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Neuroprotection of retinal ganglion cell function and their central nervous system targets

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Abstract

Technological advances have enabled the observation of a large number of retinal ganglion cells (RGCs) in an objective manner. In animal models, it has been shown how retinal ischaemia induces profound functional and structural alterations of the inner retinal and RGC layers by 3 months. These findings reflect degeneration of the inner retinal layers, the RGC population and of the retinotectal projection. Functionally, this implies a permanent disconnection of the retina from its main retinorecipient target region in the brain. Brimonidine, a selective α-2 adrenergic agonist, has been shown to activate α-2 adrenergic receptors in the retina and promote the survival and function of RGCs post-injury. This agent may prevent or diminish ischaemia-induced alterations in the inner and RGC areas as well as in the main retinofugal projection. Understanding the pattern of degeneration that occurs in the major retinofugal pathway following retinal ischaemia will benefit ongoing studies conducted to develop neuroprotectant-based treatment strategies for progressive neuropathies such as glaucoma.
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Keywords: glaucoma; neuroprotection; retinal ganglion cells; retinal ischemia

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Introduction

Axotomy-induced cell death provides a classic model for studying neuroprotection. This type of injury may be achieved by several methods, including optic nerve transection, complete nerve crush, partial optic nerve transection or partial optic nerve crush. An alternative injury model is transient ischaemia of the retina. This injury may be achieved either by elevation of intraocular pressure above systemic levels or by selective ligature of the ophthalmic vessels (SLOV). A third and very well used model is ocular hypertension-induced retinal injury. Neuroprotective effects of drugs have been shown for both axotomy- and ischaemia-induced retinal ganglion cell (RGC) death.[1–4] Among these, α-2 agonists like brimonidine (BMD) have been shown to have neuroprotective potential against transient ischaemia-induced RGC death by ligature of the ophthalmic vessels.[5,6] The models used can also be deployed to investigate a number of pathophysiological issues, including axonal regeneration, synapse formation,[7,8] injury-induced neuronal degeneration[9,10] and prevention of injury-induced neuronal degeneration (ie neuroprotection).[3,11] This paper examines some of the effects induced by transient ischaemia of the retina and how some of these may be prevented, diminished, or ameliorated.

Use of whole-mount retina preparations for studies of RGCs

For the quantitative estimations of RGC survival, in our studies, we have mostly used whole-mount retina preparations. Rats are anaesthetized and the RGC population is prelabelled with a fluorescent tracer, fluorogold (3%), applied to the superior colliculi, which are its main targets in the brain.[4,10] Within 1 week, the optic nerve is intraorbitally transected, and the retinas examined under fluorescence microscopy. The entire RGC population can be identified in the whole-mount retina preparation, and the labelling persists for up to 4 weeks after tracer application.[13] Over the last few years, this model has been used to study the survival of RGCs to various treatments or injuries. Currently, a computer-assisted analytical programme allows the number of

surviving RGCs to be determined in a more systematic and automatized fashion. During this research, it has become apparent that an area of the superior retina of albino rats has a particularly high density of RGCs, and overall these retinas contain a population of RGCs that, as average, amount to 79 000 RGCs.[14]

In the present studies, the injury model that we used consisted of retinal ischaemia induced by transient ligature of the left ophthalmic vessels for 30–90 min. The left optic nerve head (ONH) is exposed in the orbit and the superior aspect of its dural sheath opened longitudinally without damaging the ONH.[7] A 10-0 nylon monofilament suture is introduced between the ONH and its sheath, and tied around the sheath. The ligature is released up to 90 min later.[6] The RGCs can then be examined at different time points. In one study, 7 days after induction of retinal ischaemia for 60 or 90 min, cell death occurred in 36 or 47% of the RGC population, respectively.[6] By 21 days, RGCs loss amounted to 42 or 62%, respectively (Figure 1). This unexpected delayed effect suggested that retrograde axonal transport was impaired in some surviving RGCs or that there was RGC loss due to secondary injury. We see a potential for this loss of protracted, slow-dying wave of RGCs to be prevented with the use of neuroprotectants including some neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), some antiapoptotic substances and α-2 adrenergic-selective agonists.[11]

