Retinal Ganglion Cell Life and Death – Mechanisms and Implications for Ophthalmology

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Abstract

Retinal ganglion cell (RGC) survival is critical for vision, but these neurons are exquisitely sensitive to insults. In acute diseases such as ischaemic optic neuropathy or optic neuritis, or in chronic diseases such as glaucoma, injury to RGC axons in the optic nerve may lead to rapid RGC death. Retinal ischaemia and retinal artery or vein occlusions directly injure RGC cell bodies in the ganglion cell layer. Enhancing RGC viability (neuroprotection) or RGC function (neuroenhancement) remains a major goal of basic and translational research. In this article we review the many mechanisms that lead from such insults to RGC death in clinically relevant pathological processes, and discuss avenues being pursued to enhance RGC survival in human disease.

Keywords

Retinal ganglion cell, survival, neuroprotection, neuroenhancement, optic neuropathy, glaucoma, apoptosis, regeneration

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When Do Retinal Ganglion Cells Die?

Most of our understanding of retinal ganglion cell (RGC) death in the nervous system comes from studies of animal models, as human pathological tissues are so rare; however, the occasional access to human tissues generally tends to confirm what is seen in other mammals. During development, RGCs are produced in about a two-fold excess and about half die, according to their ability to efficiently connect their synapses to their targets in the brain and receive synapses from their neighbouring retinal neurons.[1,2]

Later in adulthood, during pathological conditions, synaptic connectivity as well as many other components may interplay to determine either death or survival of RGCs. For example, changes in electrical activity[1] and growth factor deprivation[3] likely contribute to RGC death after optic nerve injury, but other mechanisms – such as excitotoxicity, ischaemia, oxidative stress and abnormal protein trafficking – also induce RGC death. There appear to be many apparently contradictory data to explain RGC death, but these are likely attributable to different animal models studied, selective mechanisms potentially affecting only some subtypes of RGC or experimental methods that select some types of RGC for observation over others (e.g. central retina versus peripheral, ON versus OFF RGCs, alpha versus beta versus not alpha/beta RGCs, etc.). Nevertheless, there are likely many mechanisms for RGC death that are shared among different diseases.

What Are the Mechanisms of Retinal Ganglion Cell Death?

Apoptosis and necrosis are the two described mechanisms of RGC death.[4] In necrosis, an unexpected event as in ischaemia or excitotoxicity deprives the cell of the minimum required energy (intracellular adenosine triphosphate [ATP]) to support the basic cellular metabolism, or disrupts cellular metabolism in such a way that processes necessary to maintain normal membrane permeability and integrity are paralysed. Cells and mitochondria swell, with early fenestration of the membrane, scattered condensation of the nuclear chromatin[5] and loss of the ability to isolate intracellular components from the extracellular environment. In the process of dying, these cells release ions, proteins and neurotransmitters that have the potential to kill off neighbouring cells. This extension of necrosis to (and induction of apoptosis in) cells that were not originally injured can lead to considerable secondary damage. The process finishes with extensive phagocytic clean-up (which also releases additional pro-inflammatory mediators and free radicals). Retinal ischaemia, as in retinal artery and vein occlusions, leads to RGC death by necrosis.

On the other hand, apoptosis comprises the molecular processes of programmed cell death, with sequential degradation of intracellular organelles and final clean-up by phagocytic cells. This process is thought to minimise the release of toxic intracellular components into the extracellular environment and is the mechanism observed during development and neurodegeneration to target cells for destruction without affecting neighbouring cells that are integrated to survive.[2] Most optic nerve damage – as in traumatic or ischaemic optic neuropathies or glaucoma – is thought to lead to RGC death by apoptosis.

Pro-apoptotic Signals

Apoptosis can be initiated by an 'extrinsic pathway', via receptor-mediated signalling by ligands such as tumour necrosis factor alpha

(TNF-α), which activates signalling pathways such as the JNK pathway,[6,7] or by an 'intrinsic pathway' that begins after loss of pro-survival external signals from neighbouring cells, leading to activation of an intrinsic mitochondrial pathway, usually involving intracellular calcium-activated proteins such as calcineurin[8] and calpain.[9] Both extrinsic and intrinsic initiation pathways generally depend on new gene transcription, the so-called 'cell suicide programme', and both increase mitochondrial membrane permeability and activate organelle-degrading proteins such as caspases.[10] Together, these lead to RGC death, with shrinking, blebbing, degradation of the nuclear membrane and DNA fragmentation.[5]

