Effects of optic nerve injury, glaucoma, and neuroprotection on the survival, structure, and function of ganglion cells in the mammalian retina

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Glaucoma is an optic neuropathy that originates with pressure-induced damage to the optic nerve. This results in the retrograde degeneration of ganglion cells in the retina, and a progressive loss of vision. Over the past several years, a number of studies have described the structural and functional changes that characterize ganglion cell degeneration in the glaucomatous eye, and following optic nerve injury. In addition, a variety of different strategies for providing neuroprotection to the injured retina have been proposed. Many of these are based on the use of brain-derived neurotrophic factor (BDNF), a particularly potent neuroprotectant in the mammalian eye and the basis of our research in this area. Of particular importance is the fact that BDNF not only promotes ganglion cell survival following damage to the optic nerve, but also helps to preserve the structural integrity of the surviving neurons, which in turn results in enhanced visual function. The studies presented here describe these attributes, and serve as the foundation for ongoing work that suggests a need to think beyond the eye in the development of future treatment strategies.

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Background

Glaucoma is a leading cause of blindness that affects approximately 3% of white Americans and 10% of African Americans (Quigley, 1993; Leske et al. 2001). Clinically, most cases of glaucoma are associated with higher than 'normal' (> 21 mmHg) intraocular pressure (IOP), progressive cupping of the optic nerve head, and a characteristic loss of vision. The excavated optic disc of the glaucomatous eye reflects a reduction of neural tissue, as well as pressure-induced compression, displacement and reorganization of the perforated support structure (lamina cribrosa) through which the optic nerve fibres exit the eye (Radius & Pederson, 1984; Quigley, 1993; Hernandez & Pena, 1997; Bellezza et al. 2000). Visual field changes in glaucoma are due to a progressive loss of retinal ganglion cells, whose axons are compromised as they traverse the distorted channels of the sieve-like lamina.

While the mechanism(s) underlying optic nerve injury in glaucoma remain obscure, at least three, non-mutually exclusive, theories have been suggested: mechanical, vascular and biochemical. In the mechanical theory, pressure-induced distortions of the lamina cribrosa result in shearing and compressive forces that act directly on the ganglion cell axons (Morgan et al. 1998). The vascular theory involves a similar mechanism, but here the axon damage involves ischaemia due to compression of the capillaries that supply the optic nerve head (Findl et al. 1997; Pillunat et al. 1997). The biochemical theory is based on more recent data showing that optic nerve head glia, when activated by elevated IOP, are capable of releasing factors such as nitric oxide and tumour necrosis factor α, which can be neurotoxic (Neufeld et al. 1997). Although current data do not favour one theory over another, it is clear that damage to the optic nerve is an important factor for initiation of the disease process (see Weber & Viswanáthan, 2008 for a review).
Optic nerve injury, ganglion cell death and neuroprotection

Ganglion cell death in glaucoma is due primarily to apoptosis, and is thought to result from a decrease in the level of trophic material these neurons receive from their target neurons in the visual thalamus following optic nerve injury (Nickells, 1996). Support for this hypothesis comes from studies showing that elevated IOP disrupts axonal transport within the optic nerve (Anderson & Hendrickson, 1974; Quigley & Addicks, 1980), and that exogenous application of trophic factors to the eye following axotomy or optic nerve crush is neuroprotective for ganglion cells (rat: Mansour-Robaey et al. 1994; Mey & Thanos 1993; Cohen et al. 1994; Di Polo et al. 1998; cat: Chen & Weber, 2001). Of the different trophic factors studied, brain-derived neurotrophic factor (BDNF) is one that, by itself, has shown the most promise, and therefore has been the focus of our neuroprotection studies (Chen & Weber, 2001; Weber & Harman, 2008). Neurotrophin 4/5 (NT 4/5) has shown similar potential, while ciliary neurotrophic factor (CNTF) has yielded mixed results (see Mey & Thanos 1993; Cohen et al. 1994), including only a modest level of neuroprotection when used alone in the cat (AJW: unpublished data; see also Cohen et al. 1994).

