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Does elevated intraocular pressure reduce retinal TRKB-mediated survival signaling in experimental glaucoma?

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Abstract

Reduced retrograde transport of neurotrophins (NT) and their receptors has been hypothesized to contribute directly to retinal ganglion cell (RGC) loss in glaucoma. However, strategies of supplementing NT and NT receptors have failed to avert ultimate RGC death in experimental glaucoma. This study examines the response of major components of the NT system and their interacting proteins in a rat glaucoma model. Unilateral chronic intraocular pressure (IOP) elevation was produced by episcleral vein injection of hypertonic saline (N = 99). Retinas were collected and grouped by extent of optic nerve injury. Quantitative reverse transcription PCR, western blot analysis and immunohistochemistry were used to determine mRNA and protein levels and protein localization. Out of three RGC-specific Brn3 proteins (Brn3a, b, and c), only Brn3a was significantly downregulated at the message level to 35 ± 4% of fellow values with the severest nerve injury. With IOP elevation, no significant alterations were found in retinal mRNA levels for BDNF, NGF, NT-4/5 or NT-3. The abundance of mature retinal BDNF protein was not significantly affected by elevated IOP, while proBDNF protein decreased linearly with increasing injury grade (r^2 = 0.50). In retinas with the severest nerve injury, TrkB and TrkC receptor mRNA levels significantly declined to 67 ± 9% and 44 ± 5% of fellow values, respectively. However, the levels of TRKB protein and its phosphorylated form were unchanged. Message level for $p75^{NTR}$ was linearly upregulated up to 219 ± 26% with increasing injury (r^2 = 0.46), but no alteration was detected at protein level. The mRNA expression of $p75^{NTR}$ apoptosis adaptor proteins NADE, NRIF, and Lingo1 were significantly downregulated in retinas with the greatest nerve injury. A positive correlation was found between injury extent and message levels for Jun (r^2 = 0.23) as well as Junb (r^2 = 0.27), and RGC labeling of activated JUN protein increased. Atf3 mRNA levels demonstrated a positive linear correlation to the extent of injury (r^2 = 0.53), resulting in a nearly five-fold increase (482 ± 76%) in eyes with the greatest nerve damage. Among downstream pro-survival signaling components, Erk5 mRNA expression was linearly upregulated (r^2 = 0.32) up to 157 ± 15% of fellow values in retinas with the severest nerve injury (p < 0.01). A slight positive correlation was found between NF-κB message levels and injury extent (r^2 = 0.12). Bcl-xl mRNA levels in the most severely injured retinas were significantly reduced to 83 ± 7% by elevated IOP exposure. Message levels for Erk1/2, Akt1-3 or Bcl2 appeared unaffected. Elevated IOP did not alter mRNA levels of pro-apoptotic Bim, Bax, or p53. This study demonstrates that elevated IOP exposure does not result in a dramatic decrease in retinal levels of either BDNF or its receptor, TrkB. It shows that the responses of NT pathways to elevated IOP are complex, particularly with regard to the role of $p75^{NTR}$ and Atf3. A better understanding of the roles of these proteins in IOP-induced injury is likely to suggest informed strategies for neuroprotection in glaucoma.

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Keywords

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1. Introduction

Progressive loss of retinal ganglion cells (RGCs), a characteristic of primary open-angle glaucoma, is attributed to apoptosis of the RGCs (Quigley et al., 1995; Quigley, 1999). One hypothesis to explain how RGCs are lost is that pressure-induced axonal transport obstruction within the optic nerve head inhibits retrograde delivery of neurotrophin (NT)-tropomyosin receptor kinase (Trk) receptor complexes from the superior colliculus to the RGC body, thereby triggering apoptosis (Johnson et al., 2000; Pease et al., 2000; Quigley et al., 2000). This NT deprivation theory is attractive since these growth factors are known to promote neuronal survival and regeneration. Additionally retrograde transport of NTs to the retina appears obstructed in experimental glaucoma (Johnson et al., 2000; Pease et al., 2000). When deprived of retrograde transported NTs derived from target organs, developing neurons have been shown to die by apoptosis (Raff et al., 1993). Also, RGCs have been shown to be maintained by exogenous brain-derived neurotrophic factor (BDNF) *in vitro* (Johnson et al., 1986; Rodriguez-Tebar et al., 1989; Cohen-Cory and Fraser, 1994). However, the role of target-derived NTs in maintaining mature neurons is less clear.

The NT growth factor family is composed of nerve growth factor (NGF), BDNF, NT-3, and NT-4/5. These target-derived NT form complexes with Trk receptors and $p75^{NTR}$ receptors and are retrogradely transported through axons to neuronal soma, where they exert their effects (Ginty and Segal, 2002). Although the pro-survival role of Trk receptor mediated NT signaling is well-established, and $p75^{NTR}$ has been demonstrated to signal cell apoptosis in the absence of Trks (Miller and Kaplan, 2001a), studies continue to reveal diverse functions for both receptors and identify new signaling partners (Fig. 1). For example, $p75^{NTR}$-mediated NT signaling has been reported to promote cell survival, instead of cell apoptosis, either by modifying Trk specificity and signaling (Nykjaer et al., 2005), or via activation of nuclear factor kappa B (NF-κB) (Hamanoue et al., 1999). On the other hand, Trk receptor activation has been found to induce neuronal death under certain circumstances (Kalb, 2005). ProNTs, the precursor proteins of NTs, have recently been found to be secreted from cells as well and can serve as a death signal by complexing with $p75^{NTR}$ and sortilin (Lee et al., 2001; Teng et al., 2005).

In the retina, the transport and function of NTs relies on their binding with Trk receptors. Alterations in the abundance of the receptors alone may therefore have a major impact on NT function. More importantly, NTs are also locally produced in the retina (Vecino et al., 2002; Spalding et al., 2004; Seki et al., 2005). This suggests that obstruction of retrograde transport of NTs may not significantly reduce their levels within the retina. In addition, knowledge about the actual physiologic roles of NTs from different sources is still lacking in the retina. NTs have been suggested to act differentially on neuronal compartments, i.e., axons and cell soma (Kimpinski et al., 1997; Toma et al., 1997; Kuruvilla et al., 2000). This could make it more critical to distinguish the sources of NTs in tissues like the retina where RGC bodies and axons are exposed to distinct environments.

So far, no direct evidence is present to support the assumption that NT deprivation occurs in glaucoma before RGCs are committed to die. Although intravitreal supplementation of BDNF and NT-4/5 have been reported to enhance survival of injured adult RGCs (Mey and Thanos, 1993; Mansour-Robaey et al., 1994; Unoki and LaVail, 1994; Weibel et al., 1995; Peinado-

Ramon et al., 1996; Clarke et al., 1998; Di Polo et al., 1998), the protective effect has been only temporary and failed to prevent eventual RGC death.

In this study, we systematically examined retinal levels of NTs and NT receptors in response to intraocular pressure (IOP) elevation in a rat glaucoma model. We also measured mRNAs associated with signaling pathways mediated by NT receptors to promote cell survival or death. In order to understand the changes in the larger context of impaired RGC function, we also evaluated IOP-induced damage to RGCs as well as other retinal cell types, grouping the samples by the extent of optic nerve injury.

2. Materials and methods

2.1. Experimental glaucoma model

All animal experiments complied with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

Eight-month-old adult Brown Norway rats ($N = 99$) were housed in low-level constant light to stabilize circadian IOP oscillations (40–90 lux) (Jia et al., 2000). Sustained IOP elevation was produced unilaterally by episcleral vein injection of hypertonic saline (Morrison et al., 1997). We have previously demonstrated that the rat model used here is reproducible, and results in injury in the retina and optic nerve head that bears many similarities to that found in human glaucoma (Jia et al., 2000; Johnson et al., 2000, 2006, 2007; Schlamp et al., 2001; Ahmed et al., 2004; Fortune et al., 2004; Morrison et al., 2005; Pang et al., 2005). Because it relies on unilateral, experimental obstruction of aqueous humor outflow pathways, we are able to isolate the results to the consequences of elevated IOP (Nissirios et al., 2008).

IOP was measured in awake animals 4 days per week using a TonoPen (Medtronic, Minneapolis, MN). As our standard protocol, tissues were collected at five weeks post-injection (glaucoma model), with the exception of the short-term animals described below. For RNA and western analysis, animals were deeply anesthetized with isoflurane, decapitated, the eyes enucleated, and whole retinas quickly removed, frozen and stored at −70 °C.

For immunohistochemistry studies, eyes were perfusion fixed with buffered paraformaldehyde and paraffin embedded for sectioning and immunolabeling, as previously described (Johnson et al., 2000).

2.2. Optic nerve injury evaluation

For assessment of pressure-induced optic nerve injury, retrobulbar optic nerves from all eyes were post-fixed overnight at 4° C in 0.1 M, pH 7.4 phosphate buffer containing 2.5% glutaraldehyde, 2% paraformaldehyde and 1% $CaCl_2$, embedded in Spurr's resin and cross-sectioned for light microscopy evaluation. Nerve cross-sections were graded from 1 (no injury) to 5 (active degeneration involving the total nerve area) as previously described (Jia et al., 2000). Correlation of injury grade with axon counts by transmission electron microscopy has demonstrated that the difference between each grade unit is approximately 15,000 axons (Morrison et al., 2005).

2.3. Short-term exposure to elevated IOP

To capture retinal mRNA responses earlier in the injury process, a group of glaucoma model animals ($N = 35$) with a shorter duration of IOP elevation was generated. For these animals, IOP was measured daily during the post-injection period. Retinas were collected at one week following the first IOP measurement above a TonoPen reading of 35 mmHg in the injected eye, an average of 10.2 ± 4.3 days post-injection. We use the phrase “short-term glaucoma” to

indicate message data from these animals throughout the manuscript, distinct from data derived from the five-week glaucoma model specimens.

2.4. IOP history

For each injected eye, a cumulative IOP dose was determined as the area under the curve of the plot of days post-injection vs. corresponding IOP measurement, subtracting the mean of the corresponding values for fellow eyes. A weighted mean IOP was determined by dividing the cumulative IOP by the number of post-injection days.

2.5. Message quantification (qPCR)

For evaluation of message levels following chronic IOP elevation, total RNA was extracted from whole retinas from fellow ($N = 13$) and injected ($N = 29$) eyes (Chomczynski and Sacchi, 1987). For the evaluation of message levels following short-term IOP elevation, the groups included fellows ($N = 36$), injected eyes without measurable IOP elevation ($N = 7$), and injected eyes with elevated IOP ($N = 36$). Whole RNA from each sample (150 ng) and from a pooled retinal RNA standard curve (17.5–1200 ng) were reverse transcribed and expression levels were determined by quantitative PCR using a real-time thermocycler (LightCycler, LightCycler Software 3.5) and DNA Master SYBR Green 1 (Roche, Indianapolis, IN) according to the manufacturer's protocol as previously described (Schlamp et al., 2001; Johnson et al., 2007).

Table 1 lists the primers used in this study. These specific primers were designed to span exon junctions whenever possible. Glyceraldehyde 3-phosphate dehydrogenase (Gapdh) mRNA levels were measured in triplicate analyses and average values used for housekeeping gene normalization. Gapdh levels did not differ among groups and there was no significant correlation of Gapdh mRNA level with IOP level or optic nerve injury grade.

2.6. Quantitative western analysis

Proteins were extracted from additional whole retinas (9 fellows and 20 glaucoma model eyes at five weeks post-injection) using a modified RIPA buffer (2 mM ethylene diamine tetracetic acid, 2 mM ethylene glycol tetracetic acid, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 2 mM dithiothritol, 1 mM phenylmethylsulphonyl fluoride, proteinase inhibitor cocktail (Sigma P-8340), and 50 mM Tris Buffer, pH 7.5) (Harlow and Lane, 1988; Sambrook et al., 1989; Alexander and Acott, 2003) and sonication on ice for 30 s using a microtip and 50% maximum setting with a 400-W, 20,000-Hz sonifier (model 450; Branson, Danbury, CT). The sonicate was then centrifuged at 12,000 g for 5 min, aliquoted and stored at −80° C until use. Supernatant protein was quantified using the BCA protein assay (Thermo Scientific, Rockford, IL). A standard curve composed of pooled retinas from several fellow and experimental samples was used for relative quantification. Equivalent amounts of fellow and experimental sample proteins were separated on Tris-HCl gels and transferred to nitrocellulose blots. Blocked blots were incubated in the primary antibody overnight at 4° C, washed and then exposed to the appropriate secondary antibody for detection. Intensities of the protein bands were quantified using a Licor Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE). Antibodies used were TRKB (07-225, Milli-pore Co, Billerica, MA), $p75^{NTR}$ (63231, Promega, Madison, WI), BDNF (sc-546) and pTRK (sc-8058, Santa Cruz Biotech, Santa Cruz, CA). These were coupled with Alexa Fluor (Invitrogen, Carlsbad, CA) and IRDye (Rockland, Gilbertsville, PA) secondary antibodies, or horseradish peroxidase (Cell Signaling, Danvers, MA) and SuperSignal West Pico Chemiluminescent (Pierce, Rockford, IL).

