What can we learn about stroke from retinal ischemia models?

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Retinal ischemia is a very useful model to study the impact of various cell death pathways, such as apoptosis and necrosis, in the ischemic retina. However, it is important to note that the retina is formed as an outpouching of the diencephalon and is part of the central nervous system. As such, the cell death pathways initiated in response to ischemic damage in the retina reflect those found in other areas of the central nervous system undergoing similar trauma. The retina is also more accessible than other areas of the central nervous system, thus making it a simpler model to work with and study. By utilizing the retinal model, we can greatly increase our knowledge of the cell death processes initiated by ischemia which lead to degeneration in the central nervous system. This paper examines work that has been done so far to characterize various aspects of cell death in the retinal ischemia model, such as various pathways which are activated, and the role neurotrophic factors, and discusses how these are relevant to the treatment of ischemic damage in both the retina and the greater central nervous system.

Keywords: ischemia; retina; central nervous system; cell death pathway; apoptosis; necrosis; neurotrophic factor; excitotoxicity

Introduction

Ischemia is broadly defined as the loss of blood supply to a biological tissue resulting in energy depletion and cell death, both of which are mediated by intermediate factors such as the release of excess excitatory amino acids, free-radical formation, and inflammation[1]. Ischemia is one of the key factors that determines the pathophysiology of many brain and retinal diseases[2]. As the retina is an extension of the diencephalon, retinal blood vessels share similar anatomic, physiological and embryological properties with the brain, and possess a blood-retinal barrier analogous to the blood-brain barrier[3]. Retinal ischemia is a common cause of visual impairment and blindness, and is a characteristic feature of various clinical retinal disorders such as ischemic optic neuropathies, obstructive arterial and venous retinopathies, carotid occlusive disorders, retinopathy of prematurity, chronic diabetic retinopathy and glaucoma[4]. At the cellular level, retinal ischemia consists of a self-reinforcing pro-apoptotic cascade involving neuronal depolarization, calcium influx, oxidative stress, energy depletion, and glutamatergic stimulation.

A number of animal models and analytical techniques have been used to study retinal ischemia, and an increasing number of treatments have been shown to interrupt the resulting cell death cascades and attenuate their detrimental effects; however, our knowledge remains incomplete and treatments can be improved. A more thorough understanding of the molecular mechanisms behind ischemic damage is essential to improving potential therapies, and can provide insight into the pathophysiology of other neurodegenerative conditions as well, most notably of cerebral stroke[5].

The pathophysiology of retinal ischemia

Retinal ischemia occurs when the retinal circulation is insufficient to meet metabolic demands. It can be caused by general or, more commonly, by local circulatory failure. The metabolic demands of the retina are the highest of any tissue within the body, and so maintaining a consistently high blood supply is essential[6]. The degree of damage sustained by retinal tissue during ischemia depends on the severity and the duration of the obstruction to blood flow. The area most prominently supplied by the occluded blood vessel comprises the infarct core, while areas perfused by collateral circulation form the ischemic penumbra. Areas within the ischemic core are most severely affected by the ischemic injury, while those in the penumbra are much less affected and can retain a degree of function based on the amount of damage they have sustained.
If circulation is not quickly restored to the affected area however, the penumbra is gradually incorporated into the ischemic core and becomes completely non-functional[6, 7]. The penumbra, therefore, represents the main therapeutic target for acute ischemia therapies.

There are many pathogenic mechanisms which contribute to the cell death cascades experienced during ischemia, such as energy failure, elevation of intracellular calcium, excitotoxicity and spreading depression, generation of free radicals, blood-retinal barrier disruption, inflammation and apoptosis. The latter process is a complex cascade of cellular factors which contribute to tissue injury and impair the cellular mechanisms required to maintain ionic gradients[2]. Initially, the reduction in blood flow results in the depletion of substrates such as oxygen and glucose, which in turn causes the accumulation of lactate via anaerobic glycolysis. The depletion of energy results in neuronal depolarization, causing the activation of glutamate receptors, and thereby altering the ionic gradients of Na+, Ca2+, Cl−, and K+[9]. As the intracellular concentration of Ca2+ is increased by the dysregulation of its ionic gradient, a variety of intracellular enzymes, such as lipases, proteases and endonucleases, experience increased activity. As a result, oxygen free radicals are generated and contribute to apoptotic cell death. Oxygen free radicals are also produced by the enzymatic conversion of arachidonic acid to prostanoids and the degradation of hypoxanthine during blood reperfusion of the ischemic area[9]. The formation of free radicals recruits pro-inflammatory factors, such as interleukins, platelet activating factor, and tumor necrosis factor α (TNF α). As well, during ischemic conditions, mitochondrial permeability transition pores are formed, which cause the release of free radicals and pro-apoptotic molecules[10]. The infiltration of pro-inflammatory factors also increases the formation of free radicals. The consequences of free radicals are lipid peroxidation, membrane damage, dysregulation of cellular processes and mutations of the genome[9].