The responsiveness of ganglion cells to BDNF and NT 4/5 is not surprising, since these neurons contain the tyrosine receptor kinase B (TrkB) receptor necessary for activation of intracellular signalling pathways, including those involved in cell survival (Jelsma et al. 1993; Perez & Caminos, 1995; Watson et al. 1999; Vaillant et al. 1999; Wahlin et al. 2000). BDNF and TrkB also are present within the LGN, superior colliculus, primary visual cortex and optic nerve head (Cabelli et al. 1996; Silver & Stryker, 2001; Lambert et al. 2001), emphasizing their importance in not just the retina, but throughout the entire visual system. In addition, recent work by Pease et al. (2000) and Quigley et al. (2000) has shown that elevation of intraocular pressure interferes with the retrograde transport of BDNF and the BDNF–TrkB ligand–receptor complex. This provides additional support for the neurotrophic theory of ganglion cell death in glaucoma, and perhaps more importantly, links it to BDNF and the retinogeniculate pathway. It is important to note, however, that this is not an exclusive relation; neurotrophin release by ganglion cells, lamina cribrosa cells, and optic nerve head astrocytes are considered to provide additional paracrine and/or autocrine support (Perez & Caminos, 1995; Gao et al. 1997; Lambert et al. 2001).

Structural and functional relations of injured ganglion cells and following neuroprotection

To date, studies that have focused on the potential use of trophic factors as retinal neuroprotectants following optic nerve injury have based their evaluations almost exclusively on comparisons of ganglion cell numbers in normal versus treated eyes. Exceptions are the studies of Siliprandi et al. (1993) in which pattern electroretinograms (PERG) were used to evaluate ganglion cell function in the cat following transient ischaemia and intravitreal treatment with nerve growth factor (NGF), the studied of Wood et al. (2001), who showed elevated levels of BDNF mRNA and recovery of the ERG b-wave following topical treatment of the ischaemic rat eye with 1-beta-blockol, and the studies of Shibuki et al. (2002), who found increased ERG b-wave amplitudes in ischaemic rat eyes treated with recombinant human hepatocyte growth factor. These studies aside, however, the question of whether trophic factor-based treatment strategies afford ‘rescued’ ganglion cells the ability to retain their normal dendritic morphologies and visual response properties, remained largely unanswered, thus forming the basis for our work.

Since the majority of early BDNF-based neuroprotection studies were conducted in rodents, an essential first step for us was to determine whether BDNF is an effective neuroprotectant in a primate-sized eye. To achieve this, we performed ganglion cell counts from matched retinal areas of cats that received a severe nerve crush and either no treatment, or direct treatment of the eye with different doses of BDNF (Chen & Weber, 2001). The region selected for quantitative analysis occupied 1.72 mm², and was located 3 mm above and 1.5 mm temporal to the area centralis. This region was chosen because of the relatively constant size and density of ganglion cells in this location of the cat retina (Boycott & Wässele, 1974). A stage digitizer (AccuStage, Shoreview, MN, USA) was used to properly orientate each retina (Vakkur et al. 1963) on the microscope, and to standardize the starting point and stage movements used for cell sampling. In all cases, the fellow eye served as the normal control. Although a much greater volume of drug was required to achieve a level of cell survival comparable to that reported in rodent eyes (30 μg versus 0.5 μg), the minimum effective dose for each was similar (∼0.01 μg μl⁻¹) when differences in the vitreal volumes of the cat and rodent eye (3000 μl versus 50 μl) were taken into consideration. Comparisons of the effects of the nerve injury and BDNF treatment on ganglion cell survival are shown in Fig. 1.

It is well known that ganglion cells receive all of their synaptic input through their dendritic arbors. Thus, the next logical step was to determine whether, in addition to promoting ganglion cell survival, BDNF also helps to preserve dendritic integrity. In a second group of experimental animals, the retinæe were flat-mounted into a chamber perfused with oxygenated artificial cerebral spinal fluid (Weber & Harman, 2008). Under direct microscopic visualization, individual ganglion cells were

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identified and injected with a fluorescent dye. At the end of the injection session, the retinas were fixed with 4% paraformaldehyde, and mounted onto glass slides. The intracellularly labelled neurons then were reconstructed using confocal microscopy, and the soma and dendritic arbor of each was analysed quantitatively using image analysis software (Neurolucida, MicroBrightfield, Inc.). In agreement with our previous work in the glaucomatous primate eye (Weber et al. 1998), the optic nerve injury resulted in significant decreases in the soma and dendritic field sizes of the affected neurons, as well as the overall complexity of the dendritic arbor, indicated by a decrease in number of branches, total dendritic length, and dendritic surface area. In all cases, direct treatment of the injured eye with BDNF resulted in enhanced preservation of soma and dendritic morphology, as demonstrated by the photomicrographs in Fig. 2A–D.

