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Optic nerve and neuroprotection strategies

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Abstract

Background **Experimental studies have yielded a wealth of information related to the mechanism of ganglion cell death following injury either to the mylinated ganglion cell axon or to the ganglion cell body. However, no suitable animal models exist where injury can be directed to the optic nerve head region, particularly the unmylinated ganglion cell axons. The process of relating the data from the various animal models to many different types of optic neuropathies in man must, therefore, be cautious.**

Results **Extensive studies on the isolated optic nerve have yielded valuable information on the way white matter is affected by ischaemia and how certain types of compounds can attenuate the process. Moreover, there are now persuasive data on how ganglion cell survival is affected when the ocular blood flow is reduced in various animal models. As a consequence, the molecular mechanisms involved in ganglion cell death are fairly well understood and various pharmacological agents have been shown to blunt the process when delivered before or shortly after the insult.**

Conclusions **A battery of agents now exist that can blunt animal ganglion cell death irrespective of whether the insult was to the ganglion cell body or the mylinated axon. Whether this information can be applied for use in patients remains a matter of debate, and major obstacles need to be overcome before the laboratory studies may be applied clinically. These include the delivery of the pharmacological agents to the site of ganglion cell injury and side effects to the patients. Moreover, it is necessary to establish whether effective neuroprotection is only possible when the drug is administered at a defined time after injury to the ganglion cells. This information is essential in order to pursue the idea that a neuroprotective strategy can be applied to a disease like glaucoma, where ganglion cell death appears to occur at different times during the lifetime of the patient.**

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Introduction

Retinal ganglion cells are the output neurones from the retina, extending their axons via the optic nerve to defined areas of the brain. The optic nerve consists of ganglion cell axons, supporting glial cells in the form of astrocytes, and blood vessels. Ganglion cell axons are unmyelinated while passing through the nerve fibre layer and optic nerve head but gain a myelin sheath after leaving the lamina cribrosa. In common with nerves of the CNS, ganglion cell axons are myelinated by oligodendrocytes rather than by Schwann cells, and this probably accounts for the lack of structural regeneration that occurs after optic nerve injuries.

Diseases involving ganglion cells are common. Experimental data clearly show that ganglion cell death can be initiated by insults to the cell body and dendrites within the globe, or to the axons outside the globe. It is clear that in order to facilitate effective treatment of diseases where ganglion cell loss occurs (eg glaucoma), knowledge of the initial site of injury to the cell is of some importance. However, even in a well-characterised disease like glaucoma, unambiguous identification of the initial site and type of injury to the ganglion cell has not proved easy. Nevertheless, there is strong evidence that the optic nerve head region is the site of injury to the ganglion cells in glaucomatous optic neuropathy[1–3] and discussions have centred on whether injury to the ganglion cell axons in this location is primarily caused by mechanical trauma[3] or by alterations in the dynamics of blood flow.[4–6] It should of course be noted that clinical changes

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This article is dedicated to José Melena, who tragically died in a plane crash around the time of the meeting in Cambridge.

in the optic nerve head may not necessarily reflect the site of injury to the ganglion cells. In Leber's hereditary optic neuropathy, for example, a mitochondrial defect is known to be the primary cause of the disease.[7]

The axon as the site of injury to the ganglion cell

The optic neuropathies are a diverse group of diseases which are characterised by visual loss due to optic nerve dysfunction. A variety of factors can be responsible for the disease, but in all types of optic neuropathy, the injury is manifested at the optic nerve axon and results in the loss of retinal ganglion cells. The most common optic neuropathy is that associated with glaucoma, that is, glaucomatous optic neuropathy. However, a wide variety of other optic neuropathies are known to exist as described by Biousee *et al*[8] and Levin.[9] Defined types of optic neuropathy are associated, for example, with inflammation (eg neuroretinitis), neoplasms (eg optic nerve glioma), compression (eg orbital tumor), trauma (eg traumatic optic neuropathy), ischaemia (eg ischaemic optic neuropathy), toxicity/nutritional (eg methanol, vitamin B_{12} deficiency), drugs (eg chloramphenicol, digitalis), and hereditary diseases (eg Leber's hereditary optic neuropathy, Friedriech's ataxia). The clinical findings associated with a typical optic neuropathy include optic disc oedema and atrophy, visual field abnormalities, decreased visual acuity, changes in colour perception, and relative afferent pupillary defects.

