Rod cell death and retinal degeneration

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Abstract

Retinal degenerations cause visual deficits as a consequence of rod and cone cell death. Rod photoreceptors function under dark conditions and are responsible for motion detection, and peripheral vision, while cone photoreceptors function under light conditions and participate in color and high contrast vision. The mechanisms that lead to photoreceptor cell death have not been clearly elucidated; however, photoreceptors ultimately die by apoptosis. This chapter will focus on rod cell death in retinal degeneration. We provide examples of inherited, disease-induced, injury-induced and chemical-induced retinal degenerations. These include retinitis pigmentosa, cancer-associated...
retinopathy, retinal detachment and lead toxicity, respectively. We will review what is currently known about the mechanisms that are involved in rod cell death in each of these conditions, with specific focus on events that eventually lead to rod apoptosis. Finally, we will discuss the progress that has been made in developing potential therapeutics to prevent rod cell loss.

Introduction

Apoptosis is a form of cell death that is induced under normal physiological and pathological conditions. It is critical to embryonic development and is especially predominant in the nervous system during the time of synaptogenesis [1-4]. It is also the main mechanism of cell death that is involved in neuronal cell loss after trauma, ischemia and neurodegeneration [5, 6].

The process of apoptosis is complex. A number of different apoptotic pathways can be activated within a single cell type. There are also many different external and internal stimuli that signal the cell to undergo apoptosis, and these will determine which pathways are activated.

Retinal degeneration in and of itself is also very complex. Numerous traumas, toxins and diseases cause cell death [7-10]. In addition, multiple genetic mutations have been identified in both photoreceptor and non-photoreceptor specific genes, causing inherited disorders [11]. While two genes may have very unique functions, it is not clear how, at times, deficits in each can produce similar clinical phenotypes. Notably, a study of 12 different models of photoreceptor degeneration revealed that the kinetics of photoreceptor cell loss in each was exponential, suggesting that a single event, such as calcium overload, randomly initiates cell death [12].

Retinitis pigmentosa

Retinitis pigmentosa (RP) refers to a group of heterogeneous progressive retinal degenerations that are characterized by night blindness, gradual visual impairment and bone spicule-like pigment formation in the retina. These disorders affect approximately 1.5 million people worldwide and result in primary rod cell degeneration [13]. In many cases the rod cell death is subsequently followed by cone degeneration, resulting in central vision loss and blindness. Evidence suggests that RP cell death is caused by apoptosis. Analysis of human RP tissue has revealed upregulation of apoptotic marker genes encoding clusterin and secreted frizzled-related protein-2 (SFRP2) [14, 15]. Moreover, a number of in vivo studies have demonstrated that apoptosis occurs in different rodent models of RP [16-18].

The majority of RP is caused by mutations in rhodopsin, the most abundant protein in the retina, which constitutes up to 85% of the total protein
found in rod outer segments [19]. These mutations can lead to autosomal dominant (ad) or autosomal recessive (ar) disease. In fact, mutations in rhodopsin are responsible for approximately 25% of all cases of adRP [20-22]. P23H, the most frequent mutation, causes 10% of all adRP in North America [23].

Rhodopsin is a G-protein coupled receptor that is specifically localized to the rod outer segment discs and is formed when opsin binds 11-cis-retinal. When a single photon of light is absorbed by rhodopsin, the 11-cis-retinal is converted into all-trans-retinal and dissociates from the protein. Free opsin then undergoes a conformational change and propagates the phototransduction cascade.

Researchers have developed several transgenic animal models that carry rhodopsin mutations (Q344ter, P347S, P23H, S334ter), as well as opsin-/− mice [16, 17, 24-26]. Opsin-/− mice possess a targeted disruption in the rhodopsin gene, while the other models mimic rhodopsin mutations found in human disease. Each model exhibits retinal degeneration and TUNEL positive staining [16, 17, 27, 28].