There is some debate about the point of no return for cell death by apoptosis. However, cells are practically committed to die after activation of caspases. Caspases are a family of cysteine proteases activated by cleavage of inactive pro-caspases. Caspases work in a 'cascade' of activation and amplification. For example, caspase-8 initiates the extrinsic or receptor-mediated pathway in response to TNF family ligands or Fas-ligand in combination with the adaptor protein Fas-associated death domain (FADD), and caspase-9 initiates the intrinsic or mitochondrial pathway, activated by leakage of cytochrome c from mitochondria in combination with another adaptor protein named apoptotic protease-activating factor-1 (APAF1). Cytochrome c and APAF-1 form a complex called the apoptosome, which is able to bind and activate pro-caspase-9. Initiator caspases cleave other caspases, leading to an amplification cascade, with caspase-3 serving as a central 'effector' caspase in both pathways, degrading targets inside the cell. Although these cascades broadly define two pathways to apoptotic cell death, exceptions abound. For example, the intrinsic pathway can be initiated independently of caspase-9,[11] and the extrinsic pathway can lead to caspase-9 activation.[12]

The link between optic nerve injury and mitochondrial initiation of apoptosis in RGCs is regulated by the B-cell lymphoma 2 (Bcl-2) family of proteins.[13,14] This family comprises anti-apoptotic members (Bcl-2, Bcl-xL, Bcl-W, Mcl-1 and A1),[14,15] which contain four Bcl-2 homology (BH) domains, and pro-apoptotic members, which have three BH domains (Bax, Bak and Bok) or a BH3 domain (Bid). When the pro-apoptotic family members dominate, they increase mitochondrial membrane permeability, allowing molecules such as cytochrome c to leak out and activate caspases.[14,16] Other members cannot activate apoptosis by themselves (the BH3-only proteins Bim, Bad, Puma, Noxa, Harakiri/Death protein, HRK/DP5, Bik, Mule and Bmf), but can interact through their BH3 domains to facilitate Bax- and Bak-induced apoptosis.[13,17]

Anti-apoptotic Signals

Meanwhile, the anti-apoptotic members keep the pro-apoptotic ones in check in a series of complex protein interactions. During normal cell function, Bcl-2 and Bcl-xL prevent mitochondrial channel formation by restraining Bax, which by itself or in conjunction with BH3-only proteins is able to induce permeabilisation of the outer michocodrial membrane. Under pro-apoptotic conditions, Bad and/or Bim may sequester Bcl-2 and/or Bcl-xL, releasing Bax to form mitochondrial channels and release cytochrome c to bind Apaf-1.[17–19] Simultaneously, another protein called Smac/Diablo is released from the mitochondria, binds to inhibitor of apoptosis (IAP) and prevents it from repressing the activation of procaspases. Thus, both Smac/Diablo and cytochrome c release synergise to incline the balance towards apoptosis.[20]

Finally, the Bcl-2 proteins themselves are regulated at the level of gene expression[21] and by phosphorylation. For example, Bcl-2 overexpression protects RGCs from growth factor deprivation;[22] similarly, apoptosis in various conditions is markedly reduced after genetic deletion of Bim[17] or Bax.[23] Phosphorylation cascades beginning with upstream signalling pathways such as PI3K/AKT, ERK, JNK and nuclear family kappa B (NFκB) pathways[24,25] converge on proteins such as Bad[26] or 14-3-3, a protein family that can regulate apoptosis by sequestering some Bcl-2 family proteins, such as phosphorylated Bad.[10]

Beyond these examples, the specific mechanisms for protein–protein interactions in apoptosis, especially for the Bcl-2 family and for mitochondrial pore formation, are not completely understood.[19] A better understanding of these interactions in specific pathologies may help speed the development of new specific anti-apoptotic therapies (discussed further below).

Ischaemia, Oxidative Stress and Retinal Ganglion Cell Death

A number of pathologies lead to external insults that initiate apoptosis in RGCs. First, ischaemia – the severe decrease of blood flow with consequent loss of oxygen, glucose and other nutrients[27] – can affect RGC bodies, as in retinal artery or vein occlusions, or RGC axons, as in acute ischaemic optic neuropathy (AION). Factors such as hypertension, diabetes, vascular occlusive disease, cardiac disease, arteriosclerosis, polycythemia vera and collagen vascular diseases, as well as giant cell arteritis, predispose to ischaemic retinopathy or optic neuropathy.[28] Chronic ischaemia has also been implicated in glaucoma,[29,30] either by mechanical compression of retinal blood vessels around the optic nerve head caused by increased intraocular pressure (IOP) or by ineffective vascular autoregulation, perhaps exacerbated by atherosclerotic changes, at least in a subset of patients.