The knowledge that ganglion cell axons are the site of injury in optic neuropathies has led to the development of animal models of optic nerve injury. On the whole, studies have utilised either the partially crushed[10] or completely transected[11] optic nerve with the aim of understanding the processes related to the final demise of the whole of the ganglion cell. It should be noted that in all the animal studies, the ganglion cell axon is injured outside the optic nerve head where myelin is present. The data derived from these experiments may therefore not reflect precisely what occurs when the ganglion cell axon is damaged within the optic nerve head area as is likely to occur in, for example, glaucoma. Nevertheless, some general conclusion may be drawn from such studies. Firstly, death of ganglion cells is much more rapid in very young than in adult rats.[12,13] Secondly, degeneration is more rapid when the ganglion cell axon is transected close to the optic nerve head rather than further away.[14,15] Thirdly, not all ganglion cells die at the same rate: in the adult cat, β ganglion cells are more susceptible to death after optic nerve transection than α ganglion cells.[16] Fourthly, various transcription factors, guidance molecules, extracellular matrix proteins, neurotrophic factors, and cell death regulating factors have been shown to be associated with ganglion cell apoptosis following axon damage (for a recent review see Isenmann *et al*[17]). Such studies have tended to support the view that supplementation of appropriate neurotrophic factors to the retina (eg BDNF) may provide a means to slow-down the death process of ganglion cells injured by optic nerve injury, as is proposed to occur in glaucoma.[3]

Cell body and dendrites as the site of injury to the ganglion cell

Injury to the optic nerve is not the sole means of killing ganglion cells. Ganglion cell death can also be induced through insults directed at the cell body and dendrites. A number of different methods have been identified, and these include: intravitreal injection of ionotropic glutamate receptor agonists (eg NMDA or kainate),[18,19] raising the extracellular glutamate level by blocking uptake into Müller cells,[20] and by causing retinal ischaemia.[21] The cause of cell death in all of these models is thought to essentially occur via the same process, that is, excessive depolarisation caused by overstimulation of ionotropic glutamate receptors.[22] As well as ganglion cells, however, other cells, particularly subsets of amacrine cells, are also affected in such instances.[23] This is explained by the fact that ionotropic glutamate receptors are primarily associated with ganglion cells and subsets of amacrine cells.[24]

Role of toxic mediators in ganglion cell death

Present evidence suggests that retinal astrocytes and microglia may become activated as a result of optic nerve injury and ischaemia, and can release toxic substances (eg glutamate, D-serine, nitric oxide, tumour necrosis factors α and β) into the extracellular space. These factors may contribute to the damage in ganglion cells.[25] Interestingly, in patients with glaucomatous optic neuropathy, levels of the enzymes cyclooxygenase-1 and nitric oxide synthase, which are involved in the synthesis of eicosanoids and nitric oxide, respectively, and are associated with retinal astrocytes, are elevated in glaucomatous eyes.[26,27] Likewise, in animal models of glaucoma, ganglion cell death is attenuated by treatment with inhibitors of astrocyte-associated nitric oxide synthase.[28] These data support the view that nitric oxide is one toxic factor that is released from astrocytes in glaucoma and damages ganglion cells.

Role of mitochondrial dysfunction in ganglion cell death

Mitochondrial dysfunction has been reported to occur as a result of ischaemia[29] and also in a number of optic neuropathies.[30] Mitochondria maintain the energy requirements of the cell by oxidative phosphorylation and produce significant amounts of reactive oxygen species

(ROS) as a by-product. It is generally thought that impairments in oxidative phosphorylation and metabolism (eg oxidative stress, ischaemia) and increased production of ROS may both contribute to the opening of the mitochondrial permeability transition pores (mtPTP), thus releasing factors (such as cytochrome *c*) into the cellular cytoplasm to initiate apoptosis. This process can be blocked by overexpression of Bcl-2, which is a protein found to be associated with the outer mitochondrial membrane.[31] It may be that when ganglion cell mitochondrial impairment occurs, as in Leber's Heriditary Optic Neuropathy[32] that the reduced ATP level negatively impacts axonal transport, leading to the typical neuropathy associated with this disease (ganglion cell apoptosis). Ganglion cell mitochondria may also be affected in other ways. Excessive intracellular calcium accumulation (ischaemia, excitotoxicity), reduced extracellular zinc (see Ugarte and Osborne[33]), or damage to the axon of the cell will all result eventually in apoptosis.

