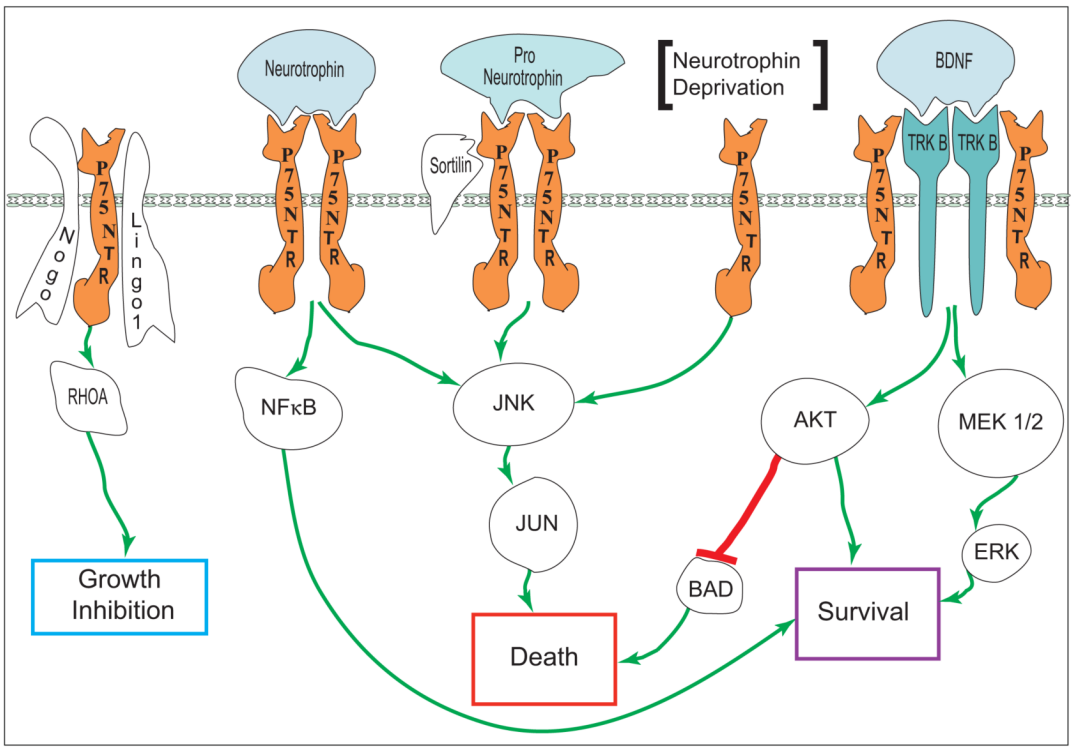

Figure 1.

A simplified summary of the NT signaling pathways discussed in this review. NT mediated RGC survival signals are principally mediated via AKT and ERK, while P75NTR is involved in multiple pathways culminating in apoptotic RGC death. However, P75NTR interaction with TRK receptors appears necessary for high affinity ligand binding and P75NTR signaling mediated via NFκB promotes cell survival. Further, P75NTR has plays a key role in RhoA-mediated axon outgrowth inhibition.