In either case, ischaemic damage leads to neuronal apoptosis and/or necrosis,[31] followed by delayed apoptosis after reperfusion in some cases.[6] Animal models of central retinal artery occlusion[32] and of ischaemic optic neuropathy[33,34] have helped to define signalling pathways that promote RGC death. As in other systems, activation of the JNK pathway likely contributes to cell apoptosis; compensatory pro-survival responses are elicited through cAMP/PKA[35] and PI3K/AKT pathways[26] and CREB phosphorylation.[32]

Both acute and chronic ischaemia contribute to oxidative stress, brought on by an unbalanced metabolic demand and associated with production of free radicals or reactive oxygen species (ROS). Oxidative stress in glaucoma may also mediate RGC death.[36] Increased ROS[37] and decreased concentration of antioxidants[38] have been found in the vitreous of glaucoma patients, as has oxidative DNA damage[39] and oxidative alterations of the trabecular meshwork.[40,41]

In these cases, RGC physiological mechanisms that bind and inactivate ROS, including production of free radical scavengers, catalase and glutathione peroxidase,[42] are overwhelmed. ROS interfere with normal cellular function not only by oxidising proteins, nucleic acids and lipids, but also by directly activating the apoptotic process of cell death.[43] Free radical scavengers such as edaravone are able to increase RGC survival *in vivo* by inhibiting ROS-induced activation of the proapoptotic JNK and P38 signalling pathways.[43] Genetic approaches to overexpress some of these intrinsic

antioxidant proteins in animal models have shown promising effectiveness in decreasing RGC death,[44] and a number of antioxidants are entering clinical trials as neuroprotectants.

Any initial RGC death, particularly from ischaemia-induced necrotic release of cellular contents, can lead to secondary cell death, which may be caused by excitotoxicity. Excitotoxicity is thought to occur when excessive activation of glutamate or other channels (e.g. from glutamate spilling into the extracellular space from dying cells), and specifically of N-methyl-D-aspartate (NMDA)-sensitive glutamate channels, leads to a deleterious increase in intracellular calcium and subsequent activation of pro-apoptotic signalling pathways.[45] However, excitotoxicity is more complex than initially thought, and depends on the duration, location and intensity of glutamate channel activation,[46] as some level of electrical activity may be protective (see below). In hippocampal neurons, neuroprotection has been shown to be related to preferential activation of synaptic NMDA receptors, and excitotoxicity with activation of non-synaptic ones,[47] but this interesting point has not been well explored in RGCs. This complexity may underlie the difficulty in using NMDA receptor blockade as a therapeutic modality. A multicentre clinical trial using the low-affinity non-competitive NMDA antagonist memantine in patients with primary open-angle glaucoma did not detect a signficant decrease in disease progression compared with placebo.[48]

It is not clear whether the main contributor to excitotoxic RGC death is necrosis or apoptosis. Studies in rats have found increased membrane permeability and cell body swelling consistent with necrosis;[49] however, it is known that calcium can trigger apoptosis mediated by the calcium-activated proteins calcineurin[50,51] and calpain,[9,52,53] both of which are able to mediate apoptotic death of RGCs.[53]

These external insults can also lead to other cellular dysregulation. For example, accumulation of abnormal proteins, such as hyperphosphorylated Tau AT8, have been described in the retina of glaucoma patients.[54] Abnormal folding of proteins inside the endoplasmic reticulum (ER) leads to 'ER stress' and severe cellular dysfunction, again associated with RGC apoptosis. ER stress is present preferentially in the ganglion cell layer 24 hours after intravitreal NMDA injection or elevation of the IOP.[55] The significance of ER stress is still under investigation to determine whether the phenomenon is a cause or just part of the process of cell death.