Studies on the isolated anoxic optic nerve and possible therapeutic implications

Ischaemia to the optic nerve is implicated in certain optic neuropathies, including carotid artery occlusive disease and posterior ischaemic optic neuropathy.[34] In order to investigate the effect of ischemia on the optic nerve, experiments have been conducted on the anoxic isolated optic nerve. The results have yielded important insights into potential ways of reducing the impact of such an insult *in vivo*. Moreover, studies on the isolated optic nerve preparation have provided major insights into how white matter can be affected by ischaemia. The optic nerve is particularly suitable for the study of white matter because it is easily accessible and is devoid of neuronal cell bodies and synaptic structures.[35,36] By recording the compound action potential (CAP), the functional integrity of the isolated optic nerve can be reliably monitored. The nerves are stimulated, and evoked responses are recorded extracellularly, using suction electrodes. This allows reproducible and quantitative measurements to be made on the effect of anoxia on the CAP and the postanoxic recovery of the CAP.[36–40] By combining CAP data with histological analysis of the optic nerve[36,41] much has been learnt about how the optic nerve is damaged by anoxia, and the types of substances that can blunt such an insult.

The central characteristic of optic nerve anoxic injury is a run-down of ionic gradients. Energy failure in the optic nerve from anoxia causes depletion of ATP and inhibition of Na,K-ATPase. Intra-axonal sodium rises through leakage of this ion through noninactivating sodium channels that remain open despite membrane depolarisation. Internal potassium is simultaneously lost through some types of nodal or internodal potassium channels.[40] The rise in internal sodium coupled with membrane depolarisation leads to the reversal of the sodium/calcium exchange mechanism, which abnormally exports sodium in exchange for calcium influx. This causes calcium-mediated damage.[42–44] The rise in intra-axonal calcium from the extracellular space is exacerbated by calcium influx through L-type voltage-dependent calcium channels,[45] and it has recently been shown that channel-mediated chloride fluxes also contribute to optic nerve injury during anoxia.[46] Should reoxygenation take place, re-energized mitochondria will import the excessive and the large amounts of calcium, that accumulate in the axoplasm during anoxia, contributing to reperfusion injury.

On the basis of these observations a number of substances have been found to reduce the impact of anoxia on the isolated optic nerve. Undoubtedly, some of these may be of potential therapeutic importance (Table 1). Sodium channel blockade, such as with local anaesthetics, antiarrhythmics, and certain anticonvulsants, has been reported to protect against optic nerve anoxia[36,37,47] as has inhibition of the sodium/calcium exchanger with bepridil or benzamil.[37] Entry of extracellular calcium contributes to the rise in intracellular calcium that ultimately causes axonal death through the activation of a number of enzymes.[36,39] Interestingly, it has been suggested that calcium predominantly enters axons through sodium channels rather than calcium channels.[44] However, other studies[45,48,49] have provided evidence that entry of calcium through calcium channels also takes place. Inhibitors of calcium-sensitive enzymes, including lipases, kinases, phosphatases, and proteases are therefore potential methods of protecting the ganglion cell axon from the effects of anoxia. In addition, potassium channel blockers are likely to be beneficial. Stys *et al*[40] showed that the potassium channel blockers, glibenclamide and tolbutamide, had no effect on CAP

Table 1 Some events leading to optic nerve anoxia and potential therapeutics interventions

Event	*Blunting the event*
Na^+ influx	Na^+ channel blockers Anticonvulsants Antiarrhythmics β-Adrenoceptor antagonists
K^+ efflux	K^+ channel blockers Inhibitors of K^+/Cl^--cotransporter
Reversal of the Na^+/Ca^{2+} exchanger	Specific inhibitors of the exchanger
Increase in intracellular Ca^{2+}	Inhibitors of calpain, PLC, PKC, NOS Inhibitors of L-type Ca^{2+} channels
Release of GABA, adenosine	GABA and adenosine agonists

recovery after isolated optic nerve anoxia, but that inward rectifier potassium channels may play an important role in the induction of anoxic injury in optic nerve axons.