2.7. Immunohistochemistry

An additional group of five-week glaucoma model animals ($N = 15$) were used for immunohistochemistry. Animals were anesthetized, perfused with buffered paraformaldehyde, and globes were paraffin embedded. Retinal proteins were localized using the brown chromogen, 3,3′ diaminobenzidine (DAB), as described previously (Johnson et al., 2000). Antibodies used were: TRKB (2 μg/ml, #G1561) and $p75^{NTR}$ (0.5 μg/ml, #G3231, from Promega, Madison, WI); pAKT (0.2 μg/ml, #9277), p^{ser63} JUN (1:100, #9261) and p^{ser73} JUN (1:100, #9164, from Cell Signaling Technology, Danvers, MA); BAX (1:800, #554106), BCL-X (1:400, #556361) and BCL-2 (1:800, #554087, BD Biosciences, San Jose, CA). For $p75^{NTR}$, p^{ser473} AKT, and the pJUN antibodies, sections were pretreated with trypsin (1 mg/ml) in 0.1 M Tris Buffer, pH 7.8 for 30 min at 37°C. The antibody to TRKB is to the full-length form of the protein. The BCL-X antibody detects both the short (BCL-XS) and long (BCL-XL) forms of the protein. The antibody for p^{ser63} JUN is specific for JUN phosphorylated at serine 63, while the p^{ser73} JUN antibody detects both JUN phosphorylated at serine 73 and JUND phosphorylated at serine-100. We used sections from approximately 6 fellow and 10 glaucoma model retinas for each antibody evaluated. All slides were graded by retinal region for stain intensity on a scale of 0-4 by two masked observers and the average grade reported. Discrepancies of more than one grade unit were resolved by mutual consensus after reexamination of sections involved.

2.8. Data analysis

All data are expressed as percentage of the mean ± SEM of the value obtained for fellow retinas. A separate analysis of 5 of the genes that were most regulated in elevated IOP eyes demonstrated that mRNA levels in control eyes from the same animal were not significantly affected by damage in the contralateral eye (data not shown). Results for retinas with elevated IOP are grouped according to optic nerve injury grade. Statistical analyses were performed using Excel (Microsoft, Redmond, WA) and Prism (GraphPad, San Diego, CA) statistical software packages. ANOVA with Dunnett's multiple comparison post-test and regression analysis were used to test for significant changes in message and protein levels.

3. Results

Glaucoma model data presented are from tissues analyzed at five weeks following injections to elevate IOP, with the exception of the changes in message level following short-term IOP elevation which are presented at the end of the results section.

3.1. IOP history and injury Grade

For the five-week retinas evaluated by qPCR, the mean IOP for uninjected, fellow eyes was 28.3 ± 0.3 mmHg and for injected eyes, 34.2 ± 0.5 mmHg. The average nerve injury grade for the injected eyes was 3.2 ± 0.2. As shown in Fig. 2A, the injury grade was highly correlated with cumulative IOP elevation ($r^2 = 0.84$, 3rd order polynomial, preferred fit compared to linear correlation, $p < 0.02$).

For these analyses, data from retinas were placed into four groups depending on the degree of nerve injury: (1) fellows ($N = 13$): uninjected eyes; (2) no nerve injury ($N = 10$): retinas from injected eyes with elevated IOP but without significant optic nerve injury (injury grade < 1.5); (3) focal injury ($N = 8$): retinas from eyes with optic nerve injury grades between 1.5 and 4.5; and (4) global injury ($N = 11$): retinas from eyes with degeneration occupying the entire area of the optic nerve (grade > 4.5). Table 2 gives the mean optic nerve injury grade and the range of cumulative exposure to elevated IOP for each of these groups. A graph of the daily mean IOP for each group is shown in Fig. 2B. Each of these groups segregates into a distinct level

of mean pressure over the experimental period, with remarkably little overlap, suggesting that our results provide an accurate reflection of the effects of elevated IOP.

3.2. Effect of elevated IOP exposure on retinal cell type markers

We quantified mRNA levels of neurofilament H (Nefh) and Brn3 POU domain genes including Brn3a (*Pou4f1*), Brn3b (*Pou4f2*), and Brn3c (*Pou4f3*). Neurofilament mRNA is produced almost exclusively by RGC and NEFH protein is localized to their axonal neurofilaments (Nixon et al., 1989; McKerracher et al., 1993). Brn3a, b, and c are transcription factors specifically localized to RGC nuclei (Xiang et al., 1995).

As shown in Fig. 3A, no significant decrease in Brn3b mRNA levels were found in glaucoma model retinas (78 ± 16% of fellow values for those most severely injured by pressure exposure). Brn3c mRNA levels did show a negative correlation to injury grade ($r^2 = 0.15$, $p = 0.01$), but it was so slight that the reduction in Brn3c mRNA level was not significant even in eyes with global nerve injury (64 ± 14% of fellow eye values, ANOVA with Dunnett's multiple comparison post-test). Only Brn3a mRNA showed a linear decrease relative to increasing nerve injury grade ($r^2 = 0.67$, $p < 0.0001$) that was severe enough to result in a significantly reduced level in the most severely injured group (35 ± 4%; $p < 0.01$, one-way ANOVA). In contrast, Nefh mRNA was significantly reduced in both retina groups with optic nerve injury grades >1.5 ($p < 0.05$, one-way ANOVA). In retinas with the highest injury grades (grade > 4.5), the Nefh mRNA levels decreased to 13 ± 2% of that in fellow eyes. The correlation between Nefh mRNA level and injury grade was highly significant ($r^2 = 0.73$, $p < 0.0001$). Considered together, the relatively unchanged levels of Brn3b and Brn3c, coupled with substantially reduced levels of Brn3a and Nefh mRNA, suggest that downregulation of some RGC genes occurs in retinas injured by elevated IOP. This provides context for interpreting the other findings of this study.

We also evaluated possible damage in other retinal cell types due to elevation of IOP by measuring mRNA levels of the following neuronal cell type markers: rhodopsin (Rho, for photoreceptors) (Organisciak et al., 1999), calbindin 1 (Calb1, for horizontal cells) (Strettoi and Pignatelli, 2000), and syntaxin 1 (Stx1a, for amacrine and horizontal cells) (Nag and Wadhwa, 2001); and the following glial cell markers: ionized calcium binding adapter molecule 1A (Iba1,for microglia) (Ohsawa et al., 2000), and glial fibrillary acidic protein (Gfap, for astrocytes and Müller cells) (Schnitzer, 1987; Ekstrom et al., 1988). As illustrated in Fig. 3B, while glaucoma model retinas demonstrated no significant change in expression of the other retinal neuronal markers, both glial markers (Iba1 and Gfap) were significantly upregulated at the message level in glaucoma model retina groups with nerve injury greater than grade 1.5 ($p < 0.05$, one-way ANOVA). The upregulation of Gfap mRNA is consistent with our previously reported microarray and immunohistochemical data (Ahmed et al., 2004).

3.3. Elevated IOP exposure and retinal NT mRNA levels

Retinal message levels for BDNF, NT-4/5, NT-3 and NGF are summarized in Fig. 4. We found no significant difference in mRNA level between fellow and glaucoma model groups in any of these NTs. While BDNF mRNA may appear decreased in the most severely affected group (74 ± 7%), this was not statistically significant and the slope of the regression line of injury grade to mRNA level was not quite significantly different from zero ($r^2 = 0.08$, $p = 0.06$). There was no significant correlation of injury grade to mRNA level for any of the other NT messages.

3.4. Elevated IOP exposure and retinal BDNF and proBDNF protein levels

In addition to retinal mRNA expression levels, we examined potential alterations in protein levels of the key NT, BDNF, by semi-quantitative western analysis. RGC apoptosis following

optic nerve injury has been suggested to result from the loss of target-derived neurotrophic support to RGC and a number of strategies to increase BDNF levels in the retina have resulted in increased RGC survival (Castillo et al.,1994; Mansour-Robaey et al.,1994; Di Polo et al., 1998; Quigley et al., 2000; Martin et al., 2003; Weber et al., 2008).

The levels of mature, 14kD BDNF protein in the retina were not significantly different from fellow eye values in glaucoma eyes with either mild to moderate (grade 1.5–3.0) or severe (grade 5) optic nerve injury (Fig. 5A), nor was there a significant linear correlation between injury grade and BDNF level. Fig. 5B illustrates a typical gel image showing bands for four experimental retinas and the standard curve used for the quantification of the BDNF doublet, normally seen in rat tissues (Fawcett et al., 1997;Katoh-Semba et al., 1997).

Using the same antibody, we also measured the levels of the precursor form of BDNF (proBDNF), which is biologically active and binds to both the BDNF receptor TrkB and $p75^{NTR}$. Based on the relative intensity of the bands on the same gel, we estimate that approximately 80% of retinal BDNF is in this proform. Unlike BDNF, retinal proBDNF was significantly decreased in both injury groups relative to the fellow group ($p < 0.05$) (Fig. 5A and C). Regression analysis also demonstrated a significant negative correlation of proBDNF levels with injury grade ($r^2 = 0.50$, $p < 0.0001$).

3.5. Elevated IOP exposure and retinal Trk receptor message levels

We then examined mRNA levels of the full-length, catalytic forms of Trk receptor family members, TrkB, TrkC and TrkA, in glaucoma model retinas. As illustrated in Fig. 6, both TrkB and TrkC mRNA levels were significantly reduced in the glaucoma model group with the severest injury ($p < 0.01$, one-way ANOVA) to 67 ± 9% and 44 ± 5% of fellow eye values, respectively. In addition, regression analysis revealed significant, negative linear correlations between injury grade and mRNA levels of both TrkB and TrkC ($r^2 = 0.22$ and 0.56, respectively, $p < 0.005$, both). There was no significant correlation between injury and the mRNA levels of the two truncated forms of the TrkB receptor, T1 and T2 (both: $r^2 < 0.05$, $p > 0.2$, data not shown), leading to the conclusion that the only TrkB expression change is in the full-length, catalytic form.

3.6. Elevated IOP exposure and activated retinal TrkB receptor protein levels

By western blot of retinas with pressured-induced injury, we found that the protein levels of full-length TRKB did not change by either ANOVA or linear correlation analysis (Fig. 7A). However, levels of activated TRKB, the tyrosine 514-phosphorylated form, demonstrated a significant, positive linear relationship to injury grade ($r^2 = 0.293$, $p < 0.05$) reaching levels that were approximately 140% of fellow eye values in the highest injury group (Fig. 7A and B). By immunohistochemistry, full-length TRKB protein appeared primarily located within the RGC and nerve fiber layer, and did not appear to change with pressure-induced optic nerve damage (Fig. 7C).

3.7. Responses of $p75^{NTR}$ to IOP elevation

We then asked if the expression of $p75^{NTR}$, the “low-affinity” transmembrane NT and proNT receptor, is regulated in the retina by pressure exposure in our model and what role this receptor might play in RGC loss. Both injury groups had significantly greater levels of $p75^{NTR}$ mRNA than the fellow eye group ($p < 0.05$, one-way ANOVA). At five weeks post-injection, retinal $p75^{NTR}$ mRNA level linearly increased ($r^2 = 0.46$, $p < 0.0001$) with increasing injury (Fig. 8A).

Although retinal $p75^{NTR}$ mRNA levels increased with IOP elevation, our western blot analysis found no change in its protein expression or correlation between $p75^{NTR}$ protein level and

injury grade (Fig. 8B). Immunolabeling of retinas from fellow eyes demonstrated an inner retinal distribution similar to that of TRKB protein (Fig. 7C). Injected eyes with nerve injury grades from 1 to 5 did not reveal any consistent difference in this pattern of labeling.

3.8. p75^{NTR}-interacting proteins

To help interpret the role of p75^{NTR} in pressure-induced RGC death, we examined mRNA expression of p75^{NTR} adaptor proteins including NT-receptor-interaction factor (NRIF), NT-receptor-interaction MAGE homolog (NRAGE) and NT-associated cell death executor (NADE), all of which have been shown to activate Jun amino-terminal kinase (JNK) and facilitate p75^{NTR}-mediated apoptosis (Yeiser et al., 2004). Sortilin will also complex with p75^{NTR} and proNTs to signal apoptosis, possibly also through the JNK pathway (Nykjaer et al., 2004; Teng et al., 2005). Finally, we measured the message level of leucine rich repeat and Ig domain containing 1 (Lingo1) and NOGO. Lingo1 is an alternative p75^{NTR} adaptor protein, and when in complex with p75^{NTR} and the NOGO receptor, is believed to inhibit the regenerative potential of axons in response to myelin protein (Mi et al., 2004).

The results (Fig. 9) revealed that NADE, NRIF, and Lingo1 mRNAs were significantly downregulated in the retinas with the greatest IOP-induced injury ($p < 0.05$, one-way ANOVA), while there was no significant change in the message level of NRAGE, NOGO or Sortilin. Significant negative linear correlations between injury grade and mRNA levels were found for NADE ($r^2 = 0.24$, $p < 0.005$), NRIF ($r^2 = 0.21$, $p < 0.005$) and Lingo1 ($r^2 = 0.29$, $p < 0.001$).

3.9. JNK activation and pro-apoptotic signaling

The further downstream signaling pathways regulating p75^{NTR}-mediated neuronal death are not clearly understood, but the JNKp53-Bax pathway is thought to play a crucial role, a process that is potentiated by Bim (Putcha et al., 2003; Nykjaer et al., 2005). JNK activation also plays important roles in cell motility and multiple cellular stress response pathways. Using qPCR, we examined retinal mRNA expression of three Jun proteins (Jun, Junb and Jund), p53, Bax and Bim in response to IOP elevation. Message levels for activating transcription factor 3 (Atf3) were also determined. This is a marker for neuronal injury (Vlug et al., 2005) that appears to also depend on JNK activation (Lindwall and Kanje, 2005; Mei et al., 2008). Atf3 has been shown to increase following optic nerve transection (Takeda et al., 2000) and can dimerize with c-Jun to influence its functional outcome (Lindwall and Kanje, 2005).