P347S, Q344ter and S334ter animals all possess C-terminal mutations that result in abnormal rhodopsin localization [29-31]. In vitro studies revealed activation of mislocalized rhodopsin causes rod cell death via apoptosis by stimulating G protein and adenylate cyclase activity, resulting in the increase of cAMP [32]. Increased cAMP levels have been identified in P347S transgenic mice and P23H and S344ter transgenic rats [33, 34]. In addition, casapase-3 has been implicated in rod cell death in S344ter rats [28].

Ongoing research is aiming to developing effective therapies for RP caused by rhodopsin mutations. Thus far a number of agents have been identified that have shown some efficacy in reducing rates of retinal degeneration in RP models; however, none have stopped the progression of the disease. For example, intraocular administration of a novel estradiol analog prevented some photoreceptor cell death in S344ter rats [35]. Several groups have also evaluated the possibility of using adeno-associated virus- (AAV) mediated delivery of growth factors to treat photoreceptor degeneration in different transgenic models. AAV-mediated delivery of basic fibroblast growth factor (FGF-2), FGF-5, FGF-18, glial cell line-derived neurotrophic factor (GDNF) or ciliary neurotrophic factor (CNTF) reduced the rate of photoreceptor degeneration in S334ter rats [36-39]. Additionally, AAV-FGF-5, AAV-FGF-18 and AAV-CNTF showed efficacy in P23H retinas, while AAV-CNTF showed significant photoreceptor preservation in opsin-/− mice [37, 39, 40]. Finally, ribozyme therapy has been explored for the treatment of adRP, as ribozymes specifically target and degrade mutant mRNA. AAV-mediated delivery of hammerhead or hairpin ribozymes directed against P23H slowed rates of retinal degeneration in rats carrying this mutation [41, 42].
While rhodopsin mutations account for the largest percentage of RP cases, mutations in at least 30 other different genes have been identified to cause adRP, arRP or X-linked RP [11]. Several lines of evidence show a correlation between non-rhodopsin gene mutations, rod cell death and apoptosis.

*PDE6B* encodes the β subunit of rod cGMP phosphodiesterase (β-PDE), a crucial player in the phototransduction cascade. Following activation, PDE hydrolyzes cGMP to 5’GMP, resulting in the closure of cGMP gated cation channels in the photoreceptor plasma membrane, hyperpolarization and a decrease in intracellular calcium levels.

Mutations in *PDE6B* cause recessive RP [43]. In order to understand the mechanism by which defects in this gene cause rod photoreceptor cell death and to develop potential therapies, researchers are studying the *rd/-* (*rd/rd*) mouse. This animal model has a nonsense mutation in codon 347 of the β-PDE gene that causes a truncation in the normal gene product and eliminates more than half of the peptide chain, including the putative catalytic domain [44]. The mouse also possesses an intronic retroviral insertion [45].

*rd/rd* mice exhibit a rapid degeneration that begins on PND8, and rod degeneration is complete by PND36 [46]. Prior to the initiation of the degenerative process, cGMP levels are elevated, suggesting that there is increased conductance through the cGMP-gated sodium and calcium cation channels, which leads to eventual metabolic overload [47]. Several studies have revealed extensive apoptosis in *rd/rd* retinas, as evidence by DNA fragmentation, chromosomal condensation in pyknotic nuclei, TUNEL staining and rapid removal of degenerative cells [16, 17, 48]. These apoptotic events occur independently of caspases 9, 8, 7, 3 and 2 and independently of the pro-apoptotic transcription factor p53 [49-51]. To further identify the pathways involved in cell death, microarray technology was used to identify changes in gene expression during the time course of photoreceptor degeneration [52]. Wildtype and *rd/rd* mice exhibit similar gene profiles initially but begin to deviate by PND10. *rd/rd* mice subsequently exhibit an increase in transcription factors and apoptosis and neuroinflammation-specific genes that corresponds with the rise in cGMP levels. Genes encoding calcium-binding proteins and proteins implicated in tissue and vessel remodeling are also elevated, supporting the hypothesis that degeneration in these animals begins with calcium toxicity, which is then followed by secondary insults that ultimately result in cell death. This cell death may be delayed by subretinal delivery of viruses encoding βPDE, trophic factors or anti-apoptotic factors [53-57].