An autoprotective mechanism involving adenosine and GABA receptors has been reported in isolated optic nerve from the rat[47,49] which suggests that $GABA_B$ and adenosine agonists may act as neuroprotectants to the anoxic optic nerve. GABA and adenosine are released during ischaemia from white matter, probably because of the reversal of the membrane potential. These substances would, therefore, cause anoxic tolerance through the stimulation of defined receptors.[50] Support for this comes from the finding that perfusion of the isolated optic nerve with the GABA uptake inhibitor nipecotic acid or the adenosine uptake inhibitor propentofylline significantly increased postanoxia survival.[47,49]

β-Adrenoceptor antagonists may also be expected to protect the isolated optic nerve preparation against anoxia. The rationale behind this statement is that some of these substances have been shown to inhibit sodium influx into neurons via an interaction with the voltage-sensitive sodium channel.[51,52] The drugs do not interfere with ion conductance directly but, rather, they modulate the gating mechanism of the sodium channel in a similar way to local anaesthetics.[53] It is of interest that some of the β-blockers that reduce sodium influx into neurones are used to reduce intraocular pressure in glaucoma (betaxolol, timolol, metipranolol). No other class of drugs used in the treatment of glaucoma is known to have sodium channel blocking properties.

Table 2 Some ways of reducing ocular blood flow in order to affect ganglion cell function

Procedures	*References*
Transient acute increase in IOP (>100 mmHg) for defined time (30–90 min) and reperfusion	23, 76
Constant chronic increase in IOP (10–20 mmHg) for 5–10 weeks	58, 77–80
Permanent occlusion of common carotid arteries	66, 68, 81
Transient or permanent occlusion of central retinal and posterior ciliary arteries	82, 83
Dye/photothrombosis: permanent occlusion of retinal blood vessels	84
Permanent ligature of the ophthalmic arteries	85, 86

Table 3 Substances shown to attenuate ganglion cell death caused by an acute increase in IOP

Substances	*References*
β-Adrenoceptor antagonists	56, 87–90
α_2-Adrenoceptor agonists	91–93
Calcium channel blockers	94–96
COX-2 inhibitor	97
Gabapentin-lactam	98
Growth factors/neurotrophins	71, 72, 99, 100
IL-1 inhibitors	101
$5\text{-}HT_{1A}$ receptor agonists	102
Free radical scavengers	55, 103, 104
Nitric oxide synthase inhibitors	105–107
NMDA receptor antagonists	105, 108–112
NMDA and AMPA antagonists	113
Sodium channel blockers	114

Studies on animal models where ocular blood flow is reduced to cause ganglion cell death, and possible therapeutic implications

Ocular blood flow in the rat can be reduced in a variety of ways to induce ganglion cell damage (Table 2). As a consequence, a lot of data exists on the mechanism of anoxia-induced ganglion cell death and potential ways of blunting the process.

When a transient acute increase in IOP (eg 110 mmHg for 30–60 min) is applied to an eye the ocular blood flow is reduced, marked by a whitening of the fundus, and a significant reduction in the a- and b-waves of the electroretinogram. During reperfusion, a limited recovery of the a- and b-waves of the electroretinogram occurs depending on the magnitude and time of the previously raised IOP.[54–56] Importantly, it is the inner retina, and particularly the ganglion cells, that are affected in these animals, and as a consequence, much has been learnt about the mechanisms of ganglion cell death and the types of substances that are able to attenuate the death process (Table 3).