The retinal blood supply

Reflecting its embryological origins, the human retina has a dual blood supply. The photoreceptors, including their cell bodies in the outer nuclear layer and the majority of the outer plexiform layer, are supplied via the choriocapillaries. These vessels are richly anastomotic and correspond to the pial- arachnoid vessels in the rest of the brain. The inner retinal layers, such as the ganglion cell layer, are nourished by the central retinal artery (CRA), which arises from the ophthalmic artery in the region of the optic foramen, and which in turn originates directly from the internal carotid artery, proximal to the origin of the middle cerebral artery[2]. The CRA runs alongside the optic nerve, until approximately 10 mm from the globe of the eye, at which point it enters the optic nerve. Once it has entered the optic disc, the retinal arteries divide irregularly and dichotomously. The main retinal vessels form 2 capillaryplexuses: the superficial capillary network, which is found within the nerve-fiber layer, and the deep capillary network lying between the boundary of the inner nuclear layer and the outer plexiform layer. Due to the connections in bloodflow between these 2 systems, complete retinal ischemia requires occlusion of the ophthalmic artery, the principal vessel supplying all vasculature of the retina[2].

Animal models of retinal ischemia

A number of in vivo and ex vivo animal models have been developed to study retinal ischemia. In order to best extrapolate the data from animal model to human clinical situation, the model which most closely resembles human retinal ischemia is preferred. An immediately limiting factor to potential model suitability is the structure of the retinal vasculature in various species. While higher primates share virtually identical retinal vascular patterns with humans, financial and ethical considerations prohibit their widespread use[11]. More commonly seen are small rodent models, and of these the rat is the most similar to humans. The pattern of vascular supply in the rat retina is holangiotic, as in primates and humans, and this makes it a suitable candidate as a model for study[12].

Certainly, differences do exist between rat and human retinas. The principal blood supply to the rat retina is via a single posterior ciliary artery which runs along the ventromedial aspect of the optic nerve, and which divides into three branches at the optic nerve head: a central retinal artery supplying the retina, and medial and lateral long retinal arteries supplying the choroid[12]. Complete transection of this vessel causes severe trauma and ocular inflammation, and widespread retinal infarction; permanent occlusion of this vessel does not correspond to CRA occlusion in humans, and even a temporary occlusion in rats probably causes more widespread injury. Hence, the degree of damage done in the process of a study must be taken into account, as it may not accurately reflect the degree of damage which occurs in the human eye. Previous work has found that in order to cause reproducible, irreversible and functional ischemic injury in the Wistar rat, at least 20 min of sustained ischemia are required[4, 13, 14].

In order to induce retinal ischemia, several methods are currently used. The high IOP (Intraocular Pressure) model of ischemia is frequently used as it is fairly simple to administer and can be used as a model to study glaucomatous injury to the eye. The basic method for this technique consists of introducing sterile fluid into the vitreous chamber of the eye. The addition of liquid into the chamber increases the pressure within the eye and compresses the vasculature passing through the optic disc and supplying the retina. Blood contained within these vessels is thereby expelled, cutting off the supply to the retinal tissue[15, 16]. Such an intervention can be accomplished via cannulation of the eye, which is then connected to an elevated pressure liquid reservoir, which increases IOP to the level of the reservoir.

Another common method of inducing retinal ischemia is vascular ligation. Carrying out this intervention requires surgical procedures very similar to those used for optic nerve transection, whereby the investigator dissects the contents of the ocular orbit to reach the optic nerve and administers the damage[17, 18]. At its simplest, this method involves placing
a suture around the optic nerve bundle, thereby ligating the posterior ciliary vessels. Due to the close association between the optic nerve and these vessels, selective compression of the vasculature is imperfect and it commonly causes the axons of the optic nerve to be compressed and damaged. A technically more demanding version of this intervention is to separate the optic nerve and posterior ciliary vessels, thereby freeing the vasculature to be ligated independently of the optic nerve. This method produces results more appropriate to retinal ischemia without the confounding effects of optic nerve damage[29].