Another gene that has been linked to RP and apoptosis is peripherin/RDS. It encodes a photoreceptor-specific membrane glycoprotein that is found in the rims of rod and cone outer segments and is essential for the formation and maintenance of these structures. Mutations in peripherin/RDS have been identified.
in autosomal dominant RP and in a digenic form of RP, caused by mutations in both peripherin/RDS and ROM1 [58-60].

Homozygous retinal degeneration slow mice (rds) have a null mutation in peripherin 2 (Prph2), lack outer segments and develop rod and cone degeneration [61, 62]. These animals also exhibit apoptosis-specific internucleosomal DNA fragmentation; however, unlike rd/rd mice, rod apoptosis is delayed in the absence of p53 [16, 17, 63]. Interestingly, minocycline, a tetracycline, that has been shown to suppress caspase-dependent apoptosis, can also delay apoptosis in this animal model; however, it has no effect on preserving the thickness of the outer nuclear layer nor can it prevent rod cell death [64]. In contrast, adenovirus-mediated and AAV-mediated delivery of CNTF to rds retinas can reduce photoreceptor loss and improve electroretinogram (ERG) responses, while AAV-mediated delivery of Prph2 can restore outer segment structure and disc formation [39, 65-67]. Nevertheless, these treatments do not cause long-term rescue of the rds phenotype.

Recently, two novel RP-causative genes have been identified, and both have been linked to apoptosis. A mutation in CA4 (carbonic anhydrase IV) causes autosomal dominant RP while a mutation in CERKL (CERK-like protein) causes autosomal recessive RP [68, 69]. CA4 encodes a glycosylphosphatidylinositol-anchored zinc metalloenzyme that is highly expressed in the choriocapillaris and in the epithelial and fiber cells of the lens [70]. It has also been identified in the plasma membrane of kidney epithelial cells, pulmonary endothelial cells and endothelial cells of other microcapillary beds [71-74].

Patients with RP17 have a C to T transition in base 40 of the CA4 cDNA [68]. This causes a mutation in the gene’s signal sequence (R14W). Transfection of the mutant cDNA into COS-7 cells reveals a reduction in steady state levels of carbonic-anhydrase IV, an upregulation of proteins that are markers for the unfolded protein response (UPR), an upregulation of proteins that are markers for endoplasmic reticulum (ER) stress and an induction in apoptosis, which is evident by annexin V binding and TUNEL staining [68]. Based on these findings, it has been hypothesized that expression of the mutant protein in the choriocapillaris activates UPR, leading to ER stress and apoptosis. This will cause damage to the capillaries and result in eventual ischemia in the retina, ultimately causing cell death. Notably, carbonic anhydrase inhibitors prevent the adverse effects observed in COS-7 cells expressing the R14W mutation, including the induction of apoptosis, suggesting a possible pharmacological approach to treat this form of RP in the future [71].

CERKL encodes a ceramide kinase homolog that is expressed in human fetal and adult retina [69]. In situ hybridization in murine tissue localized Cerkl message primarily to ganglion cells with faint signals also detected in the inner
and outer nuclear layers [69]. RT-PCR also detected message in human kidney, lung, pancreas, brain, placenta and liver [69, 75]. Live cell imaging revealed that CERKL is localized to many cellular compartments, including the cytoplasm and the nucleus, where it is specifically enriched in the nucleoli [75].

Ceramide kinases function to convert ceramide, a sphingolipid metabolite, into ceramide-1-phosphate [75]. Evidence suggests that ceramide can cause cell growth arrest and apoptosis, while ceramide-1-phosphate is antiapoptotic and neuroprotective [76, 77]. Notably, photoreceptor degeneration was inhibited in drosophila mutants by decreasing intracellular ceramide pools [78].