We found that both Jun ($r^2 = 0.23$, $p < 0.002$, linear regression) and Junb ($r^2 = 0.27$, $p < 0.001$, linear regression) mRNAs were linearly upregulated with increasing injury grade (Fig. 10A), suggesting JNK activation. As a result, both mRNAs were significantly higher in the severest injury group compared to fellow retina values: 142% for Jun and 171% for Junb ($p < 0.01$ and $p < 0.05$, respectively, one-way ANOVA). In contrast, Jund mRNA levels were unaffected. Atf3 mRNA demonstrated the most robust response to elevated IOP, with an increase to 482 ± 76% (compared to fellow retinas) in eyes with injury grade greater than 4.5 ($p < 0.01$). This increase was also linearly correlated to the extent of injury ($r^2 = 0.53$, $p < 0.0001$). Message levels for p53 also showed a significant positive correlation to nerve injury grade ($r^2 = 0.15$, $p < 0.01$, linear regression), however, the small rate of increase did not result in a significant difference between injury groups. No significant changes were found in the message levels of Bax and Bim.

Immunolabeling for p^{ser63} JUN demonstrated a marked increase in intensity in the RGC layer in glaucoma model eyes with little or no labeling of other regions of the retina (Fig. 10B) and a masked evaluation of intensity grading of 5 fellow and 11 glaucoma model retinas showed a linear correlation to injury grade ($r^2 = 0.60$, $p < 0.001$). This activated form of JUN has been associated with apoptosis following withdrawal of trophic support (Li et al., 2004;Ribera et

al., 2007). A similar pattern was seen when retinal sections were immunostained for p^{ser73} JUN. Immunolabeling for BAX demonstrated a generally uniform staining pattern that did not differ between fellow and glaucoma model retinas (not shown).

3.10. NT receptor mediated pro-survival signaling

Several pathways have been implicated in Trk receptor mediated survival signaling in neurons. These include PI3K-Akt (Atwal et al., 2000; Brunet et al., 2001; Miller and Kaplan, 2001b), Erk1/2 (Xia et al., 1995; Bonni et al., 1999; Hetman et al., 1999) and Erk5 pathways (Kamakura et al., 1999; Cavanaugh et al., 2001). Akt activation inhibits pro-apoptotic p53 and enhances expression of Bcl2 and Bcl-xl, two typical anti-apoptotic Bcl2 family proteins. Erk5, the newest member of the MAP kinase family (Lee et al., 1995; Zhou et al., 1995), has a unique role mediating retrograde survival signaling in response to NTs (Watson et al., 2001).

In our qPCR study, we found that mRNA levels of all three rat Akt isoforms (Akt1, Akt2, and Akt3), Erk1, Erk2 as well as Bcl2 were unchanged with elevated IOP in the whole retina, both by linear regression analysis and ANOVA. Bcl-xl, the anti-apoptotic Bcl family member most associated with the RGC survival (Levin et al., 1997; Malik et al., 2005), was slightly, but significantly decreased ($p < 0.05$, ANOVA) to 83 ± 7% of fellow retina values in the glaucoma model retinas with the greatest nerve injury (Fig. 11). For Erk5, a positive correlation was found between its message levels and injury grade ($r^2 = 0.32$, $p < 0.0001$), and the most injured group displayed a significant upregulation (157 ± 15%) compared to fellow retinas ($p < 0.01$, one-way ANOVA).

Immunolabeling for active, serine-473 phosphorylated AKT (pAKT) was detected in the RGC and inner nuclear layer of both fellow eye and glaucoma model retinas, with only a suggestion of increased label in the glaucoma eyes ($N = 14$). While labeling intensity and distribution of BCL-2 seen in fellow eyes did not change with exposure to elevated IOP, BCL-X immunostaining appeared less intense in the RGC and plexiform layers of glaucoma model retinas (data not shown).

NT-signaled neuronal survival can also be mediated by $p75^{NTR}$. In addition to interacting with Trk receptors to enhance their affinity for NTs, $p75^{NTR}$ can activate pro-survival signaling via the NF-κB pathway (Carter et al., 1996). To further examine the role of $p75^{NTR}$ in the signaling pathways regulating RGC survival in retinas with elevated IOP, we determined retinal NF-κB mRNA levels and found a small, positive correlation to injury grade ($r^2 = 0.12$, $p < 0.05$). However, this increase was not enough to result in a significant elevation of NF-κB mRNA in the severest injury group (123 ± 14% of fellow eye values) (Fig. 11).

3.11. Message level changes following short-term exposure to elevated IOP (short-term glaucoma)

To attempt to capture early, potentially important, gene expression responses to IOP elevation, we examined the same message levels in retinas collected at one week following the initial IOP measurement of 35 mmHg or more. Description of the cumulative IOP history and optic nerve injury grades associated with these retinas is shown in Table 3. Fig. 12 summarizes the mRNA responses in these retinas, which confirmed the response patterns found at five weeks following injections to elevate IOP. Short-term glaucoma retinas had similar upregulation of $p75^{NTR}$, Junb, Jun, Gfap and Iba1 mRNA levels. Also confirmed was the down-regulation of Nefh and TrkC mRNA in the group with the greatest injury. For Lingo1, a significant negative correlation between injury grade and message level was detected (r^2 0.13, $p < 0.02$), but only resulted in values in the most injured group that were 73 ± 10% of fellow eye values ($p > 0.05$, ANOVA). No significant alteration of Brn3b, BDNF, TrkB, Sortilin, NOGO, Bad, Bax, or Bcl2 mRNA levels was detected in these retinas.

4. Discussion

The NT deprivation theory of glaucoma hypothesizes that RGC viability depends on a steady supply of trophic NT. Originating in the superior colliculus, NT complex with their Trk receptors and are ultimately delivered by retrograde transport to the RGC body. Because the receptors are produced in the RGC, and must first be transported in orthograde fashion to the superior colliculus, it seems reasonable to expect that elevated IOP, which can obstruct both retrograde and orthograde axonal transport (Pease et al., 2000; Quigley et al., 2000), could contribute to RGC death in glaucoma.

As illustrated in Fig. 1, the major NT pathways and their relationships to cell death and survival are diverse and complex. Because of this, an analysis of the simultaneous responses of its major components in an intact glaucoma model is essential in order to determine if predictions based on this theory are indeed supported.

If all pertinent RGC NT were derived from the superior colliculus, we would expect that their retinal levels would be diminished in eyes with chronic IOP elevation. However, we found no significant alteration in the mature form of BDNF protein in the whole retina. Although we previously found immunohistochemical evidence for reduced inner retinal labeling in specimens shortly after IOP elevation, we also noted a return of RGC label in eyes with prolonged pressure elevation (Johnson et al., 2000). These results strongly suggest that depletion of mature BDNF is not seen in eyes with chronic moderate pressure elevation. Although proBDNF was significantly decreased, this likely represents ongoing conversion to the mature, active form.

We also found that retinal message for BDNF, as well as other NTs, was not significantly changed by chronic IOP elevation. Other investigators have reported an increase in retinal message for BDNF with elevated IOP (Rudzinski et al., 2004; Kim et al., 2007). Both studies, along with other work (Vecino et al., 2002; Seki et al., 2004), demonstrate that retinal sources for the local production of NT do exist, and could explain the lack of significant changes in retinal NT in our model.

Chen and colleagues have reported that intravitreal application of BDNF can decrease retinal message and protein levels for full-length TrkB (Chen and Weber, 2004). This suggests that production of TrkB receptor is sensitive to feedback based on retinal levels of NT. Thus, if elevated IOP decreased retrograde delivery of BDNF to the RGC, we might expect to see an increase in TrkB message. Instead, we found that TrkB message levels were reduced. However, there was no change in full-length TRKB protein, while active, phosphorylated TRKB levels were increased, suggesting active interaction with BDNF and an attempt to promote survival. Rudzinski, using the vein cautery model, found no change in retinal TrkB (Rudzinski et al., 2004). Again, neither model produced the result predicted by the NT hypothesis.

Our finding of a concurrent reduction in message for TrkC as well as TrkB is interesting. However, its significance is uncertain, since message for its specific NT, NT-3, did not change. Rudzinski and colleagues found an increase in TrkC in Mueller cells, but not in RGC (Rudzinski et al., 2004). These results suggest that further understanding of the responses and function of this NT and its receptor with regard to RGC survival in glaucoma should be pursued.

Our observation of a significant increase in message for the $p75^{NTR}$ receptor, with no apparent change in protein level, provides an additional indication of the complexity of the role of NT signaling in glaucoma. The $p75^{NTR}$ receptor is capable of triggering multiple pro-apoptotic and pro-survival pathways and interactions, as well as regulating axon regenerative potential (Barker, 2004; Mandemakers and Barres, 2005). NT binding to $p75^{NTR}$ has been shown to activate apoptotic pathways, particularly in association with the adaptor proteins NRIF,

NRAGE, and NADE (Roux and Barker, 2002). We found significant downregulation in message for NADE and NRIF, as well as Lingo1, which in complex with $p75^{NTR}$ can inhibit axon regeneration. These results suggest that RGC death may not be the primary consequence of $p75^{NTR}$ upregulation following chronic IOP elevation. Rudzinski has also noted a slight increase in message for $p75^{NTR}$ and suggested that this increase may contribute to RGC death in glaucoma (Rudzinski et al., 2004). However, specific attempts to protect RGC using intravitreal injection of $p75^{NTR}$ antagonists have been unsuccessful (Shi et al., 2007). $p75^{NTR}$ is also recognized to have pro-survival activities. $p75^{NTR}$ interaction with Trk receptors is essential for high-affinity NT binding (Huang and Reichardt, 2003). This results, in part, in activation of PI3K-Akt signaling pathway, which mediates signaling of neuronal survival (Miller and Kaplan, 2001b) as well as enhanced expression of anti-apoptotic Bcl2 and Bcl-xl. Previous work in the vein cautery model has demonstrated upregulation of message for both Bcl2 and Bcl-xl (Kim et al., 2007) as well as increased retinal labeling with antibodies to pAKT (Kanamori et al., 2004; Kim and Park, 2005). While we did not find a change in message for Bcl2 and Akt, there appeared to be a slight decrease in retinal Bcl-xl message and protein, with only a suggestion of increased immunolabeling for AKT in the RGC layer in eyes with elevated IOP, even after five weeks. Again, these discrepancies may reflect differences in the methods used to elevate IOP (Nissirios et al., 2008).

We did not identify significant message changes for Erk1/2, which have been demonstrated to play an important role for RGC survival in experimental glaucoma (Zhou et al., 2005). We did, however, detect a significant increase in Erk5 message, representing what we believe to be the first report on this signaling molecule in the retina. While this is consistent with increased survival signaling by retrograde transport (Watson et al., 2001), message data alone of these and the other signaling molecules investigated here do not necessarily reflect their function. Although beyond the scope of the present study, detailed evaluations of potentially altered levels, intracellular distribution or activation states of these and other intriguing proteins should stimulate future investigations.

$p75^{NTR}$ can also enhance neuronal survival via upregulation of the transcription factor, NF-κB (Choi et al., 2000). Interestingly, we did find a significant upregulation of NF-κB, which supports this possibility. Our finding of an increase in the activated form of TRKB protein further suggests that, following chronic IOP elevation, the NT receptor system is tilted in favor of survival, rather than apoptotic pathways.

$p75^{NTR}$-mediated apoptosis is thought to involve the JNK-p53-Bax pathway (Putcha et al., 2003; Nykjaer et al., 2005). Our results reveal increased immunohistochemical labeling of the RGC layer with p^{ser63} JUN, as well as an increase in message for both Jun and Junb. This agrees with previous demonstrations of increased labeling with antibodies to p^{ser73} JUN within RGC (Levkovitch-Verbin et al., 2005, 2007), suggesting that this particular pathway is involved in mediating RGC death following elevated IOP.

However, these authors also acknowledge that Jun may contribute to cell survival as well as cell death (Levkovitch-Verbin et al., 2005), and the outcome may depend on the activity of other transcription factors. Along these lines, we found a nearly 5-fold increase in message for Atf3, representing the most dramatic response of all of the messages that we evaluated. While Levkovitch and colleagues reported no change in the related Atf2 in their glaucoma model, our study appears to represent the first report of retinal Atf3 expression changes following chronic IOP elevation.

Atf3 is an immediate early response gene induced by a wide variety of stresses in neuronal and non-neuronal cells. It can contribute to apoptosis as well as cell survival, depending on the cell type (Mei et al., 2008) and on whether it forms homodimers, which function as a transcription

repressor (Chen et al., 1994; Vlug et al., 2005) or heterodimers, which are capable of either transcription activation or repression (Chu et al., 1994). In the eye, Takeda and colleagues have demonstrated increased expression of Atf3 in RGC, colocalized with phosphorylated Jun, following intraorbital optic nerve crush (Takeda et al., 2000). Because this expression appears mostly during the initial regenerative period, these authors suggest that Atf3 heterodimerizes with Jun and functions in a supportive, or anti-apoptotic role. The marked upregulation that we observed in our model suggests that Atf3 deserves closer scrutiny to identify its cellular localization and potential relationship to Jun and its role in RGC death or survival.