A relatively non-invasive method of retinal vessel occlusion involves the intravenous injection of Bengal Rose, a photore sponsive dye, followed by intense retinal illumination. The principle behind this method was introduced by Watson et al in 1985[20], when they proposed that by inducing a photochemical reaction within the vasculature, a thrombosis could be created. This method has since been used to study ischemic injury, particularly in the brain[20], as well as in the retina[22–24]. There are several advantages to this method, namely that animal preparation does not require mechanical manipulation of vasculature or parenchyma. As well, the lesion size and location can be modulated by altering the irradiating intensity, duration of light exposure, beam position and dye concentration[20, 29]. However, this method produces variable histologic injury, which is difficult to quantify[29], and it may also cause secondary damage due to neurotoxicity in addition to ischemic damage[29]. It is also worth noting that it is not an ideal method for the study of ischemia/reperfusion injury as the lesion created is resistant to the reintroduction of blood flow[29].

**Molecular analysis of retinal ischemia**

The intensity of damage which occurs due to an ischemic injury is critically dependent on the duration of the insult. Several prominent molecular changes are observed within the retina over the course of such an injury, altering the protein expression patterns of the cellular population. The resulting protein signatures have many similarities with those resulting from optic nerve crush or transection injuries[27, 29]. One of the prominent molecular changes found in retinal ischemic injury, as well as optic nerve crush and transection, is the transient increase in growth-associated factor 43 (GAP-43)[29]. Specifically, after ischemic injury GAP-43 was found to be increased in retinal ganglion cells (RGCs) at 3 and 7 days following reperfusion[29]. GAP-43 is most recognized for its expression during CNS synaptogenesis: it is a membrane-associated protein which is up-regulated in neuronal growth cones, but which is down-regulated after synaptogenesis in almost all brain regions, except for those few which preserve plasticity[31]. Throughout the CNS, GAP-43 expression is increased in neurons with damaged axons[32]. In the retina, GAP-43 is normally localized to the inner plexiform layer due to its expression by RGCs and a subset of amacrine cells[33], and its increased expression in response to ischemic injury suggests structural remodeling in the inner plexiform layer of the retina in order to preserve retinal function[34].

**Mechanisms of cell death during retinal ischemia**

Following retinal ischemia, there are two modes of cell death which occur: necrosis and apoptosis[35]. Both are often found playing parts in insults to the CNS, and each has discrete biochemical and histological features[35]. Necrosis, long considered a form of caspase-independent cell death (CID), or “accidental” cell death, is the pathological process that occurs when cells are exposed to an extreme physical or chemical insult or any other serious disruption to their normal physiology[35]. It is characterized by a rounding of the cell, a gain in cell volume, mitochondrial swelling, dissolution of organelles, condensation of chromatin around the nucleus, and irreversible damage to the plasma membrane both by external influences and by the release of intracellular lytic enzymes in response to the insult[35–38]. The crux of necrotic damage appears to be a compromised plasma membrane due to ATP-mediated energy depletion. The process begins with an impairment of the cell’s ability to maintain homeostasis, leading to the influx of water and extracellular ions, thus drastically altering intracellular ion concentrations and severely disrupting the ionic gradient which exists across the plasma membrane[35]. Intracellular organelles, most notably mitochondria, become inactive, and the entire cell becomes dysfunctional. Owing to all of these disruptions, the cell eventually lyse and the cytoplasmic contents, including lysosomal enzymes, are released into the extracellular space. As a result, necrotic cell death is often associated with extensive tissue damage and inflammation[35]. Recent investigations into the processes of necrosis, however, have yielded evidence indicating that at least a part of the damage attributable to this process may be executed by a mechanism termed “necroptosis”[39, 40].