In contrast, a modest (10–20 mmHg) increase in IOP sustained for many weeks is not thought to cause retinal ischaemia, yet still causes ganglion cell death (Table 2). Many authors consider that ganglion cell death induced by such a constant 'chronic' small rise in IOP simulates a process that occurs in glaucoma.[57–60] It is, however, worth noting that a modest change in the electroretinogram (ERG) does occur in such animals.[61] Such ERG alterations are not normally associated with glaucoma and suggest that ocular blood flow is affected. There are, at present, few data on the types of substances that can directly protect ganglion cells in animal models of glaucoma. One reason why this may be the case is that there is significant variability in relating the rise in IOP with the degree of ganglion cell death (see Chauhan *et al*[62]). Nevertheless, work by the group of Neufeld[28,63] has shown that nitric oxide synthase inhibitors are able to blunt ganglion cell death significantly in a rat model of chronic glaucoma, and in another study the NMDA antagonist, MK-801, was shown to attenuate ganglion cell injury caused by a constant ocular hypertension.[64] Unsurprisingly, an earlier study has also shown that drugs that lower the elevated IOP, for example, betaxolol, also protect against ganglion cell death in chronic hypertensive animals.[65]

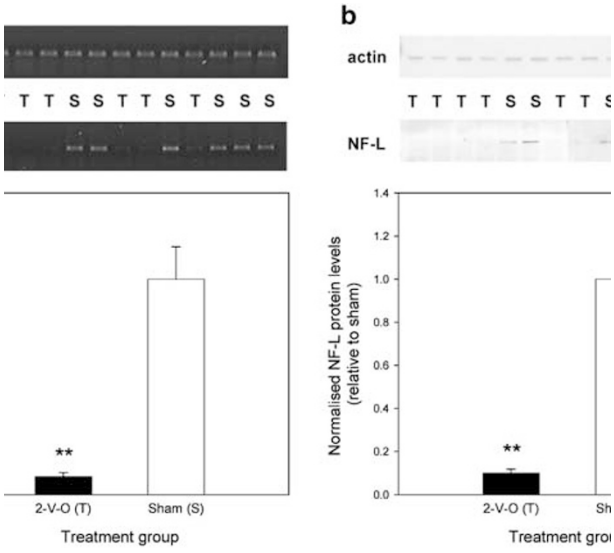

Figure 1 The effect of permanent occlusion of two carotid arteries (2-V-O) on the expression of neurofilament light (NF-L; 70 kDa) mRNA (a) and protein (b) expression in rat retina as detected by RT-PCR and immunoblotting. (a) Effect of 2-V-O on levels of NF-L mRNA in rat retina. The data are normalised for the housekeeping gene GAPDH, and are shown relative to sham-operated rats. The insets show the scanned images for the ethidium bromide-stained agarose gel on which the densitometry was performed. (b) Effect of 2-V-O on levels of NF-L protein in rat retina. The data are normalised for the housekeeping gene actin and shown relative to sham-operated rats. The insets show the scanned images for the immunoblot on which the densitometry was performed. The effect of permanent vessel occlusion is striking, with a marked loss of NF-L mRNA and protein. $^{**}P<0.01$, by unpaired *t*-test analysis comparing sham *vs* occluded eyes, where $n=6$–7. T = two vessel occlusion (2-VO) or common carotid occlusion. S = Sham operated control.