Patients with RP26 possess a nonsense mutation (R257X) in exon 5 of the CERKL gene, which causes a premature truncation within the predicted catalytic domain of the protein [69]. This mutation may increase ceramide levels, causing the cells to be more sensitive to apoptotic stimuli. Interestingly, in vitro studies could not readily confirm that wildtype CERKL phosphorylates ceramide [75]. In addition, the R257X mutant does not associate with the nucleoli and accumulates in the nucleus; consequently, abnormal localization of the mutant protein may be responsible for the clinical phenotype [75].

Cancer-associated retinopathy

Patients with non-ocular cancers can develop retinal degeneration. These acquired retinopathies are often termed paraneoplastic syndromes, unique conditions that arise in a specific tissue as a result of a cancer that has metastasized in another remote site. Although the cause of these diseases has not been clearly defined, many paraneoplastic syndromes are thought to develop as a consequence of an auto-immune response. Tumor cells can produce proteins that are found elsewhere in the body and subsequently release them into the systemic circulation. The host’s immune system mounts a response to these foreign antigens by producing antibodies. These auto-antibodies are unable to distinguish between the protein which is being aberrantly expressed and that which is required for normal cell function. As a result, otherwise normal, healthy tissue is attacked and damaged.

Cancer-associated retinopathy (CAR) is one of the best characterized paraneoplastic syndromes. It has primarily been associated with small-cell lung carcinoma; however, it has also been found in patients with other malignancies including prostate, ovarian, breast and gastric cancers [79, 80]. Patients with CAR have a number of clinical symptoms. These include sudden and progressive visual loss with ring scotoma, photopsia, attenuated retinal arterioles and abnormal a- and b- wave ERGs, without evidence of optic nerve head damage, inflammation or neurological symptoms [81].

Scientific evidence suggests that antibodies against recoverin (CAR antigen) play a major role in the development of CAR [80]. Recoverin is a 23 kDa calcium-binding protein that is primarily expressed in retinal
photoreceptors and some retinal bipolar cells. In the presence of high calcium, recoverin interacts with rhodopsin kinase, decreasing its catalytic activity and inhibiting rhodopsin phosphorylation and deactivation [82]. Epitope mapping experiments revealed that recoverin has two linear amino acid stretches that are immunodominant [83]. These correspond to residues 48-52 and 64-70. Interestingly, the peptide encoding residues 64-70 has been found to be pathogenic and causes retinal degeneration in Lewis rats following immunization [84-86]. In addition, this amino acid stretch is located near recoverin’s calcium binding site. Calcium binding induces conformational changes in recoverin and facilitates antibody binding [87].

High levels of circulating anti-recoverin antibodies have been detected in CAR patients, sometimes several months before the diagnosis of cancer [88]. When rats are immunized with purified recoverin protein, they also develop anti-recoverin antibodies and photoreceptor degeneration [85, 86, 89, 90].

Patient-derived antibodies are cytotoxic to retinal cells in vitro [91, 92]. In vivo studies revealed they induce retinal photoreceptor cell apoptosis [89, 93]. Retinal analysis unmasked DNA fragmentation, nuclear chromatin condensation, and increased vacuolization of photoreceptor outer segments [93]. Studies were performed to administer recoverin antibodies directly to the retina via intravitreal injections, and photoreceptor cell loss and decreased ERG responses were observed, providing strong evidence that the antibodies can directly penetrate both photoreceptor and bipolar cells [89, 93]. Furthermore, it has been shown that anti-recoverin antibodies cross the blood retinal barrier in a number of different animal models [87].

In vitro studies in retinal cells revealed that anti-recoverin antibodies upregulate pro-apoptotic bcl-2 proteins, cause cytochrome c to be released from the mitochondria and significantly increase caspase 9 and caspase 3 enzymatic activities [94]. Thus, a mechanism for anti-recoverin antibody-induced retinal degeneration has been proposed [7, 94]. It has been suggested that photoreceptors initially internalize anti-recoverin antibodies via endocytosis. The antibodies then compete with calcium for binding sites on recoverin, ultimately leading to an increase in intracellular calcium. The rise in calcium triggers an upregulation in the expression of bcl-2 proteins and a release of cytochrome c and Apaf-1 from the mitochondria into the cytosol. Cytochrome c and Apaf-1 form a multisubunit protease activation complex (apoptosome) with pro-caspase 9 and dATP. The apoptosome auto-catalytically cleaves caspase 9, and caspase 9 can then cleave downstream caspases, resulting in the activation of caspase 3, DNA fragmentation and cell death.