Although our data do not support the concept that reduced delivery of NT plays a major role in RGC damage due to elevated IOP, other trophic factors could still contribute to this process. Jiang et al. has reported that intravitreal injection of biodegradable microspheres containing glial cell line-derived neurotrophic factor (GDNF) improved survival of RGC layer cells and axons (Jiang et al., 2007). Similarly, ciliary neurotrophic factor (CNTF), either through direct intravitreal injection (Ji et al., 2004) or by increased RGC expression via injection of adenoassociated viral vectors containing CNTF (Pease et al., 2009) has been shown to reduce RGC and axonal loss in the rat laser glaucoma model. Deficits of these, or other trophic factors, may contribute to glaucomatous RGC loss.

It is also important to note that these results reflect findings from the whole retina, and that responses unique to RGCs may be diluted by contributions from non-RGC layers of the retina. More refined analyses, based on analysis of the RGC layer alone, are likely to reveal changes on the NT signaling system more specific to RGC damage in glaucoma and are currently in progress in our laboratory.

This study of the NT system in an intact model of chronic IOP elevation indicates a higher than anticipated level of complexity and suggests that responses of many of its components enhance survival, rather than apoptosis. This may explain why interventions based on our current understanding of the role of NT in RGC survival and the assumption that an NT deficit underlies glaucomatous RGC loss so far have not provided lasting benefits. Inappropriate NT therapy may result in unexpected and, potentially undesirable consequences.

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References

Ahmed F, Brown KM, Stephan DA, Morrison JC, Johnson EC, Tomarev SI. Microarray analysis of changes in mRNA levels in the rat retina after experimental elevation of intraocular pressure. Invest. Ophthalmol. Vis. Sci 2004;45(4):1247–1258. [PubMed: 15037594]

Alexander JP, Acott TS. Involvement of the Erk-MAP kinase pathway in TNFalpha regulation of trabecular matrix metalloproteinases and TIMPs. Invest. Ophthalmol. Vis. Sci 2003;44(1):164–169. [PubMed: 12506070]

Atwal JK, Massie B, Miller FD, Kaplan DR. The TrkB-Shc site signals neuronal survival and local axon growth via MEK and P13-kinase. Neuron 2000;27(2):265–277. [PubMed: 10985347]

Barker PA. p75NTR is positively promiscuous: novel partners and new insights. Neuron 2004;42(4): 529–533. [PubMed: 15157416]

Bonni A, Brunet A, West AE, Datta SR, Takasu MA, Greenberg ME. Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. Science 1999;286(5443):1358–1362. [PubMed: 10558990]

Brunet A, Datta SR, Greenberg ME. Transcription-dependent and -independent control of neuronal survival by the PI3K-Akt signaling pathway. Curr. Opin. Neurobiol 2001;11(3):297–305. [PubMed: 11399427]

Carter BD, Kaltschmidt C, Kaltschmidt B, Offenhauser N, Bohm-Matthaei R, Baeuerle PA, Barde YA. Selective activation of NF-kappa B by nerve growth factor through the neurotrophin receptor p75. Science 1996;272(5261):542–545. [PubMed: 8614802]

Castillo B Jr. del Cerro M, Breakefield XO, Frim DM, Barnstable CJ, Dean DO, Bohn MC. Retinal ganglion cell survival is promoted by genetically modified astrocytes designed to secrete brain-derived neurotrophic factor (BDNF). Brain Res 1994;647(1):30–36. [PubMed: 8069702]

Cavanaugh JE, Ham J, Hetman M, Poser S, Yan C, Xia Z. Differential regulation of mitogen-activated protein kinases ERK1/2 and ERK5 by neurotrophins, neuronal activity, and cAMP in neurons. J. Neurosci 2001;21(2):434–443. [PubMed: 11160424]

Chen BP, Liang G, Whelan J, Hai T. ATF3 and ATF3 delta Zip. Transcriptional repression versus activation by alternatively spliced isoforms. J. Biol. Chem 1994;269(22):15819–15826. [PubMed: 7515060]

Chen H, Weber AJ. Brain-derived neurotrophic factor reduces TrkB protein and mRNA in the normal retina and following optic nerve crush in adult rats. Brain Res 2004;1011(1):99–106. [PubMed: 15140649]

Choi J, Kim J, Kim D, Chun M, Gwag BJ, Yoon SK, Joo C. Failure to activate NF-kappaB promotes apoptosis of retinal ganglion cells following optic nerve transection. Brain Res 2000;883(1):60–68. [PubMed: 11063988]

Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem 1987;162(1):156–159. [PubMed: 2440339]

Chu HM, Tan Y, Kobierski LA, Balsam LB, Comb MJ. Activating transcription factor-3 stimulates 30′, 50′-cyclic adenosine monophosphate-dependent gene expression. Mol. Endocrinol 1994;8(1):59–68. [PubMed: 8152431]

Clarke DB, Bray GM, Aguayo AJ. Prolonged administration of NT-4/5 fails to rescue most axotomized retinal ganglion cells in adult rats. Vis. Res 1998;38(10):1517–1524. [PubMed: 9667016]

Cohen-Cory S, Fraser SE. BDNF in the development of the visual system of Xenopus. Neuron 1994;12 (4):747–761. [PubMed: 8068082]

Di Polo A, Aigner LJ, Dunn RJ, Bray GM, Aguayo AJ. Prolonged delivery of brain-derived neurotrophic factor by adenovirus-infected Muller cells temporarily rescues injured retinal ganglion cells. Proc. Natl. Acad. Sci. U.S.A 1998;95(7):3978–3983. [PubMed: 9520478]

Ekstrom P, Sanyal S, Narfstrom K, Chader GJ, van Veen T. Accumulation of glial fibrillary acidic protein in Muller radial glia during retinal degeneration. Invest. Ophthalmol. Vis. Sci 1988;29(9):1363–1371. [PubMed: 3417421]

Fawcett JP, Aloyz R, McLean JH, Pareek S, Miller FD, McPherson PS, Murphy RA. Detection of brain-derived neurotrophic factor in a vesicular fraction of brain synaptosomes. J. Biol. Chem 1997;272 (14):8837–8840. [PubMed: 9082996]

Fortune B, Bui BV, Morrison JC, Johnson EC, Dong J, Cepurna WO, Jia L, Barber S, Cioffi GA. Selective ganglion cell functional loss in rats with experimental glaucoma. Invest. Ophthalmol. Vis. Sci 2004;45(6):1854–1862. [PubMed: 15161850]

Ginty DD, Segal RA. Retrograde neurotrophin signaling: Trk-ing along the axon. Curr. Opin. Neurobiol 2002;12(3):268–274. [PubMed: 12049932]

Hamanoue M, Middleton G, Wyatt S, Jaffray E, Hay RT, Davies AM. p75-mediated NF-kappaB activation enhances the survival response of developing sensory neurons to nerve growth factor. Mol. Cell Neurosci 1999;14(1):28–40. [PubMed: 10433815]

Harlow, E.; Lane, D. Antibodies: a Laboratory Manual. Vol. xiii. Cold Spring Harbor Laboratory; Cold Spring Harbor, NY: 1988. p. 726

Hetman M, Kanning K, Cavanaugh JE, Xia Z. Neuroprotection by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol 3-kinase. J. Biol. Chem 1999;274(32):22569–22580. [PubMed: 10428835]

Huang EJ, Reichardt LF. Trk receptors: roles in neuronal signal transduction. Annu. Rev. Biochem 2003;72:609–642. [PubMed: 12676795]

Ji JZ, Elyaman W, Yip HK, Lee VW, Yick LW, Hugon J, So KF. CNTF promotes survival of retinal ganglion cells after induction of ocular hypertension in rats: the possible involvement of STAT3 pathway. Eur. J. Neurosci 2004;19(2):265–272. [PubMed: 14725620]

Jia L, Cepurna WO, Johnson EC, Morrison JC. Patterns of intraocular pressure elevation after aqueous humor outflow obstruction in rats. Invest. Ophthalmol. Vis. Sci 2000;41(6):1380–1385. [PubMed: 10798653]

Jiang C, Moore MJ, Zhang X, Klassen H, Langer R, Young M. Intravitreal injections of GDNF-loaded biodegradable microspheres are neuroprotective in a rat model of glaucoma. Mol. Vis 2007;13:1783–1792. [PubMed: 17960131]

Johnson EC, Cepurna WO, Jia L, Morrison JC. The use of cyclodialysis to limit exposure to elevated intraocular pressure in rat glaucoma models. Exp. Eye Res 2006;83(1):51–60. [PubMed: 16530758]

Johnson EC, Deppmeier LM, Wentzien SK, Hsu I, Morrison JC. Chronology of optic nerve head and retinal responses to elevated intraocular pressure. Invest. Ophthalmol. Vis. Sci 2000;41(2):431–442. [PubMed: 10670473]

Johnson EC, Jia L, Cepurna WO, Doser TA, Morrison JC. Global changes in optic nerve head gene expression after exposure to elevated intraocular pressure in a rat glaucoma model. Invest. Ophthalmol. Vis. Sci 2007;48(7):3161–3177. [PubMed: 17591886]

Johnson JE, Barde YA, Schwab M, Thoenen H. Brain-derived neurotrophic factor supports the survival of cultured rat retinal ganglion cells. J. Neurosci 1986;6(10):3031–3038. [PubMed: 2876066]

Kalb R. The protean actions of neurotrophins and their receptors on the life and death of neurons. Trends Neurosci 2005;28(1):5–11. [PubMed: 15626491]

Kamakura S, Moriguchi T, Nishida E. Activation of the protein kinase ERK5/BMK1 by receptor tyrosine kinases. Identification and characterization of a signaling pathway to the nucleus. J. Biol. Chem 1999;274(37):26563–26571. [PubMed: 10473620]

Kanamori A, Nakamura M, Nakanishi Y, Nagai A, Mukuno H, Yamada Y, Negi A. Akt is activated via insulin/IGF-1 receptor in rat retina with episcleral vein cauterization. Brain Res 2004;1022(1–2):195–204. [PubMed: 15353229]

Katoh-Semba R, Takeuchi IK, Semba R, Kato K. Distribution of brain-derived neurotrophic factor in rats and its changes with development in the brain. J. Neurochem 1997;69(1):34–42. [PubMed: 9202291]

Kim HS, Park CK. Retinal ganglion cell death is delayed by activation of retinal intrinsic cell survival program. Brain Res 2005;1057(1–2):17–28. [PubMed: 16139821]

Kim HS, Chang YI, Kim JH, Park CK. Alteration of retinal intrinsic survival signal and effect of alpha2-adrenergic receptor agonist in the retina of the chronic ocular hypertension rat. Vis. Neurosci 2007;24(2):127–139. [PubMed: 17640403]

Kimpinski K, Campenot RB, Mearow K. 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 1997;33(4):395–410. [PubMed: 9322157]

Kuruvilla R, Ye H, Ginty DD. Spatially and functionally distinct roles of the PI3-K effector pathway during NGF signaling in sympathetic neurons. Neuron 2000;27(3):499–512. [PubMed: 11055433]

Lee JD, Ulevitch RJ, Han J. Primary structure of BMK1: a new mammalian map kinase. Biochem. Biophys. Res. Commun 1995;213(2):715–724. [PubMed: 7646528]

Lee R, Kermani P, Teng KK, Hempstead BL. Regulation of cell survival by secreted proneurotrophins. Science 2001;294(5548):1945–1948. [PubMed: 11729324]

Levin LA, Schlamp CL, Spieldoch RL, Geszvain KM, Nickells RW. Identification of the Bcl-2 family of genes in the rat retina. Invest. Ophthalmol. Vis. Sci 1997;38(12):2545–2553. [PubMed: 9375574]

Levkovitch-Verbin H, Harizman N, Dardik R, Nisgav Y, Vander S, Melamed S. Regulation of cell death and survival pathways in experimental glaucoma. Exp. Eye Res 2007;85(2):250–258. [PubMed: 17586494]

Levkovitch-Verbin H, Quigley HA, Martin KR, Harizman N, Valenta DF, Pease ME, Melamed S. The transcription factor c-jun is activated in retinal ganglion cells in experimental rat glaucoma. Exp. Eye Res 2005;80(5):663–670. [PubMed: 15862173]

Li L, Feng Z, Porter AG. JNK-dependent phosphorylation of c-Jun on serine 63 mediates nitric oxide-induced apoptosis of neuroblastoma cells. J. Biol. Chem 2004;279(6):4058–4065. [PubMed: 14617628]

Lindwall C, Kanje M. The Janus role of c-Jun: cell death versus survival and regeneration of neonatal sympathetic and sensory neurons. Exp. Neurol 2005;196(1):184–194. [PubMed: 16126201]

Malik JM, Shevtsova Z, Bahr M, Kugler S. Long-term in vivo inhibition of CNS neurodegeneration by Bcl-XL gene transfer. Mol. Ther 2005;11(3):373–381. [PubMed: 15727933]

Mandemakers WJ, Barres BA. Axon regeneration: it's getting crowded at the gates of TROY. Curr. Biol 2005;15(8):R302–R305. [PubMed: 15854897]

Mansour-Robaey S, Clarke DB, Wang YC, Bray GM, Aguayo AJ. Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. Proc. Natl. Acad. Sci. U.S.A 1994;91(5):1632–1636. [PubMed: 8127857]

Martin KR, Quigley HA, Zack DJ, Levkovitch-Verbin H, Kielczewski J, Valenta D, Baumrind L, Pease ME, Klein RL, Hauswirth WW. Gene therapy with brain-derived neurotrophic factor as a protection: retinal ganglion cells in a rat glaucoma model. Invest. Ophthalmol. Vis. Sci 2003;44(10):4357–4365. [PubMed: 14507880]