Necroptosis is a recently discovered, caspase-independent form of regulated cell death. It shares morphological features with necrosis, such as membrane and organelle swelling followed by cell lysis, and is activated by death receptors such as TNF α, FasL, and TRAIL, the very same ligands which can activate the extrinsic apoptotic pathway[43–45]. Thus, the activation of these receptors may initiate alternative death pathways[42–44]. Research indicates that the key modulators between necrosis and apoptosis are receptor-interacting protein kinase 1 (RIPK1)[39, 45, 46], and RIPK3[47–51]. The ability of RIPK1 to switch between these 2 pathways appears to rest on its serine/threonine kinase activity; its activation is essential for the activation of necroptosis, but it is dispensable for both NF-κB activation and initiation of the apoptotic pathways[45]. The small molecule inhibitor necrostatin-1 (Nec-1), has been shown to be a potent inhibitor of RIPK1 and of necroptosis[46, 52]. Treatment with Nec-1 has demonstrated a reduction in infarct volume in mouse models of middle cerebral artery (MCA) occlusion, suggesting the importance of necroptosis in CNS ischemic injury[39]. As well, recent work has supported the impact of necroptosis in the retina as Nec-1 treatment was able to attenuate retinal thinning and RGC loss after ischemic injury[40].
While necrosis is more dominant in the ischemic core, apoptosis becomes more common in the penumbra as the cells are found further away from the core\textsuperscript{[23]}. Apoptosis is a normal process during development, and is also a defense mechanism which occurs during immune reaction or when cells are damaged; it is the other primary method by which cells die during ischemia\textsuperscript{[2,30]}. In contrast to the uncontrolled degeneration which occurs by necrosis, apoptosis is a strictly regulated process. It plays a significant role in both acute and chronic neurodegenerative conditions, such as glaucoma, retinitis pigmentosa, cataracts, and retinoblastoma\textsuperscript{[38,54]}. Many studies have found that following ischemia-reperfusion injury, treatment with anti-apoptotic agents is effective at preserving cellular populations throughout the retina\textsuperscript{[35]}. There are many distinct morphological changes which are common to apoptosis: early on there is a reduction in cell volume and chromatin condensation occurs, followed by extensive plasma membrane blebbing and the detachment of cell fragments to form apoptotic bodies\textsuperscript{[36,57]}. Macrophages or microglia then engulf the apoptotic bodies and degrade them\textsuperscript{[58]}. In contrast with necrosis and necroptosis, there is no inflammatory reaction associated with apoptosis because the degraded cells are quickly phagocytosed and therefore do not release their contents into the extracellular space, and there is no release of pro-inflammatory cytokines\textsuperscript{[39,40]}. There exist 2 main apoptotic pathways: the extrinsic death receptor pathway, and the intrinsic, or mitochondrial, pathway. Both are linked, and considerable interplay occurs between them\textsuperscript{[61]}. There is also an additional pathway which relies on the infiltration of immune cells into the tissue undergoing apoptosis. In this final case, the granzyme and perforin released by the invading immune cells degrades the cellular proteins and chromatin\textsuperscript{[42]}. Both the extrinsic and intrinsic pathways involve an energy-dependent cascade of molecular events which result in biochemical modifications throughout the cell, such as protein cross-linking, DNA breakdown and phagocytic breakdown\textsuperscript{[43]}. Caspases, a family of cysteine proteases, have been found to be major regulators in the degeneration of RGCs by apoptosis\textsuperscript{[64-66]}. Initially, caspases are expressed as inactive proenzymes, which are cleaved into their active form. This allows them to in turn activate other caspases downstream, and thus initiate a protease cascade. Traditionally, the primary caspases involved in the apoptotic degeneration of RGCs after axotomy have been caspase-3 and caspase-9\textsuperscript{[67-72]}. While neither caspase-3 nor caspase-9 was found to be involved in axonal degeneration\textsuperscript{[73,74]}, it now appears that caspase-6 and caspase-8 both play prominent roles in this process, as well as in RGC apoptosis\textsuperscript{[75,76]}. As well, caspase-2 has recently been shown to be involved in RGC apoptosis following optic nerve damage, most likely at the stage of apoptosis initiation\textsuperscript{[77]}. Once caspases have been initiated, there appears to be an irreversible commitment to cell death\textsuperscript{[58]}. To date, 10 major caspases have been identified and broadly categorized into initiator caspases (caspase-2, -8, -9, and -10), executioner caspases (caspase-3, -6, and -7), and inflammatory caspases (caspase-1, -4, and -5)\textsuperscript{[78,79]}. The extrinsic pathway of apoptosis involves transmembrane receptor-mediated interactions in order to initiate apoptosis. Most notably, it involves receptors that are part of the TNF superfamily\textsuperscript{[80]}. Members of the TNF superfamily share a similar cysteine-rich intracellular domain, called the “death domain”, which is essential for transmitting the death signal to intracellular pathways\textsuperscript{[81]}. So far, the majority of the research into this pathway’s ligand/receptor combinations has been directed towards FasL/FasR, TNFα/TNFRI, Apo3L/DR3, Apo2L/DR4, and Apo2L/DR5\textsuperscript{[82-84]}. The extrinsic phase of apoptosis is defined by the binding between these cell-surface receptors and their specific ligand. There is clustering of the receptors, and through this, cytoplasmic adapter proteins exhibiting the corresponding death domains are recruited. Among the recruited proteins are FADD, TRADD, RIP, and pro-caspase-8\textsuperscript{[85]}. The resulting structure is termed the death-inducing signaling complex (DISC), and results in the autocatalytic activation of pro-caspase-8, and the triggering of the execution phase of apoptosis\textsuperscript{[86]}. At this point, there is also a regulator of the process, the protein c-FLIP, which will bind to FADD and caspase-8, rendering them ineffective\textsuperscript{[87,90]}. Activation of apoptosis by TNFα is a 2 step process. Under apoptosis-competent conditions, TNFα stimulation sequentially induces the formation of 2 protein complexes, complex I and complex IIa, which stimulate NF-κB activation and apoptosis, respectively\textsuperscript{[91,92]}. Initially, TNFα binding to the TNF receptor 1 (TNFRI) induces the recruitment of TRADD, RIP1 and TRAF2 to the receptor’s intracellular domain, thus forming complex I\textsuperscript{[93]}. Subsequently, these RIP1 and TRADD undergo posttranslational modifications and the entire complex I dissociates from TNFRI\textsuperscript{[93]}. The addition of Fas-associated death domain (FADD) and caspase-8 forms complex IIa. The signal which stimulates the transition from complex I to complex IIa is currently unclear, however, it is known that an alternate multiprotein aggregate, Complex IIb, may form in apoptosis-deficient conditions, particularly in the presence of caspase-8 inhibitors. This contains at least one additional component: RIPK3. Interaction between RIPK1 and RIPK3 plays a critical role in mediating downstream apoptotic events\textsuperscript{[93]}. Treatment with Nec-1 prevents recruitment of both RIPK1 and RIPK3 to Complex IIb, indicating the importance of RIPK1 in the apoptotic process\textsuperscript{[47,48]}. The intrinsic pathway involves a diverse array of non-receptor mediated stimuli that produce intracellular signals that act directly on intracellular targets, most notably mitochondria. This results in the opening of the mitochondrial permeability transition pore, loss of mitochondrial transmembrane potential, and the release of pro-apoptotic proteins which are normally sequestered within the mitochondria\textsuperscript{[84]}. Among these proteins are cytochrome c, Smac/DIABLO, and HtrA2/Omi, which activate the caspase-dependent mitochondrial pathways\textsuperscript{[85]}. Other pro-apoptotic proteins released by the mitochondria are AIF, endonuclease G, and CAD, however this only occurs once the cell is irrevocably committed to die\textsuperscript{[58]}.
The regulation of these pro-apoptotic mitochondrial events is through members of the Bcl-2 family of proteins[86]. This family of proteins regulates mitochondrial membrane permeability. They can be either pro-apoptotic or anti-apoptotic, and therefore have a crucial role to play as they can either enhance the signals inducing cell death, or inhibit them.