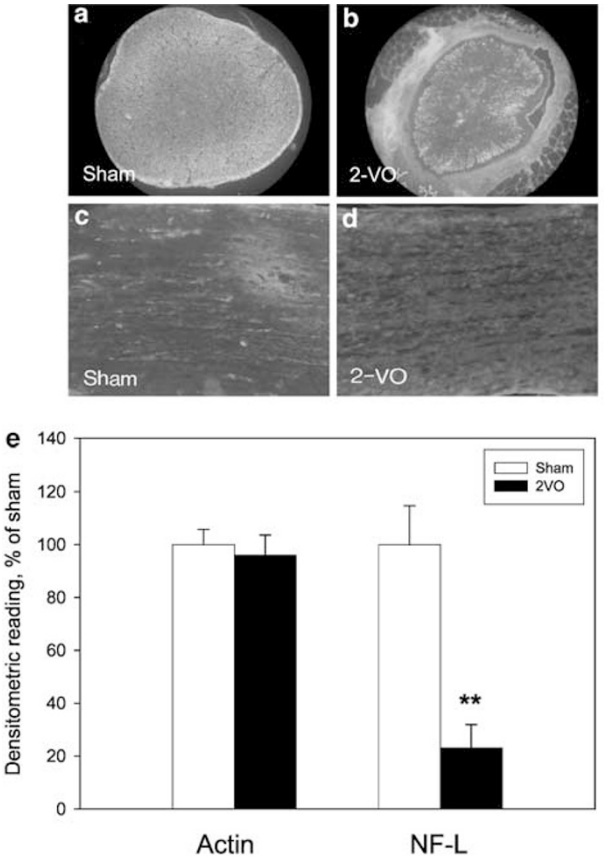

Figure 2 The effect of permanent occlusion of two carotid arteries on neurofilament (70 kDa) expression as detected by immunohistochemistry in rat optic nerve. (a) and (b) show transverse sections through the optic nerve, and (c) and (d) show longitudinal sections through the same tissue. (a and c) sections from sham operated animals whereas (b) and (d) are from eyes subjected to permanent two vessel occlusion (4 weeks after surgery). The effect of permanent vessel occlusion is striking, with a marked loss of neurofilament (70 kDa) obvious in both transverse (b) and longitudinal (d) sections. (e) Densitometric analysis of immunoblots for neurofilament (70 kDa) and actin (positive control) following two vessel occlusion or sham operations in rat optic nerves. Again, there is an evident decrease in expression of NF-L protein in occluded *vs* sham operated animals. $^{**}P<0.01$, by unpaired *t*-test analysis comparing sham *vs* occluded eyes, where $n=3$.

Ischaemic damage to the optic nerve and death of retinal ganglion cells also occurs in animals subjected to permanent carotid occlusion.[66–68] Loss of the pupil light reflex as an indicator of ganglion cell dysfunction occurs within 2 weeks in approximately 58% of albino rats given permanent carotid occlusion.[68] Maintenance of these animals for 90 days or longer also results in significant photoreceptor death[68,69] possibly because a greater amount of light reaches the retina than in animals which still have a functional pupillary reflex. We have confirmed that the observation made by Stevens *et al*[68] is correct, and that animals can effectively be screened for ganglion cell death after carotid artery occlusion simply by analysing their pupillary reflex. In our hands, animals subjected to permanent carotid occlusion have lost their pupillary reflex by 7 days postsurgery, and have dramatically reduced mRNA (Figure 1a) and protein (Figure 1b) levels of the ganglion cell marker neurofilament light (NF-L) 4 weeks after carotid occlusion. In contrast, the pupillary reflex is intact and ganglion cell markers are unchanged in sham-operated animals (Figure 1). The NF-L protein level is also reduced in the optic nerve of these animals, as revealed by immunohistochemistry and immunoblotting experiments (Figure 2). The potential, therefore, for using this animal model in neuroprotection studies is evident, although to date no data on the subject have been reported.

Role of peptide factors in the protection of injured ganglion cells

Neurones are thought to be dependent upon a supply of certain peptide factors (growth factors, cytokines,

neurotrophic factors, neurotrophins), which allow these cells to reach their targets during growth and development, and subsequently allow their continued survival. It was previously thought that ganglion cells derived all of their trophic support from the target cells, but recent evidence suggests that the true situation is more complicated and glial cells and neighbouring neurons are also involved.[70] Nevertheless, it is logical to assume that removal of trophic support to ganglion cells, whether by axonal damage to prevents retrograde transport of factors from the brain, or from other cells in the retina, the ganglion cells will suffer and ultimately die. Although the precise combination of peptide factors needed for survival of healthy ganglion cells has not yet been delineated, a large body of work has established that administration of various trophic factors can delay and even partially prevent gangion cell death after not only optic nerve injury,[70] but also following high-pressure ischaemia,[71,72] or injection of NMDA.[73] Many trophic factors, including brain-derived neurotropic factor, ciliary neurotropic factor, glial cell line-derived neurotropic factor, neurotrophins 3 and 4, fibroblast growth factor-2, and nerve growth factor have been shown to exert beneficial effects on ganglion cell survival after injury, but none of these compounds have been able to stimulate significant neuronal regeneration after optic nerve injury. It will be interesting to determine the influence of these peptide factors on the rate and extent of optic nerve and ganglion cell injury after permanent occlusion of the carotid arteries, since the injury is vascular rather than mechanical in nature.