McKerracher L, Essagian C, Aguayo AJ. Temporal changes in beta-tubulin and neurofilament mRNA levels after transection of adult rat retinal ganglion cell axons in the optic nerve. J. Neurosci 1993;13 (6):2617–2626. [PubMed: 8501527]

Mei Y, Yuan Z, Song B, Li D, Ma C, Hu C, Ching YP, Li M. Activating transcription factor 3 up-regulated by c-Jun NH(2)-terminal kinase/c-Jun contributes to apoptosis induced by potassium deprivation in cerebellar granule neurons. Neuroscience 2008;151(3):771–779. [PubMed: 18178318]

Mey J, Thanos S. Intravitreal injections of neurotrophic factors support the survival of axotomized retinal ganglion cells in adult rats in vivo. Brain Res 1993;602(2):304–317. [PubMed: 8448673]

Mi S, Lee X, Shao Z, Thill G, Ji B, Relton J, Levesque M, Allaire N, Perrin S, Sands B, et al. LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. Nat. Neurosci 2004;7(3):221–228. [PubMed: 14966521]

Miller FD, Kaplan DR. Neurotrophin signalling pathways regulating neuronal apoptosis. Cell Mol. Life Sci 2001a;58(8):1045–1053. [PubMed: 11529497]

Miller FD, Kaplan DR. On Trk for retrograde signaling. Neuron 2001b;32(5):767–770. [PubMed: 11738023]

Morrison JC, Johnson EC, Cepurna W, Jia L. Understanding mechanisms of pressure-induced optic nerve damage. Prog. Retin. Eye Res 2005;24(2):217–240. [PubMed: 15610974]

Morrison JC, Moore CG, Deppmeier LM, Gold BG, Meshul CK, Johnson EC. A rat model of chronic pressure-induced optic nerve damage. Exp. Eye Res 1997;64(1):85–96. [PubMed: 9093024]

Nag TC, Wadhwa S. Differential expression of syntaxin-1 and synaptophysin in the developing and adult human retina. J. Biosci 2001;26(2):179–191. [PubMed: 11426054]

Nissirios N, Chanis R, Johnson E, Morrison J, Cepurna WO, Jia L, Mittag T, Danias J. Comparison of anterior segment structures in two rat glaucoma models: an ultrasound biomicroscopic study. Invest. Ophthalmol. Vis. Sci 2008;49(6):2478–2482. [PubMed: 18515586]

Nixon RA, Lewis SE, Dahl D, Marotta CA, Drager UC. Early post-translational modifications of the three neurofilament subunits in mouse retinal ganglion cells: neuronal sites and time course in relation to subunit polymerization and axonal transport. Brain Res. Mol. Brain Res 1989;5(2):93–108. [PubMed: 2469928]

Nykjaer A, Willnow TE, Petersen CM. p75NTR – live or let die. Curr. Opin. Neurobiol 2005;15(1):49–57. [PubMed: 15721744]

Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P, Nielsen MS, Jacobsen C, Kliemannel M, Schwarz E, Willnow TE, et al. Sortilin is essential for proNGF-induced neuronal cell death. Nature 2004;427 (6977):843–848. [PubMed: 14985763]

Ohsawa K, Imai Y, Kanazawa H, Sasaki Y, Kohsaka S. Involvement of Iba1 in membrane ruffling and phagocytosis of macrophages/microglia. J. Cell Sci 2000;113(Pt 17):3073–3084. [PubMed: 10934045]

Organisciak DT, Li M, Darrow RM, Farber DB. Photoreceptor cell damage by light in young Royal College of Surgeons rats. Curr. Eye Res 1999;19(2):188–196. [PubMed: 10420189]

Pang IH, Johnson EC, Jia L, Cepurna WO, Shepard AR, Hellberg MR, Clark AF, Morrison JC. Evaluation of inducible nitric oxide synthase in glaucomatous optic neuropathy and pressure-induced optic nerve damage. Invest. Ophthalmol. Vis. Sci 2005;46(4):1313–1321. [PubMed: 15790897]

Pease ME, McKinnon SJ, Quigley HA, Kerrigan-Baumrind LA, Zack DJ. Obstructed axonal transport of BDNF and its receptor TrkB in experimental glaucoma. Invest. Ophthalmol. Vis. Sci 2000;41(3): 764–774. [PubMed: 10711692]

Pease ME, Zack DJ, Berlinicke C, Bloom K, Cone F, Wang Y, Klein RL, Hauswirth WW, Quigley HA. Effect of CNTF on retinal ganglion cell survival in experimental glaucoma. Invest. Ophthalmol. Vis. Sci 2009;50(5):2194–2200. [PubMed: 19060281]

Peinado-Ramon P, Salvador M, Villegas-Perez MP, Vidal-Sanz M. Effects of axotomy and intraocular administration of NT-4, NT-3, and brain-derived neurotrophic factor on the survival of adult rat retinal ganglion cells. A quantitative in vivo study. Invest. Ophthalmol. Vis. Sci 1996;37(4):489–500. [PubMed: 8595949]

Putcha GV, Le S, Frank S, Besirli CG, Clark K, Chu B, Alix S, Youle RJ, LaMarche A, Maroney AC. JNK-mediated BIM phosphorylation potentiates BAX-dependent apoptosis. Neuron 2003;38(6): 899–914. [PubMed: 12818176]

Quigley HA. Neuronal death in glaucoma. Prog. Retin. Eye Res 1999;18(1):39–57. [PubMed: 9920498]

Quigley HA, Nickells RW, Kerrigan LA, Pease ME, Thibault DJ, Zack DJ. Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis. Invest. Ophthalmol. Vis. Sci 1995;36(5):774–786. [PubMed: 7706025]

Quigley HA, McKinnon SJ, Zack DJ, Pease ME, Kerrigan-Baumrind LA, Kerrigan DF, Mitchell RS. Retrograde axonal transport of BDNF in retinal ganglion cells is blocked by acute IOP elevation in rats. Invest. Ophthalmol. Vis. Sci 2000;41(11):3460–3466. [PubMed: 11006239]

Raff MC, Barres BA, Burne JF, Coles HS, Ishizaki Y, Jacobson MD. Programmed cell death and the control of cell survival: lessons from the nervous system. Science 1993;262(5134):695–700. [PubMed: 8235590]

Ribera J, Ayala V, Casas C. Involvement of c-Jun-JNK pathways in the regulation of programmed cell death of developing chick embryo spinal cord motoneurons. Dev. Neurosci 2007;29(6):438–451. [PubMed: 17119319]

Rodriguez-Tebar A, Jeffrey PL, Thoenen H, Barde YA. The survival of chick retinal ganglion cells in response to brain-derived neurotrophic factor depends on their embryonic age. Dev. Biol 1989;136 (2):296–303. [PubMed: 2573550]

Roux PP, Barker PA. Neurotrophin signaling through the p75 neurotrophin receptor. Prog. Neurobiol 2002;67(3):203–233. [PubMed: 12169297]

Rudzinski M, Wong TP, Saragovi HU. Changes in retinal expression of neurotrophins and neurotrophin receptors induced by ocular hypertension. J. Neurobiol 2004;58(3):341–354. [PubMed: 14750147]

Sambrook, J.; Fritsch, EF.; Maniatis, T. Molecular Cloning: a Laboratory Manual. Vol. second ed.. Cold Spring Harbor Laboratory; Cold Spring Harbor, NY: 1989.

Schlamp CL, Johnson EC, Li Y, Morrison JC, Nickells RW. Changes in Thy1 gene expression associated with damaged retinal ganglion cells. Mol. Vis 2001;7:192–201. [PubMed: 11509915]

Schnitzer J. Retinal astrocytes: their restriction to vascularized parts of the mammalian retina. Neurosci. Lett 1987;78(1):29–34. [PubMed: 3614770]

Seki M, Fukuchi T, Tanaka T, Nawa H, Takei N, Abe H. Quantitative analyses of mRNA and protein levels of neurotrophin-3 in the rat retina during postnatal development and aging. Jpn. J. Ophthalmol 2004;48(5):460–464. [PubMed: 15486768]

Seki M, Tanaka T, Sakai Y, Fukuchi T, Abe H, Nawa H, Takei N. Muller cells as a source of brain-derived neurotrophic factor in the retina: noradrenaline upregulates brain-derived neurotrophic factor levels in cultured rat Muller cells. Neurochem. Res 2005;30(9):1163–1170. [PubMed: 16292510]

Shi Z, Birman E, Saragovi HU. Neurotrophic rationale in glaucoma: a TrkA agonist, but not NGF or a p75 antagonist, protects retinal ganglion cells in vivo. Dev. Neurobiol 2007;67(7):884–894. [PubMed: 17506493]

Spalding KL, Rush RA, Harvey AR. Target-derived and locally derived neurotrophins support retinal ganglion cell survival in the neonatal rat retina. J. Neurobiol 2004;60(3):319–327. [PubMed: 15281070]

Strettoi E, Pignatelli V. Modifications of retinal neurons in a mouse model of retinitis pigmentosa. Proc. Natl. Acad. Sci. U.S.A 2000;97(20):11020–11025. [PubMed: 10995468]

Takeda M, Kato H, Takamiya A, Yoshida A, Kiyama H. Injury-specific expression of activating transcription factor-3 in retinal ganglion cells and its colocalized expression with phosphorylated c-Jun. Invest. Ophthalmol. Vis. Sci 2000;41(9):2412–2421. [PubMed: 10937548]

Teng HK, Teng KK, Lee R, Wright S, Tevar S, Almeida RD, Kermani P, Torkin R, Chen ZY, Lee FS, et al. ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. J. Neurosci 2005;25(22):5455–5463. [PubMed: 15930396]

Toma JG, Rogers D, Senger DL, Campenot RB, Miller FD. Spatial regulation of neuronal gene expression in response to nerve growth factor. Dev. Biol 1997;184(1):1–9. [PubMed: 9142978]

Unoki K, LaVail MM. Protection of the rat retina from ischemic injury by brain-derived neurotrophic factor, ciliary neurotrophic factor, and basic fibroblast growth factor. Invest. Ophthalmol. Vis. Sci 1994;35(3):907–915. [PubMed: 8125754]

Vecino E, Garcia-Grespo D, Garcia M, Martinez-Millan L, Sharma SC, Carrascal E. Rat retinal ganglion cells co-express brain derived neurotrophic factor (BDNF) and its receptor TrkB. Vis. Res 2002;42 (2):151–157. [PubMed: 11809469]

Vlug AS, Teuling E, Haasdijk ED, French P, Hoogenraad CC, Jaarsma D. ATF3 expression precedes death of spinal motoneurons in amyotrophic lateral sclerosis-SOD1 transgenic mice and correlates with c-Jun phosphorylation, CHOP expression, somato-dendritic ubiquitination and Golgi fragmentation. Eur. J. Neurosci 2005;22(8):1881–1894. [PubMed: 16262628]

Watson FL, Heerssen HM, Bhattacharyya A, Klesse L, Lin MZ, Segal RA. Neurotrophins use the Erk5 pathway to mediate a retrograde survival response. Nat. Neurosci 2001;4(10):981–988. [PubMed: 11544482]

Weber A, Harman CD, Viswanathan S. Effects of optic nerve injury, glaucoma, and neuroprotection on the survival, structure, and function of ganglion cells in the mammalian retina. J. Physiol. 2008

Weibel D, Kreutzberg GW, Schwab ME. Brain-derived neurotrophic factor (BDNF) prevents lesion-induced axonal die-back in young rat optic nerve. Brain Res 1995;679(2):249–254. [PubMed: 7543356]

Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 1995;270(5240):1326–1331. [PubMed: 7481820]

Xiang M, Zhou L, Macke JP, Yoshioka T, Hendry SH, Eddy RL, Shows TB, Nathans J. The Brn-3 family of POU-domain factors: primary structure, binding specificity, and expression in subsets of retinal ganglion cells and somatosensory neurons. J. Neurosci 1995;15(7 Pt 1):4762–4785. [PubMed: 7623109]

Yeiser EC, Rutkoski NJ, Naito A, Inoue J, Carter BD. Neurotrophin signaling through the p75 receptor is deficient in traf6−/− mice. J. Neurosci 2004;24(46):10521–10529. [PubMed: 15548667]

Zhou G, Bao ZQ, Dixon JE. Components of a new human protein kinase signal transduction pathway. J. Biol. Chem 1995;270(21):12665–12669. [PubMed: 7759517]

Zhou Y, Pernet V, Hauswirth WW, Di Polo A. Activation of the extracellular signal-regulated kinase 1/2 pathway by AAV gene transfer protects retinal ganglion cells in glaucoma. Mol. Ther 2005;12(3): 402–412. [PubMed: 15975850]