Preserved retinal function by neurotrophic factors

Neurotrophic factors are recognized as playing key roles in the development and survival of tissues within both the central and peripheral nervous system[5]. As well, they play an important role in countering the complex mechanisms of apoptotic neuron death in the retina[97-99]. Several investigations have been carried out to determine the efficacy of growth factors in promoting survival following ischemic injury in the retina. Recently, bFGF (basic fibroblast growth factor) has been found to support neuronal survival and promote neurite outgrowth, as well as play an essential role in the maintenance of neurons within the spinal cord and cerebral cortex[100]. The factor bFGF has previously been found to induce both mesodermal and neuroectodermal tissue regeneration, as well as induce the outgrowth of fibers in cultured retinal ganglion cells in cultured RGCs[101], and it has also been shown to delay the degeneration of rat photoreceptors[102]. It has also been demonstrated that bFGF is effective at rescuing RGCs, as well as other cellular populations in the retina from ischemic injury induced by elevated intraocular pressure[103]. Other neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF) have also been studied and have been found to protect the retina from pressure-induced ischemic injury[104]

Another neurotrophic factor that has protective effects following retinal ischemia is glial cell line-derived neurotrophic factor (GDNF). In retinas subjected to ischemic injury, intracellular administration of virus encoding recombinant GDNF has yielded a preservation of retinal thickness, indicating that cellular populations were better preserved; and specifically, it was found that RGCs survived in greater numbers. In agreement with these findings, eyes receiving increased GDNF retained more functionality following ischemic injury, as measured by electroretinogram[105].