Neurotrophic Deprivation, Decreased Electrical Activity and Retinal Ganglion Cell Death

RGCs depend on trophic signals from their neighbours in the retina, in the optic nerve and in their targets in the brain for survival.[56] Target-derived growth factors[3] such as brain-derived growth factor (BDNF) are taken up by RGC axon terminals and travel retrogradely through the axon, down the optic nerve and back to the cell body in the retina.[57] RGC dependence on target-derived BDNF seems to be more important during formation of early axon connections with their targets in the brain,[58,59] but may switch to dependence on other sources, such as other retinal cells, including amacrine cells and Muller glia during development and into adulthood.[60,61] Similarly, other trophic factors, such as fibroblast growth factor-2 (FGF2), glial-derived neurotrophic factor (GDNF), insulin-like growth factors (IGFs) and ciliary neurotrophic factor (CNTF), strongly support RGC survival. For example, lentiviral-mediated transfer of CNTF into schwann cells placed in a peripheral nerve graft to repair rat optic nerve after injury also significantly increased RGC survival.[62] Indeed, a number of these have demonstrated efficacy in animal models of RGC degeneration and have been examined in clinical trials for other degenerative diseases of the nervous system. There is a strong justification to transition such molecules to human trials for RGC neuroprotection.

Not only do RGCs depend on peptide trophic factors from their targets and other neighbours to survive, but they may also depend on physiological levels of electrical activity to survive. During development, RGCs wire into the retinal network and are depolarised through gap junctions and chemical synapses. Considerable evidence points to a role for electrical activity in maintaining RGC survival in the adult as well. RGC death is increased if electrical activity is blocked with tetrodotoxin.[63] Conversely, RGC survival is enhanced with physiological levels of electrical activity *in vitro*[22] and *in vivo*.[64] In a rat model of optic nerve injury, a single two-hour session of transcorneal electrical stimulation (TES) of the retina with a bipolar contact lens electrode promotes RGC survival one week after injury.[65] TES can stimulate RGCs in human patients[66,67] and is dependent on the pattern of electrical stimulation used,[68] raising the possibility of human clinical trials.

How does electrical activity enhance RGC survival? A number of mechanisms may be at play. Activity can upregulate the production of growth factors,[3,57,69] mainly by activating gene expression.[70] Depolarisation can elevate RGC levels of cAMP,[1,71] and both depolarisation and cAMP elevation have the ability to recruit TrkB receptors to the RGC membrane[72] and to enhance RGC responsiveness to peptide trophic factors *in vitro* and *in vivo*.[72,73] These data raise the hypothesis that one of the reasons RGCs die after injury is that they are less electrically active.[74]

How does electrical activity increase cAMP in RGCs? Increases in cAMP and subsequent enhancement of neurotrophic responsiveness likely depend on calcium-sensitive adenylyl cyclases (ACs).[75] Most attention has focused on the transmembrane ACs, which are activated pharmacologically by forskolin, a drug that can potentiate RGC trophic responsiveness in a manner similar to depolarisation.[22,56]

Recently, however, an alternative intracellular, soluble source of cAMP was described in somatic mammalian cells, called soluble AC (sAC).[76] sAC is activated by several factors, including calcium, bicarbonate, CO_2 and, probably, ATP.[77–80] sAC is ideally positioned to play a role in survival, being localised around and inside mitochondria,[81–83] and its calcium responsiveness makes it a good candidate to mediate activity-dependent survival. Inhibition of sAC activity in RGCs using 2-hydroxyestradiol[80] decreases RGC survival, while the sAC agonist bicarbonate[84] increases survival and axon growth in RGCs.[85] These data suggest that sAC may be mediating some of the pro-survival responses to electrical activity that were previously attributed exclusively to transmembrane adenylyl cyclase-produced cAMP.

Other signalling pathways are also modulated in activity-dependent neuroprotection, including the NFκB signalling pathway,[86,87] which mediates the effect of activity-dependent neurotrophic factor (ADNF) and activity-dependent neuroprotective protein (ADPN). These peptides can rescue neurons from death after tetrodotoxin-induced activity blockage,[88] and peptides derived from ADNF (ADNF-9) and ADNP (NAP) increase survival and axonal growth in RGCs cultured in

growth-factor-free media.[89] Currently, clinical trials are ongoing to determine a possible therapeutic effect of intranasal and intravenous formulations of some of these peptides in Alzheimer's disease and other neurodegenerative diseases.[90]

Conclusion

In summary, it is clear that many positive and negative signals balance against each other to determine whether RGCs will survive or die after an insult to the retina or optic nerve. Combinatorial approaches are more likely to maximise RGC neuroprotection, and many such approaches have demonstrated efficacy in animal models. As we continue to decode the molecular mechanisms of apoptosis in RGCs, other important potential pro-survival therapies may be incorporated into a multifaceted approach. It will be important moving forward to transition as many of these as possible into well-structured clinical trials in humans. ■

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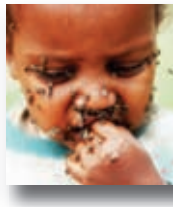

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