Investigation of potential neuroprotective effects on RGCs

The possibility of neuroprotective effects was investigated using the injury model involving retinal ischaemia. Two groups of animals were prepared for comparison, one being treated with saline (0.9% NaCl) and the second with BMD (0.5% in saline). Treatments were applied to the left eye as two 5-μl drops 1 h before ischaemia.[15] Two drops of 0.5% BMD in these animals elicited enough response to upregulate the expression of BDNF and ciliary neurotrophic factor (CNTF) in the retina of the animals' left eye.[16] Both neurotrophic factors are known to reduce RGC death.[1,2,4]

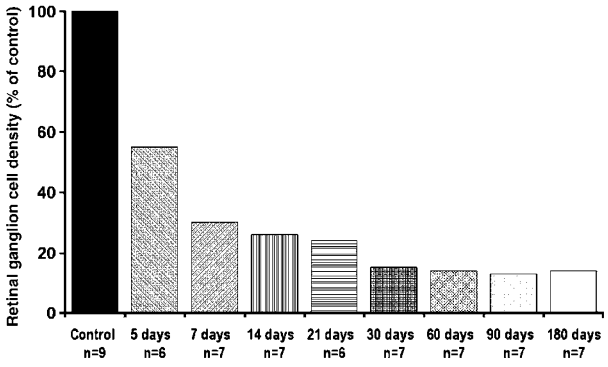

Figure 1 Transient ligature of the ophthalmic vessels (LOV) for 90 min results in progressive RGC loss. Data represent mean densities (expressed as percentages of control retinas) of di-ASP labelled RGCs in the left experimental retinas of groups of rats at increasing survival periods of time (5–180 days) after 90 min LOV. RGC death begins during the first 5 days and progresses for up to 2 months. (Reproduced from Lafuente *et al*[12] with permission of *Neuroscience* from Elsevier.)

In our earlier studies, topical application of BMD was found to confer a dose-dependent protective effect on RGCs.[17] BMD, according to the topical doses administered, prevented up to 90% of RCG loss. Between 7 and 21 days after ischaemia, there was an additional slow RGC loss, which could be significantly reduced by pretreatment with the highest BMD dose. The administration of BMD up to 2 h, but no later, after ischaemia still provided a protective effect to RGCs, presumably because it was administered at a time when a large proportion of RGCs were already committed to die.[6]

A method of examining the functional aspects of RGCs that survive injury is to investigate axonal transport. Cells that survive ischaemia should have their retrograde axonal transport preserved. Our findings indicate that, in a proportion of RGCs that survive transient ischaemia of the retina, this injury induces an impairment of retrograde axonal transport.[15] This damaged axonal transport can be prevented, almost fully, by BMD administered before induction of ischaemia.[15] Our group could also show that BMD protects against ischaemia-induced degeneration of the retinotectal projection[18] (Figure 2), and preserves anterograde axonal transport as well as inner retinal layer function[19] (Figure 3).

Conclusion

In summary, retinal ischaemia induces profound functional and structural alterations of the inner retinal and RGC layers. These findings reflect degeneration of the inner retinal layers, and of the RGC population and retinotectal projection. Functionally, this implies a permanent disconnection of the retina from its main retinorecipient target region in the brain. BMD, an α-2 adrenergic-selective agonist, may prevent or diminish the ischaemia-induced alterations in the inner and RGC areas as well as in the main retinofugal projection. Understanding the pattern of degeneration that occurs in the major retinofugal pathway following retinal ischaemia will benefit ongoing studies conducted to develop neuroprotectant-based treatment strategies, and may provide insights into other