The dendritic changes described above suggest that, in addition to early degenerative changes in ganglion cell morphology, there also are early defects in visual function. Previously, we demonstrated a decrease in visual responsiveness to patterned and high temporal frequency stimuli by single ganglion cells in the glaucomatous primate eye (Weber & Harman, 2005). In order to examine the effect that optic nerve injury and BDNF treatment have on visual function in the cat, we performed electoretinographic (ERG) analyses on cats within each experimental group. Because our initial studies suggested that treatment of the injured eye with 30 μg BDNF resulted in the greatest amount of ganglion cell survival, but that treatment with 90 μg might be more beneficial toward the rescue of the large, α-type, ganglion cells, we compared animals using each dose. The ERG data were collected from ketamine/xylazine anaesthetized cats.

Figure 1 A, bar graph comparing ganglion cell survival in the normal retina, following optic nerve injury and no treatment, and with treatment of the injured eye with different levels of BDNF. B–D, photomicrographs showing ganglion cell densities in the sample regions of a normal retina (B), an injured retina that did not receive treatment (C), and a retina that received BDNF treatment at the time of the nerve injury (D). Sample region: 1.72 mm², located 3 mm above and 1.5 mm temporal to the area centralis – see text.
using an Espion Electrophysiology System and DTL Plus fibre electrodes (Diagnosys, LLC) moistened with 1% carboxymethylcellulose sodium, centred across the cornea of each eye and covered with a contact lens. Full-field flash ERGs consisted of 750 ms red (610 nm) flashes of 0.56–75 cd m$^{-2}$ delivered on a rod-saturating blue (440 nm) background of 75 cd m$^{-2}$, while the pattern ERGs (PERG) from central retina comprised 0.016–2 cpd square wave gratings (98% contrast and 50 cd m$^{-2}$ mean luminance), counterphase modulated at either 1 Hz (transient) or 4 Hz (steady-state). In all cases, a prominent b-wave in response to the flash stimulus indicated that the nerve injury did not have a significant affect at the photoreceptor level of the retina (data not shown). The PERG responses under each stimulus and treatment condition are summarized in the graphs of Fig. 3. In brief, the responses from the eyes receiving the 30 μg BDNF treatment were significantly better than those of the untreated eyes for the 1 Hz but not the 4 Hz stimuli, and they did not reach normalcy under either temporal modulation condition. They also did not differ significantly from the transient and steady-state responses.
measured in the eyes treated with 90 μg BDNF. By contrast, the responses from eyes receiving the 90 μg treatment were significantly better than those of the untreated eyes at both the 1 Hz and 4 Hz levels, and they were not significantly different from normal under each condition. Thus, it appears that the higher dose of BDNF is effective in preserving retinal function at both the transient and steady-state levels of stimulation, while the lower dose is most effective in preserving only the transient response.

Future studies: neuroprotection beyond the eye

Neuron-target dependence is a well-known phenomenon that affects developing and mature components of both the central and peripheral nervous systems. With respect to the visual system, several studies have demonstrated that reducing the number of target neurons in the LGN, either by early damage to visual cortex (Pearson et al. 1981; Kalil, 1984; Weber et al. 1989), or direct application of kainic acid to the LGN (Pearson et al. 1991; Pearson & Stoffler, 1992), results in a significant loss of ganglion cells in the retina. This loss is thought to result from a decrease in the level of trophic materials transported retrogradely from the LGN target region to the retina, in the absence of any direct insult to the retinogeniculate axons themselves. While target cell loss represents perhaps the most extreme case of a reduction in trophic supply, decreases due to reduced synaptic connectivity or decreased LGN activity might also play an important role, especially during early stages of degeneration. Our data, and those of others, showing that glaucomatous neuronal damage is not restricted to the retina, but also involves decreases in the size and number of neurons in the LGN (Chaturvedi et al. 1993; Weber et al. 2000; Yucel et al. 2001) suggest that restricting neuroprotective treatment to only the retina might be short-sighted. Indeed, studies in the rat have indicated that peripheral nerve grafts attached to the cut optic nerve enhance retinal ganglion cell survival, but that a more permanent level of survival is achieved only when the nerve graft is used to link the regenerating retinal axons with target neurons in the tectum (Vidal-Sanz et al. 1991). Presumably the restoration of neuron–neuron connections provides not only trophic support, but also mutual electrical stimulation between the pairs of neurons (Shen et al. 1999).