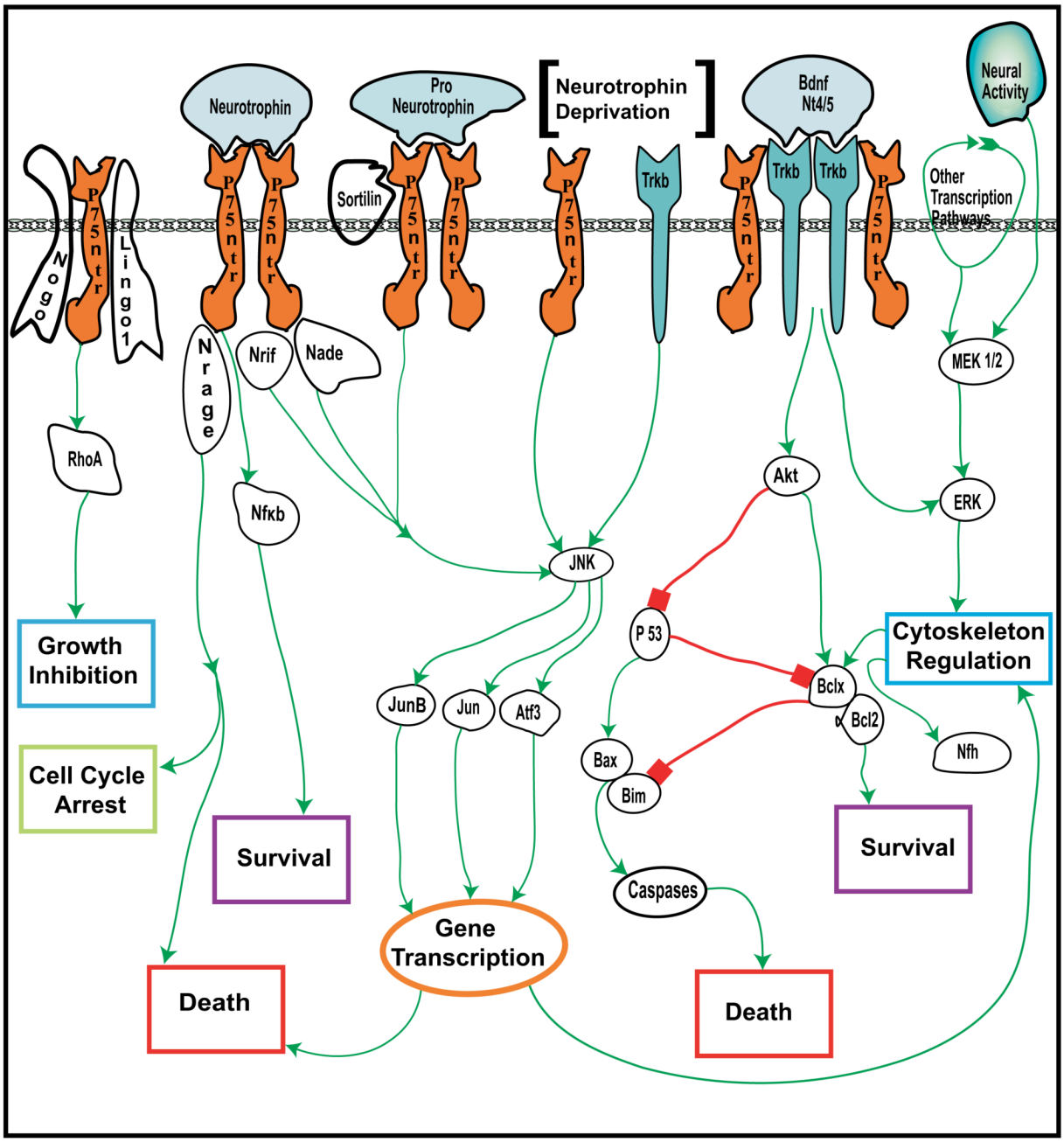

Fig. 1.

Overview of the major NT pathways and their relationships to cell death and survival, summarizing the relationships between the molecules that are analyzed in this report.

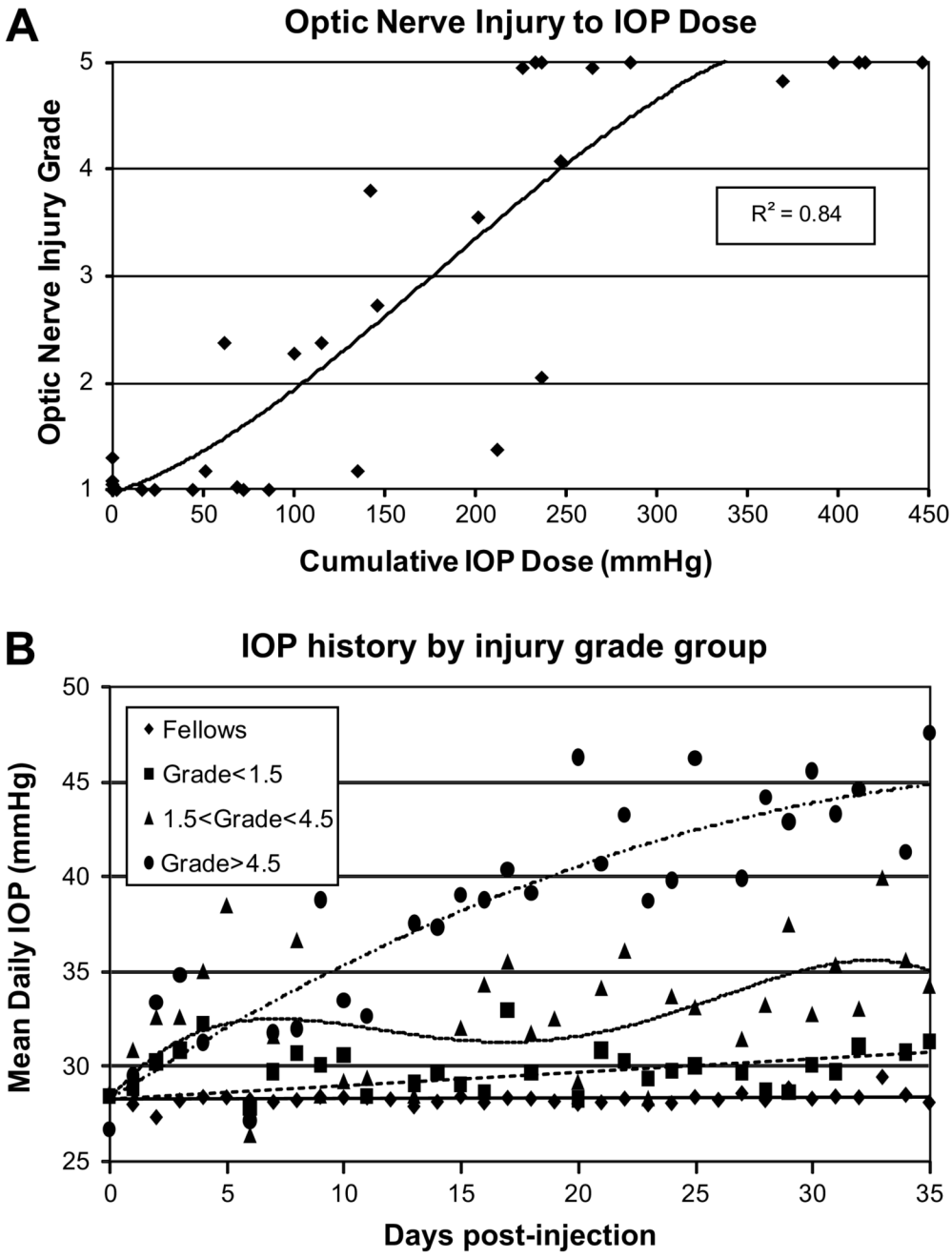

Fig. 2.

IOP histories and correlation of optic nerve injury grade to pressure exposure. A. Graph showing the high correlation between cumulative IOP and optic nerve injury grade for all eyes evaluated for mRNA expression at five weeks post-injection. The best fit line is a 3rd order polynomial ($r^2 = 0.84$, $p < 0.01$). B. Graph of the daily mean IOP for the fellow eyes and each of the three injury grade groups, with best fit lines superimposed. For the injury groups, Grade < 1.5, 1.5 < Grade < 4.5, and Grade > 4.5 indicate no, focal and global optic nerve injury, respectively. The lines demonstrate little, if any, overlap between the groups after day 10, when pressure elevation is generally established in this model.

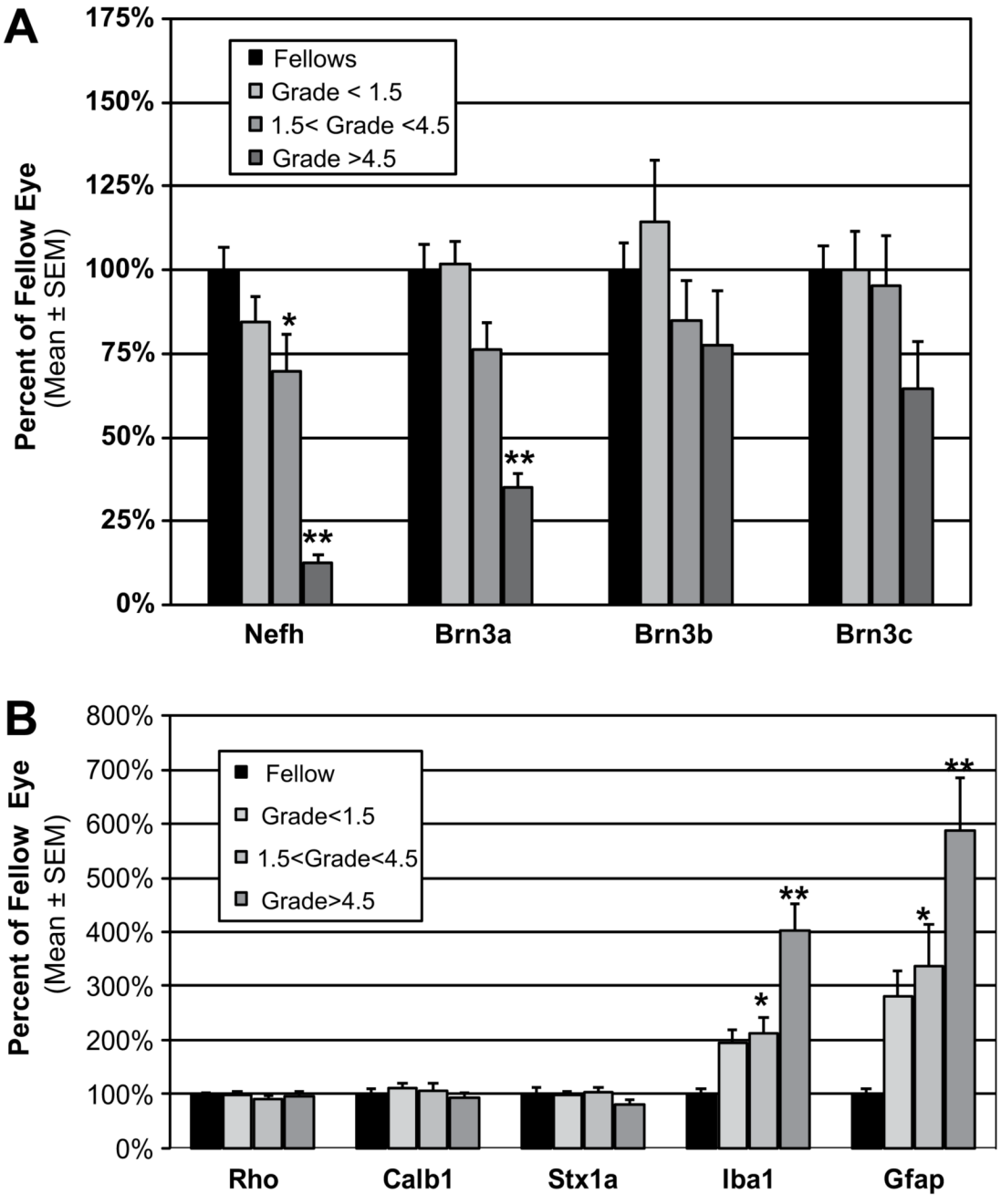

Fig. 3.

Responses of retinal neuronal and glial marker mRNA in glaucoma model eyes. A. Levels of messages highly expressed in RGC, Brn3a, Brn3b, Brn3c and Nefh, compared to fellow eyes. Brn3a mRNA levels demonstrate a negative linear correlation to injury grade ($r^2 = 0.67$, $p < 0.0001$). This leads to a significant reduction of the Brn3a level in the global injury group (35 ± 4%, $^{**}p < 0.01$). Brn3c mRNA levels show a slight negative correlation to injury grade ($r^2 = 0.15$, $p = 0.01$) but not enough to cause a significant reduction in the injury groups ($p > 0.05$). No change is found in Brn3b mRNA levels either by linear regression or ANOVA, although it appears depressed in eyes with global injury. By contrast, Nefh mRNA is significantly depressed in retinas with both focal (1.5 < grade < 4.5, $^{*}p < 0.05$) and global injury (grade >

4.5, $^{**}p < 0.01$), and there is a very significant negative correlation between Nefh mRNA levels and injury grade ($r^2 = 0.73$, $p < 0.0001$). B. No significant alterations are observed at any extent of injury in markers for photoreceptors (Rho), horizontal cells (Calb1) or amacrine and horizontal cells (Stx1A). However, messages for glial markers Iba1 and Gfap are both significantly upregulated in focal ($^{*}p < 0.05$) and global ($^{**}p < 0.01$) nerve injury eyes.