Neurturin (NRTN) is a member of the GDNF family of ligands. It is one, among many, factors which interacts with members of the GDNF family of receptors (GFRαs), and activates intracellular signaling via the Ret receptor tyrosine kinase[106]. The Ret receptor tyrosine kinase then activates essential pro-survival intracellular signaling pathways such as the mitogen activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)-Akt, and phospholipase Cγ pathways[107]. It has been shown that GFL-mediated Ret signaling plays a critical role in the maintenance of multiple central and peripheral neurons[108], and that within the retina, it is expressed in the ganglion cell layer and inner nuclear layer[109]. Through these studies, it was shown that NRTN, and the activity it induces in Ret, are important for the normal function of the retina, specifically for its actions on horizontal cells, amacrine cells, and RGCs[106].

Many studies have concentrated on the efficacy of BDNF in promoting the survival of RGCs following injury to the retina or optic nerve, and in using it to develop therapeutic strategies for ocular diseases[110]. So far, research has indicated that, among all the neurotrophic factors, BDNF is the most effective at directing injured RGCs towards survival, a fact attributed to the high levels of expression of tyrosine receptor kinase B (TrkB; the BDNF receptor) which is expressed by these cells[111-113]. BDNF was found to be beneficial to RGCs in several injury models, including in promoting survival both in vitro and in vivo[114, 115], and in stimulating the growth of regenerating neurites[116]. Intravitreal injections of BDNF have proven to slow the loss of RGCs in a rat chronic hypertension model[117], and to support the survival of retinal ganglion cells for up to 1 week following axotomy[112, 118]. Most recently, BDNF was incorporated into rat mesenchymal stem cells (rMSCs), which were then administered to the retina following axotomy or increased intraocular pressure[119]. In both experimental models, levels of BDNF were successfully increased by the treatment, and surviving RGC populations were significantly larger. Whether this effect could be increased by also increasing expression of TrkB in the target cell population remains to be seen, however it has been shown that following axotomy, combined upregulation of BDNF along with TrkB does have additive effects on retinal ganglion cell survival[120].

The neurotrophic factor Nerve Growth Factor (NGF) has been shown to be present normally within the eye, along with its receptors TrkA and p75. All 3 factors have, in fact, been shown to be expressed by the rat lacrimal gland tissue[121, 122] in vivo, and in vitro it has been shown that conjunctival cells (epithelial cells, goblet cells, immune cells and fibroblasts) all produce, store, release, and utilize NGF. The diverse activity of NGF appears to modulate the activity of these cells, and therefore affect the secretion of cytokines and other growth factors[123-125]. Due to its wide-ranging effects, NGF is believed to be implicated in a variety of ocular diseases[126]. As well, it has previously been shown that NGF is able to enhance RGC survival following optic nerve transection[115], as well as following ischemic insult. In the latter situation, NGF was also able to promote the functional recovery of RGCs[127]. More recently, studies have demonstrated that in a model of elevated intraocular pressure, NGF is effective at preventing RGC death in a rat model[128]. While this study used elevated intraocular pressure to mimic the hallmark symptom of glaucoma, it must also be noted that increased intraocular pressure also reduces blood-flow to the retina by increasing pressure on retina vasculature. Therefore, it may be the case that some of the effects of NGF are due to its impact on ischemic injury. NGF has also been examined in conjunction with novel neuroprotective strategies to combat ischemia-induced excitotoxicity[129]. It was found that the neurosteroid dehydroepiandrosterone (DHEA) is able to protect RGCs from excitotoxicity, a main mechanism by which cells die as a result of ischemia[40]. The results also showed that DHEA works via the NGF/TrkA pathway to promote RGC survival via a cascade of events which are as yet
Neurotrophin-3 (NT-3) is a neurotrophin which controls neuronal survival in both the peripheral and central nervous systems. It, along with BDNF and NGF, is recognized as one of the principal neurotrophic factors in the central nervous system; the principal ligand is TrkC. Currently, evidence points towards TrkC actually inducing apoptosis in the absence of NT-3, as the lack of its binding partner allows the intracellular domain of TrkC to become susceptible to cleavage by locally activated caspases, especially caspase-9. Neurotrophins, including BDNF, GDNF and NT-3, are known to be shuttled anterogradely in RGC axons. During ischemia, it is possible that the drop in oxygen supplied to neurons can result in a lack of ATP, which can induce a loss of the cell’s ability to maintain its axonal transport system, and this loss of pro-survival signaling by neurotrophic factors will induce apoptosis. Recent work in cerebral ischemia has shown that increased levels of NT-3 improve cell survival and neurological status following transient middle cerebral artery occlusion by reducing the initial damage caused by the ischemic event.