Aside from a reduction in the level of target-derived trophic factor available following optic nerve injury, there are several additional reasons that extending treatment beyond the retina might enhance ganglion cell survival. These concern the effects that optic nerve injury and BDNF application have on TrkB receptor levels within the retina, as well as differences in the intracellular signalling pathways activated by application of the drugs to different regions of the neuron (e.g. soma versus axon terminal). Previous studies by us (Chen & Weber, 2004) and Cheng et al. (2002) have shown that optic nerve injury alone results in a decrease in the level of TrkB receptors within the retina. While our data suggest that the decrease in TrkB receptors coincides with the retrograde loss of ganglion cells, those of Cheng et al. (2002) suggest that a decrease in retinal receptor levels might actually precede ganglion cell loss. Furthermore, additional work by us (Chen & Weber, 2001, 2004) and Sommerfeld et al. (2000) indicates that BDNF limits its own neuroprotective capacity by causing a down-regulation of retinal TrkB receptors. With respect to intracellular signalling, Watson et al. (2001) have shown that application of neurotrophins to the cell soma versus the axon terminal results in the activation of different neuronal survival pathways; neurotrophin stimulation of the cell soma activates the extracellular signal-regulated 1/2 (Erk1/2) and Erk 5 cascades, while receptor-ligand binding at the nerve terminal results only in Erk 5 activation within the cell soma. Taken together, these various studies indicate that there are distinct differences with respect to how neurons obtain and respond to neurotrophic factors. In the visual system, it might be the case that TrkB receptors within the normal retina serve a minor role in supporting ganglion cells during periods of limited stress, while long-term survival depends on the retention of stable connections and activation of retrograde intracellular signalling via their association with target neurons in LGN (e.g. Vidal-Sanz et al. 1991; Shen et al. 1999). This might explain why previous studies that have focused exclusively on optic nerve section and treatment to the eye alone have not been able to achieve long-term ganglion cell survival; TrkB receptors on ganglion cells might be easily saturated by exposure to the levels of drug necessary to provide the initial neuroprotection.

More recent studies in our lab have indicated that application of BDNF to both the eye and visual cortex results in levels of ganglion cell survival and function that exceed those seen following treatment of the eye alone, and that this enhanced response is sustained for up to 2 weeks. While ongoing work is aimed at determining whether this dual treatment strategy might support an even more prolonged period of ganglion cell survival and function, it seems quite clear that the development of new treatment strategies will require one to 'think outside the eye'.

Summary

Over the past several years considerable strides have been made in our understanding of the relation between glaucoma-related optic nerve injury and the resulting degeneration of ganglion cells in the retina. In particular, we now understand that the mechanism of optic nerve injury is a complex process that involves mechanical,
vascular and biochemical events. We also understand that most ganglion cells in the glaucomatous eye die by means of apoptosis, and that without some form of intervention, this cell death is progressive. Our intracellular studies have shown that, at the single cell level, the earliest morphological signs of ganglion cell degeneration relate to structural changes in the distal regions of the dendritic arbor, and that these changes result in functional deficits in the response properties of these neurons. Although the time course of this process remains to be defined, this progressive pattern of degeneration reflects a ‘window of opportunity’ during which different forms of neuroprotection might be applied. Direct application of neuroprotectants, either alone or in combination, to the injured eye represent the primary strategy used in most studies, although additional work is focused on the potential role of the immune system as a means of neuroprotection (Bakalash et al. 2005; Ben-Simon et al. 2006; Tezel et al. 2007). As indicated here, BDNF by itself is capable of not only enhancing ganglion cell survival, but also preserving the dendritic integrity of the surviving neurons, which in turn results in enhanced visual responses relative to no treatment. The different results seen with respect to low versus high doses of the drug indicate clearly that more work is needed in order to determine whether this reflects a general inability of low doses to preserve ganglion cell function at high temporal frequencies, regardless of cell class, or if it represents a differential ability of BDNF to promote α- versus β-cell survival at low versus high doses. Development and optimization of any therapeutic treatment will require determination of the sensitivity and responsiveness of the different classes of ganglion cells to the various compounds selected. In addition, our ongoing studies indicate the need to develop treatment strategies that are beneficial to the entire central visual pathway, and not just the eye.

References


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