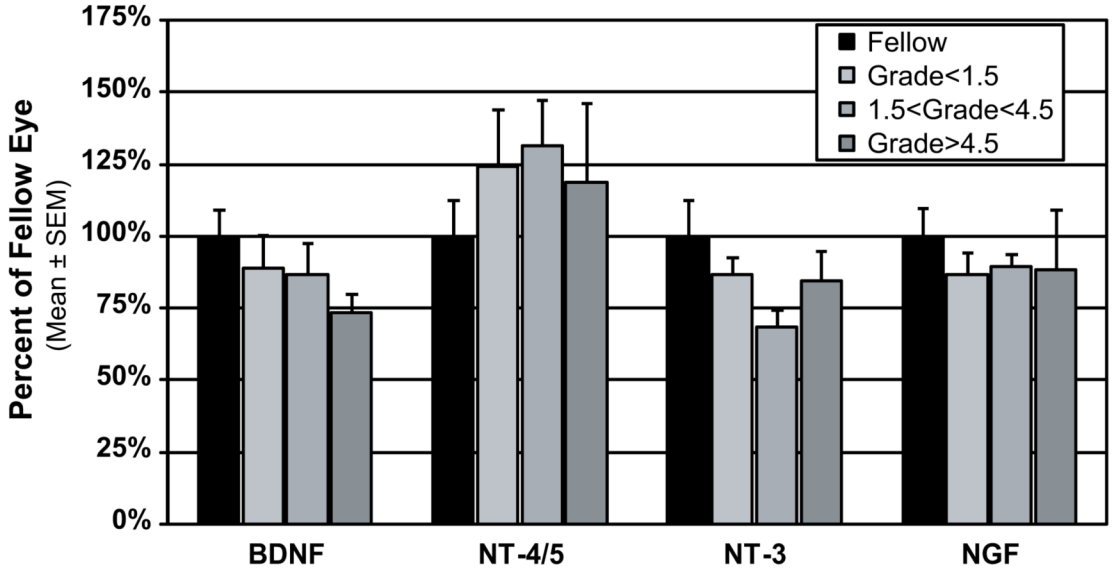

Fig. 4.

NT mRNA levels in glaucoma model retinas demonstrate no significant correlation to injury grade for any member of the NGF family of NT. Although BDNF mRNA levels appear to decrease with greater amounts of nerve damage, this correlation was not quite significant by linear regression analysis ($r^2 = 0.08$, $p = 0.06$).

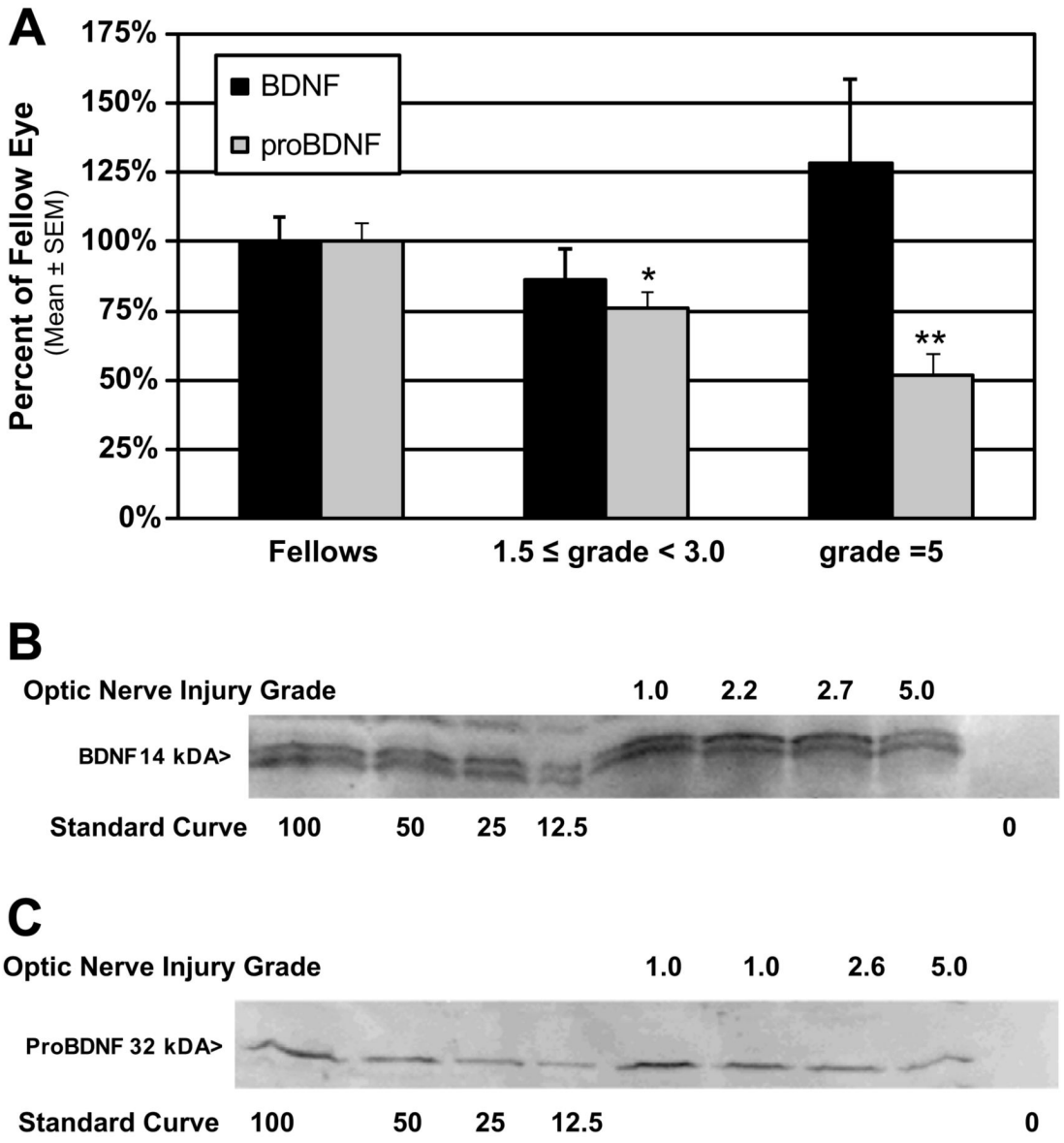

Fig. 5.

Retinal BDNF protein levels in glaucoma model retinas. A. Mature BDNF protein levels in either focal or global model eyes are not significantly different from those in fellow eyes, although the precursor form (proBDNF) is significantly decreased in both injury groups (ANOVA with Dunnett's post-test, $*p < 0.05$, $**p < 0.01$). B. Representative gel for quantification of mature BDNF, showing standard curve (units reflect relative protein load) and results from 4 retinas with increasing injury grades. C. Representative gel for 32 kD proBDNF demonstrating reduced protein levels with increasing nerve injury. ProBDNF is quantitated on a separate set of gels from an electrophoresis of lesser amounts of total protein, due to the relative abundance of proBDNF in the retina.

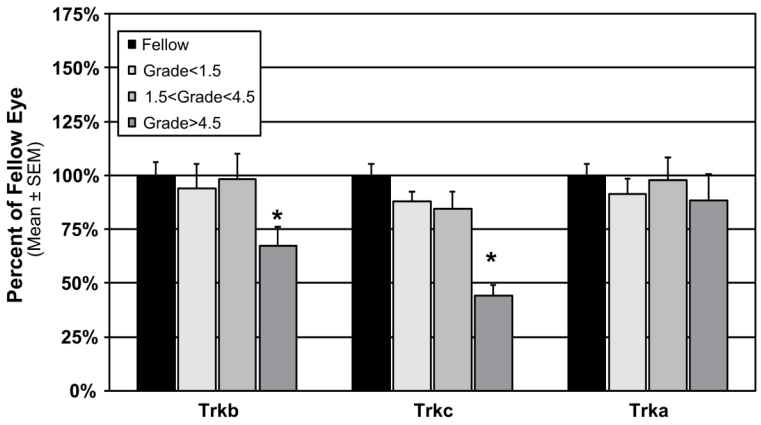

Fig. 6.

Response of retinal mRNA levels of full-length Trk receptors in a glaucoma model. Message for TrkB and TrkC are both significantly decreased in eyes with global nerve injury compared to fellow eyes (*$p < 0.01$), and both demonstrated a significant, negative correlation with nerve injury ($r^2 = 0.22$, $p < 0.005$, TrkB, and 0.56, $p < 0.0001$, TrkC).

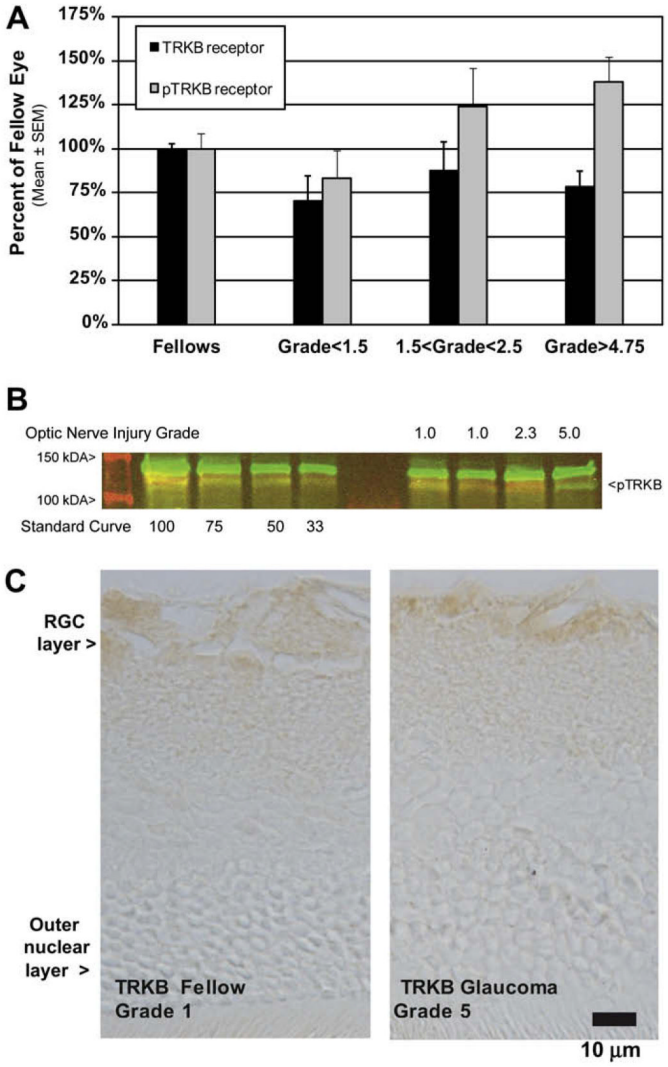

Fig. 7.

Response of TRKB receptor protein in experimental glaucoma. A. Full-length TRKB receptor protein does not change significantly with increasing optic nerve damage, while the activated form, pTRKB, increases linearly with increasing optic nerve damage. B. A representative gel illustrating co-labeling of TRKB (red) and pTRK (green), with the greatest intensity for activated TRKB corresponding to the greatest optic nerve injury. Upper green band is pTRKA, which is more abundant in the retina. Standard curve units reflect relative protein load. C. Immunohistochemistry for TRKB, which is localized to RGC soma and NFL, and does not appear to change despite advanced pressure damage. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

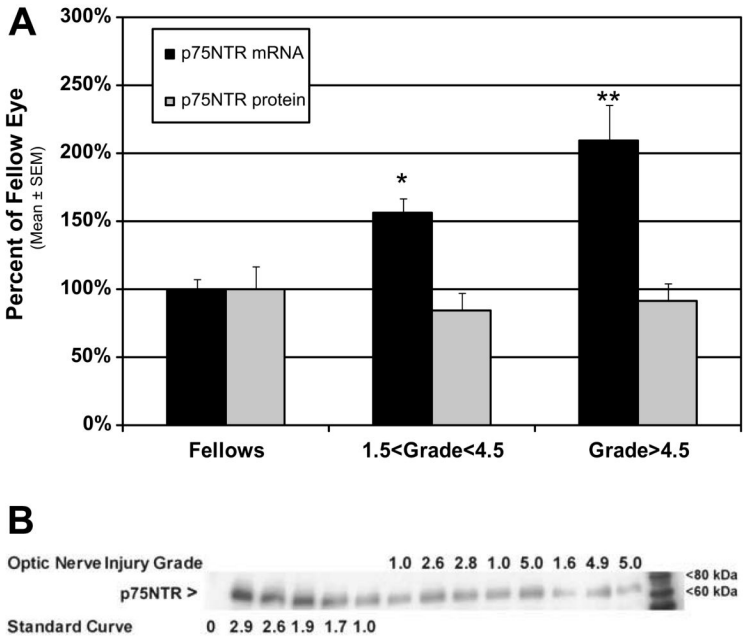

Fig. 8.

Retinal $p75^{NTR}$ mRNA and protein response to experimental glaucoma. A. $p75^{NTR}$ mRNA increased significantly to 157 ± 10% (*$p < 0.05$) and 210 ± 26% (**$p < 0.01$) for focal and global injury eyes, respectively. Protein levels were unchanged in glaucoma model eyes. B. Representative example of western blot documenting lack of consistent change in protein levels with increasing pressure-induced nerve damage. Standard curve units reflect relative protein load.

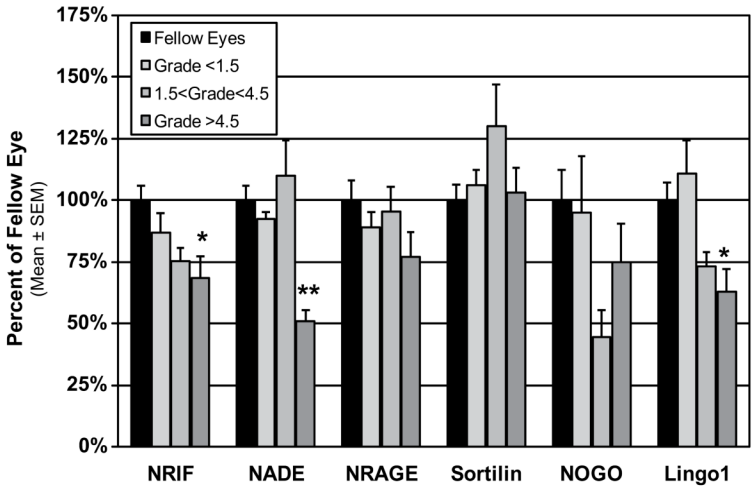

Fig. 9.