Neurotrophin 4 (NT-4) is also commonly known as Neurotrophin-5 (NT-5), or as NT-4/5. NT-4/5 forms part of the complex network of growth factors, along with BDNF, NGF, and NT-3, of retrogradely transported neurotrophins which orchestrate the generation and maintenance of neuron populations. NT-4/5 binds selectively to the cellular receptor TrkB and NT-4/5 is known to have roles in the stimulation of GAP-43 and T-α1-tubulin to induce axon regeneration. More recent work has also shown that, along with promoting the regeneration of axons, NGF, BDNF and NT-4/5 all play a role in the formation of dendrites, and the establishment of synapses within the sympathetic ganglia. In support of this, transgenic mice with a knockout of NT-4/5 showed a marked decrease in axon elongation during regeneration. Studies of this factor have demonstrated that it does command some neuroprotective abilities, such as when it is administered to rubrospinal tract neurons following cervical axotomy. As well, treatment with NT-4/5 has been shown to reduce infarct size in rats with middle cerebral artery occlusion, however, unlike BDNF, it was unable to prolong survival of damaged RGCs over a prolonged period.

Ciliary Neurotrophic Factor (CNTF) is known to play an essential, cooperative role in motoneuron survival and function. It is expressed almost exclusively within the nervous system, however at much higher levels within the PNS than in the CNS, and in the latter is produced mostly by astrocytes. It has demonstrated protective abilities in multiple sclerosis, and has been previously used in a therapeutic trial for the treatment of motor neuron disease and amyotrophic lateral sclerosis. CNTF, along with other neurotrophic factors such as GDNF and BDNF, has become widely recognized for its capacity to rescue RGCs following a variety of different lesions, such as ischemia, traumatic, or metabolic injury. In addition to its anti-apoptotic effects within the retina, CNTF has been established to have regeneration-promoting properties, as it appears to stimulate neurogenesis when adenovirally delivered to injured RGCs. More recently, CNTF gene transfer via adeno-associated virus (AAV) has been found to protect RGCs in the rat from a variety of acute ischemia models. Recent work indicates that expression of CNTF by CNS astroglia may depend on the contact between astroglial cells and neurons; binding of astroglial integrin receptors would suppress CNTF expression, while loss of this contact would induce its production.

Insulin growth factor (IGF) is found both systemically and in the CNS. Most studies on its effects have been conducted systemically, and it has been found to inhibit apoptosis in various cell types such as cardiomyocytes. While it is acknowledged that the intracellular pathways activated by IGF may vary based on cell type and applied stress, its activity appears to involve both the Erk and PI3K/Akt pathways. Current research also suggests that the IGF-1 pathway is a promising avenue for therapeutic use to improve repair after ischemia/reperfusion events in cardiac tissue, raising the possibility of potential applications regarding retinal ischemia. Supporting this perspective is work showing that IGF contributes to retinal neovascularization following diabetic retinopathy.

Epidermal Growth Factor (EGF) binds to EGFR to induce cellular proliferation, differentiation and survival. The binding of EGF to EGFR has been shown to have a major impact on determining pluripotent stem cell fate within the retina. More recent work has further examined this concept and has found that in zebrafish, heparin-binding epidermal-like growth factor (HB-EGF) is necessary and sufficient to induce the dedifferentiation of Mueller glia into multipotent progenitors capable of regenerating other cell types within the retina. Studies on de-differentiation of Mueller glia in mammals, however, are not able to produce cells which can then re-differentiate into any cell type, only into myelinating oligodendrocytes. Further work has also suggested that p53 plays a role in the limited ability of these “false MSCs”, halting them from fully re-entering the mitotic cycle. However, recent studies indicate that EGF may have a function in aiding RGC survival; it has been found that Nell2, an EGF-related gene, supports RGC survival after optic nerve injury.