Conclusions

Animal models for studying ganglion cell death and potential neuroprotection therapies *in situ* are restricted to those where the optic nerve has been transected or crushed, where neurotoxins like NMDA have been injected into the vitreous humour, or where the retina has been subjected to a defined ischaemic insult. Whether ganglion cells die by the same or different mechanisms in each of these animal models remains to be established. It also remains unclear whether only certain substances can unequivocally protect ganglion cells in all of these animal models. Assessing the merits of published data on the subject is difficult, since it is not uncommon that conflicting data are reported for similar experiments. This is partly because of the difficulty in obtaining an accurate measure for ganglion cell death. Moreover, some authors measure ganglion cell numbers as a marker for cell death. However, such a measure reflects only end-stage changes and may considerably underestimate the degree of ganglion cell injury. In addition, these methods invariably involve counting ganglion cells in very limited regions of the retina, and assume that cell death is uniform. However, there is evidence that at least one form of ganglion cell injury (ischaemic) results in heterogenous zones of ganglion cell loss.[74] Although ganglion cell death following optic nerve transection may be expected to be uniform, this has not been reported, nor have possible regional differences in ganglion cell death following optic nerve crush. Therefore, methods based on the analysis of whole retinal extracts (eg measurement of the total retinal levels of a protein or mRNA specific for ganglion cells eg NF-L) provide less variability, and thereby maximize the ability to detect small changes relative to methods based on the histological analysis of defined areas of the retina.

Nevertheless, it is possible to make a few general conclusions from studies that have been conducted on animal models *in vivo*. Insults such as ischaemia, NMDA-induced toxicity, or optic nerve transection all result in at least one population of ganglion cells dying by apoptosis. Moreover, the same substance can sometimes protect against ganglion cell death in all animal models. Thus, α_2-adrenoceptor agonists, NMDA antagonists and BDNF have been shown to blunt damage to the retina caused by optic nerve injury, NMDA-induced toxicity or ischaemia (Table 4). A possibility worth considering is that the fundamental pathways leading to ganglion cell death are common, varying perhaps only in the speed of the process (in the different animal models). If this is the case then many of the neuroprotective substances for ganglion cells in animal studies are likely also to blunt the death process of ganglion cells in man. Neglecting the questions of ethics, drug side effects, and drug delivery

Table 4 Effects of different substances on retinal damage caused by optic nerve injury, NMDA-induced toxicity or ischaemia

Substances	*Optic nerve injury*	*NMDA*	*Ischaemia*
α_2-Adrenoceptor agonists	Protective[115]	Unknown	Protective[91,92,116]
BDNF	Protective[117–119]	Protective[73]	Protective[71,72]
CNTF	Protective[117,120,121]	Protective[122]	Protective[71]
bFGF	Protective[123]	Unknown	Protective[71,99]
Caspase-1 inhibitors	Not protective[124]	Protective[125]	Protective[126]
Caspase-3 inhibitors	Protective[16,127,128]	Unknown	Protective[126]
NMDA antagonists	Protective[115,129]	Protective	Protective[105,108–112]

problems, it should be possible, therefore, to predict that a substance which can protect against neuronal death in an animal model will act similarly in the various human ocular or brain diseases. This has yet to be shown to be the case. One possible reason for this is timing. In laboratory experiments, the neuroprotectant is usually delivered at, or before, the onset of the insult. In the human situation, this is obviously impractical. Indeed, in general, experimental evidence suggests that ganglion cell death cannot be attenuated in animal experiments where the neuroprotectant has been administered a few hours after an insult such as ischaemia, optic nerve cut, or intravitreal injection of NMDA. A study does exist, however, where the neuroprotectant was administered 18 h after retinal ischaemia and found to be effective,[75] but this result remains to be confirmed. It seems sensible to conclude, therefore, that for effective neuroprotection in a clinically acute disease, such as stroke, success is likely to be more difficult than for a chronic disease, such as glaucoma. This is because treatment has to begin in the early stages of the disease and in glaucoma possibly before characteristic optic nerve and/or visual field changes allow an unequivocal diagnosis of glaucomatous optic neuropathy.

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