Retinal mRNA levels of $p75^{NTR}$-interacting proteins, demonstrating significant decreases in message for NRIF, NADE and Lingo1 in the most affected retinas (*$p < 0.05$, **$p < 0.01$, ANOVA with Dunnett's multiple comparison post-test).

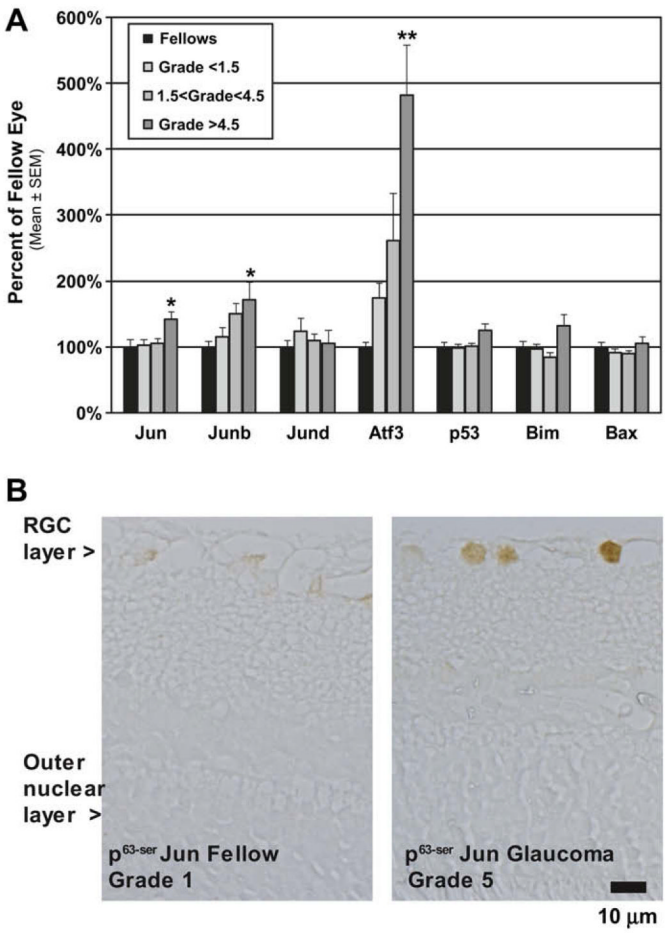

Fig. 10.

Signaling via JNK activation. A. Analysis of messages compared to injury grade demonstrates a significant upregulation of the mRNA levels of Jun and Junb, but not Jund, in eyes with global injury (*$p < 0.05$). Atf3 mRNA demonstrates a striking increase of 482 ± 76% (**$p < 0.01$) in retinas with the greatest damage, and a positive correlation with injury grade ($r^2 = 0.53$, $p < 0.0001$). Message level for p53, although significantly correlated to injury, is not significantly different between injury groups. B. Representative immunostaining for p^{ser63} JUN pattern, comparing a fellow and grade 5 retina, illustrating a clearly heavier label of RGCs in the glaucoma model retina.

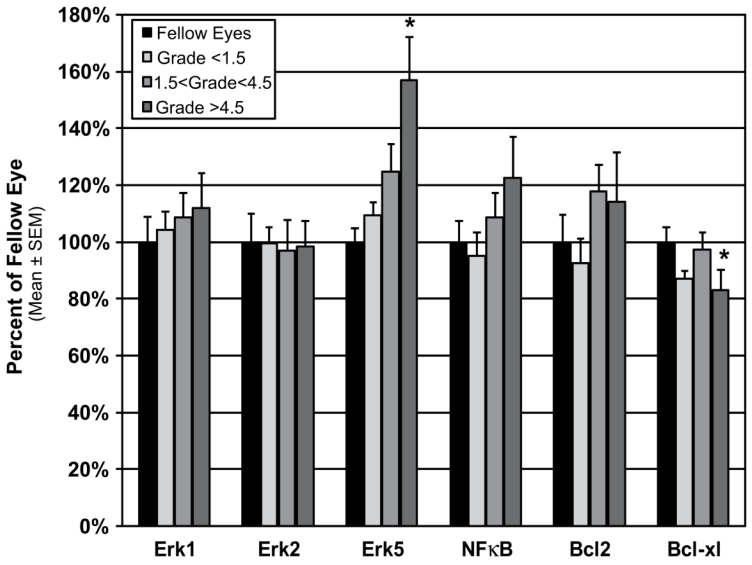

Fig. 11.

Responses to elevated IOP of mRNAs for pro-survival proteins. Downstream of Trk receptor activation are survival pathways mediated by Akt and Erk kinases. Message levels for Erk1 and Erk2 are unchanged, as are those for Akt1, Akt2, and Akt3 (not shown). However, Erk5 levels demonstrate a positive correlation with injury grade ($r^2 = 0.32$, $p < 0.0001$) that results in a significant increase in Erk5 message level in retinas with the greatest nerve injury (*$p <$ 0.01). Message levels for NF-κB, a transcription factor mediating $p75^{NTR}$ survival pathways, are significantly increased with injury grade by linear correlation ($r^2 = 0.12$, $p < 0.05$). Mitochondrial pro-survival mRNA levels for Bcl2 are unchanged, while levels of Bcl-xl are slightly, but significantly reduced in retinas with injury greater than 4.5 (*$p < 0.05$).

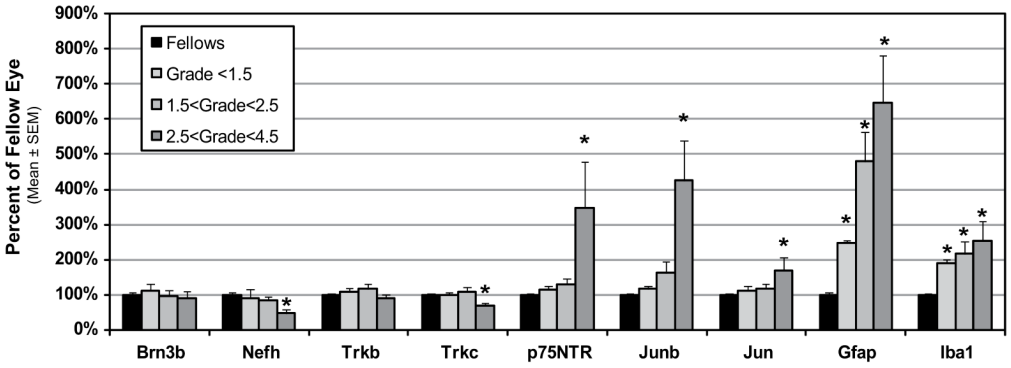

Fig. 12.

Short-term mRNA data. Many of the changes noted in glaucoma eyes five weeks following injections to elevate IOP are confirmed to occur shortly after IOP elevation (*$p < 0.05$).

Table 1

Primers used for qPCR.

Message (gene name[a])	Forward primer	Reverse primer	Product length
Akt1	CCATCACACCGCCTGATCAAG	AATTCCGAGGCTGGCGAGGTT	232
Akt2	GCCCAGTCCATCACAATCAC	ACAGGGAAGGGAACGCAAAC	218
Akt3	AATGACTATGGCCGAGCTGT	ATACGTCCTGCCAGTTTACTCC	280
Atf3	GACTGGTATTTGAAGCCAGGAGTG	GGACCGCATCTCAAAATAGC	96
Bad	CTATGGCCGTGAGCTCCGAA	GGATAATGCGCGTCCAACTG	125
Bax	GTGGCAGCTGACATGTTTG	ATCAGCTCGGGCACTTTAG	116
Bcl2	CTGAACCGGCATCTGCACAC	GCAGGTCTGCTGACCTCACT	195
Bcl-xl (*Bcl2L1*)	GAGATGCAGGTATTGGTGAG	TGGTCACTTCCGACTGAAGA	234
Bdnf	TTGGCTGGCGGTTCATAA	TTCTTGGCAACGGCAACA	323
Bim (*Bcl2L11*)	GCTTCCATAAGGCAGTCTCAG	TCTTCCGCCTCTCGGTAAT	143
Brn3a (*Pou4F1*)	CAGGAGTCCCATGTAAGA	ACAGGGAAACACTTCTGC	133
Brn3b (*Pou4F2*)	ACCGCGAGAAGCTCACCAAG	TGGCTGGATGGCGAAGTAGG	116
Brn3c (*Pou4F3*)	ACTCTGCTTTCCCTGCCCGACT	CAGAAGGGTCCGGTTCTGTGGT	96
Calb1	ATCAGGATGGCAACGGATAC	CCGACAAGGCCATTATGTTC	132
Erk1 (*Mapk3*)	AGGAGCTGATCTTCCAAGAG	TGCAGAGAAGGAGCAGGTAG	120
Erk2 (*Mapk1*)	ACCTTGACCAGCTGAATCAC	TGTTCCACGGCACCTTAT	132
Erk5 (*Mapk7*)	AGCTTGTGCCTTGCTGCCAGGAC	AGGCGGCTGCTTAAGGCTGAACG	150
Jun	CCAGAAGATGGTGCAGTGTT	GCGCATGCTACTTGATATGG	116
Gapdh	TGCCACTCAGAAGACTGTGGATG	GCCTGCTTCACCACCTTCTTGAT	249
Gfap	GCGGAGAACAACCTGGCTGTGTA	GCAGTTGGCGGCGATAGTCATTA	376
Iba1(*Aif1*)	TCCGAGGAGACGTTCAGTTA	CTGGCTCACAACTGCTTCTT	237
Junb	CGCCTGGAGGACAAGGTGAA	AACTGGCAGCCGTTGCTGAC	131
Jund	CGACATGGACACGCAGGAAC	TCTCCTCCAGGCGCGAGATA	113
Lingo1	CTTCCTGGGCGTGGTCCTATT	GATGCCTGCGTCCGATTTC	118
Nade (*Ngfrap1*)	GTTCATGGAGGAGATGAGAGAG	AGGAATACAGCGGGAATCAC	224
Nefh	GAGGACCGTCATCAGGTAGACAT	GGAGACGTAGTTGCTGCTTCTTC	446
NF-κB (*Nfκb1*)	CTCTCGTCCTCCTCCACAAG	GGACTCCGAGAAGCTGAGTT	108
Ngf	CACAGCCACGGACATCAA	GTGAGTCGTGGTGCAGTATGAG	175
NOGO (*Rtn4*)	CAGAAAGAACCGCACCCGTAGC	TACCAGTGCAAGGCCCAGAGGA	136
NRAGE (*Maged1*)	CGGTGCCATTGGCTTCTTCT	TGCCACTCTCAGTCAACAGG	145
NRIF (*Zfp110*)	TTGCAGCAAGGAGAGGAT	GGTTCGAAGGCAAAGGTT	164
NT-3 (*Ntf3*)	CGGATGCCATGGTTACTTCT	GATATCCGCCTGGATCAACT	176
NT-4/5 (*Ntf5*)	TTCTTCGAGACGCGCTGCAA	CAGACGCAAGCGGTGTCGAT	197
p53 (*Tp53*)	CATCATCACGCTGGAAGACT	TTCAGCTCTCGGAACATCTC	276
$p75^{NTR}$ (*Ngfr*)	GCGACAGTGGCATCTCTGTG	GCAGTGGACTCGCTGCATAG	235
Rho	CTAAGACCGCCTCCATCTAC	CTCCTACAGTCAGCCACAGT	196
Sortilin (*Sort1*)	CCTATCATCCTGGCCATCGT	TGCTGCTGAAGCACCGACTA	131
Stx1A	ATGTGGAACACGCTGTGGACTA	GCGATGATGATGCCCAGAAT	133
TrkA (*Ntrk1*)	GCCACACGCAACTGTCTGGT	TCGCCTCAGTGTTGGAGAGC	256
TrkB (*Ntrk2*)	TCCTAGCGGAGTGCTATAACCT	CACAGACACCGTAGAACTTGAC	165
TrkB T1	ACCACGCCAACTGACATCG	CTACCCATCCAGGGGGATCTT	231
TrkB T2	GAATATGGGAAGGACGAG	AGCACACTTCTGCTTACC	315
TrkC (*Ntrk3*)	GCATCATGTACCGGAAGT	GCCAAGAATGTCCAGGTAGA	296

[a]Gene name provided if an alternative designation is used in the text.

Table 2

Histories for five-week glaucoma model retinas analyzed by qPCR.

	Cumulative IOP elevation above fellows mmHg[a]	Optic nerve injury grade[a]	*N*
Fellows	1.03 ± 0.08		13
No injury	64 ± 611.09 ± 0.13		10
Focal injury	146 ± 852.90 ± 0.78		8
Global injury	354 ± 964.98 ± 0.04		11

[a] mean ± sd.

Table 3

Histories for short-term glaucoma retinas analyzed by qPCR.

	Cumulative IOP elevation above fellows, mmHg[a]	Optic nerve injury grade[a]	*N*
Fellows		1.0 ± 0.0	36
Grade < 1.5	27 ± 3	1.1 ± 0.0	21
1.5 < Grade<2.5	42 ± 8	1.8 ± 0.1	8
2.5 < Grade<4.5	85 ± 11	3.5 ± 0.3	6

[a] mean ± sd.