Vascular endothelial growth factor (VEGF) exists as several isoforms, VEGF-A, -B, -C, and -D; the most well characterized of which is VEGF-A. Administration of VEGF-A has been shown to enhance the formation of blood vessels following traumatic and hypoxic damage. As well, previous studies have shown neuroprotective and neuroproliferative properties for VEGF-A. The major anti-apoptotic pathways activated by VEGF-A are the MAPK and PI3K pathways. It is important to note that in order to achieve this protection, VEGF-A splice variants must be expressed at biological ratios, as improper ratios result in hyper-permeable vasculature and increased edema. Doing so, it has been shown that an increase of VEGF-A enhances recovery after spinal cord compression injury and increases RGC survival in the retina after ischemic injury.
Excitotoxicity and ischemic injury in the retina

In the normal physiological state, neurotransmitters are only found at very low levels in the extracellular space. This is due to the restricted ion gradient maintained across the neuronal membrane, as well as the efficient and effective removal of neurotransmitters from the synaptic clefts by glia. During retinal ischemia, however, the concentration of neurotransmitters, notably glutamine, in the extracellular space increases dramatically[184, 185]. It is also worth noticing that during reperfusion, the increased levels of neurotransmitters in the extracellular space will activate their receptors and contribute to the death of RGCs[186].

While glutamate has long been recognized as the major excitatory neurotransmitter in the CNS, it has also been notable for its ability to kill neurons under certain conditions[187, 188]. This ability has been attributed to the large presence of NMDA receptors on susceptible cells, such as RGCs. However, there has been work suggesting that excitotoxicity following ischemia does not directly affect RGCs after all[189]. Rather, this has been work suggesting that excitotoxicity following ischemia is the imbalance of intracellular K+ and high concentration of Na+ without, thus a gradient exists for the loss of intracellular K+ and the gain of extracellular Na+. This gradient is maintained by various channels and ionic transporters which cross the cell membrane, most notably the Na+/K+ ATPase[208]. Research has revealed that the loss of cell volume during apoptosis is largely dependent on a loss of intracellular K+[209, 210].

A large family of voltage-gated potassium channels (Kv) have been found in mammalian brains. They are involved in the mediation of K+ efflux upon membrane depolarization, and have been shown to play a role in mediating apoptotic cell death; of these, the most prominent appear to be Kv1.1, Kv1.3, and Kv2.1[211-213]. Inhibition of Kv1.1 and Kv1.3 by use of siRNAs was able to successfully inhibit their expression, and protect RGCs from apoptosis in optic nerve transection. As well, it was noted that Kv1.1 depletion increased levels of the antiapoptotic gene Bcl-xL, whereas depletion of Kv1.3 reduced expression of caspase-3, caspase-9 and Bad, all of which are pro-apoptotic[211, 212].

Kv2.1 expression has also been shown to increase following damage, but before the appearance of apoptosis[208, 214]. Inhibition of Kv2.1 has been shown to inhibit apoptosis in vitro[215], as well as in vivo[216]. Following cerebral ischemia, it was also found that Kv2.1 participates in promoting apoptosis[217].

Conclusions

Ischemia in the CNS is undoubtedly a complex problem with many facets. It is made all the more challenging to study due to the nature of CNS tissues, which are difficult to reach and to submit to consistent injury and treatment. The retinal model of ischemic injury addresses these problems as the retina is much more accessible than other CNS tissues and yet conserves the features that characterize neuron degeneration. The retina develops from the diencephalon, and so remains part of the CNS; retinal ischemia activates the same pathways as ischemic injury in other CNS areas, and can therefore offer strong evidence regarding the pathological processes following injury. This makes the study of retinal ischemia useful for discovering the ways in which ocular diseases such as glaucoma and diabetic retinopathy affect retinal cell populations, but also for building our knowledge of the processes of ischemic damage in other CNS areas. Much has already been discovered using the model of retinal ischemia, and its continued use will only serve to increase our understanding of ischemic injury and further characterize the complex cascade of mechanisms which contribute to this, including the opening of receptor-operated, and voltage-operated Ca2+ channels, efflux from intracellular stores, and a breakdown of Ca2+ buffering mechanisms[195-198]. One process which has been hypothesized to help in the prevention of cell death due to Ca2+ de-regulation is preconditioning. Preconditioning is accomplished by introducing small amounts of the supposed stressor to a group of cells prior to an insult[199]. It has been shown that preconditioning is an effective method of preventing cell death during hypoxic and ischemic insults in liver tissue[200], as well as in myocardial tissue[201]. As well, the effects of drug-induced preconditioning against NMDA or glutamate were shown to induce neuroprotection in rat hippocampal tissue[202]. More recent work has followed up on the hypotheses that acetylcholine (ACh) and nicotine may be neuroprotective against excitotoxicity in the retina[199, 200, 204]. Work on the neuroprotective abilities of ACh and nicotine indicate that it hinges on their activation of nAChRs, which in turn activates the pro-survival PI3K/Akt, Bcl-2, NF-kB, and MAPK pathways[205].
of processes and factors involved.

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