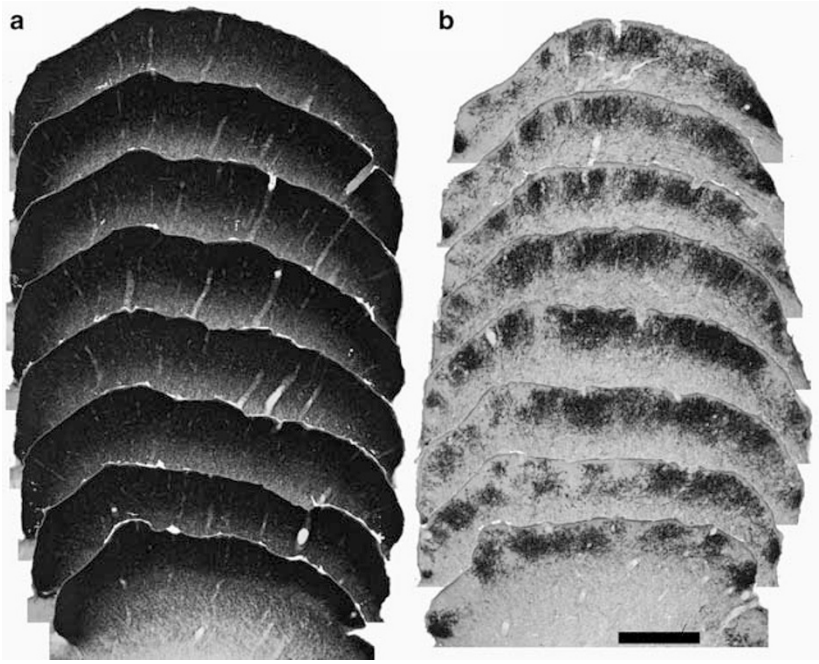

Figure 2 Light micrographs illustrating every fourth 40-μm-thick cryostat serial coronal section through the mid-SC in its rostro- (top) caudal (bottom) extent, from one representative control intact rat (a) or an experimental rat that was treated 1 h before 90 min of retinal ischaemia in the left eye with two 5-μl drops of saline (b). To identify retinotectal afferents, the left eye was injected with the tracer cholera toxin B subunit (CTB) 4 days before animal processing and 2 months after retinal ischaemia. The side contralateral to the eye injection shows density and distribution of CTB-labelled retinal afferents in the superficial layers of the SC. Dark areas correspond to RGC terminals anterogradely labelled with CTB. In the right SC of the control rat, very densely labelled retinal axons fill the superficial layers (a). In contrast, note the reduction of CTB-labelled profiles in the superficial layers of the right SC in the vehicle-treated rat (b), which results in reduced thickness and in areas virtually lacking retinal axons. Dorsal is to the top and middle is towards the left. Scale bar=500 μm. (Reproduced from Mayor-Torroglosa *et al*[19] with permission of *Invest Ophthalmol Vis Sci* via Copyright Clearance Center.)

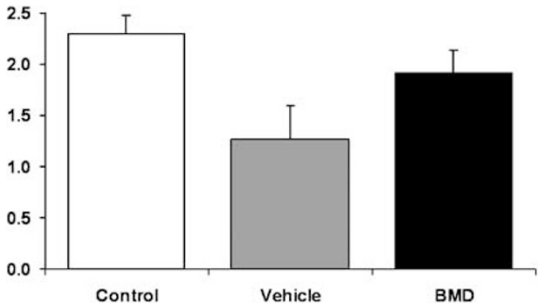

Figure 3 Average volumes of the retinotectal projection. Histograms representing mean ($\pm$SD) volume (mm^3) occupied by retinal afferents in the visual layers of the contralateral SC orthogradely labelled with CTB applied to the ischemic eye for five days, in control, vehicle-treated or BMD-treated groups of rats 3 months after 90 min of retinal ischaemia to the left eye. In the vehicle-treated group of animals, mean volume had decreased to approximately one half of control volumes, whereas in the BMD-reated group the mean volume corresponded to approximately 84% of the control values. (Reproduced from Mayor-Torroglosa *et al*[19] with permission of *Invest Ophthalmol Vis Sci* via Copyright Clearance Center.)

progressive diseases that may also cause ischaemia, such as glaucoma.

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