There are currently no effective therapies for CAR. Corticosteroid administration and plasmapheresis have been performed in conjunction with anti-neoplastic therapy clinically, resulting in variable effects [95, 96]. Additionally, intravenous immunoglobulin treatment showed efficacy in some patients [96].
A number of novel treatment options have also been explored in vitro and in vivo. One study revealed that anti-recoverin antibody-induced photoreceptor cell death was photodependent. ERG responses were reduced under cyclic and continuous light conditions in a rat model of CAR; however, changes in ERG responses were not observed under constant dark conditions [97]. This study also reported that systemic administration of nilvadipine, a calcium antagonist, suppressed anti-recoverin antibody-induced retinal dysfunction in rats, presumably by decreasing calcium stores. Interestingly, an in vitro study speculated that increasing calcium stores might protect retinal neurons from anti-recoverin mediated cell death [98]. Thus, rat retinal neurons were exposed to anti-recoverin antibodies and potassium. Potassium administration, decreased TUNEL-positive cells, prevented antibody-induced DNA fragmentation and antibody-induced rod cell death [98].

Another approach described in the literature utilized AAV to deliver CNTF to treat anti-recoverin antibody-induced degeneration in rats [99]. CNTF is a cytokine that has been previously shown to promote photoreceptor survival in animal models of retinal degeneration and modulate apoptosis [39, 40, 100]. Subretinal delivery of AAV-CNTF in a rat model of CAR significantly protected photoreceptors from apoptotic death [99]. Treatment activated the STAT3 pathway and reduced caspase 3 activity, providing a possible mechanism by which it elicited its anti-apoptotic effects. Notably, caspase inhibitors have also been successful in blocking the effects of anti-recoverin antibodies on the retina [101].

While it is clear anti-recoverin antibodies can cause photoreceptor degeneration, not all patients with CAR possess these antibodies [7]. Moreover, some patients with small cell lung carcinoma have anti-recoverin antibodies, but do not develop visual symptoms [102]. Similarly, there are patients with idiopathic retinopathy that also possess anti-recoverin antibodies [7]. This body of evidence suggests other factors must also play a role. Indeed, autoantibodies to other retinal antigens have been detected in CAR patients. These include α-enolase, heat shock cognate protein 70, 40 kDa protein, 45-kDa protein, TULP1 and neurofilament proteins [103-108]. Further research needs to be performed to elucidate the role of these autoantibodies in the disease process and to discern why certain patients do not develop visual symptoms, even in their presence. Likewise, it is necessary to determine what causes CAR in patients which lack autoantibodies, as these findings will allow for the development of novel efficacious treatments in the future.

**Retinal detachment**

Retinal detachment is a prevalent and serious clinical problem that is most commonly caused by trauma and myopia; however, it may also develop in patients with a pre-existing retinal degeneration. This condition results when the neural retina becomes separated from the RPE and choroidal blood supply.
Normally, the RPE functions in recycling the all-trans form of vitamin A to the 11-cis form, so that it can bind opsin in the photoreceptor disc outer segments and initiate the phototransduction cascade. In addition, the apical side of the RPE extends microvilli that surround the photoreceptor outer segments. Nutrients pass from the choroidal blood supply through the RPE to reach the photoreceptor cells. In a detachment, the outer retina’s major blood supply is removed, and vitamin A recycling is dramatically hindered, leading to photoreceptor death. Typically, detachment affects the peripheral retina, resulting in rod death; however, fovea detachments can also occur with significantly more deleterious consequences.

Retinal detachment has been modeled in rats, rabbits, ground squirrels, and cats [109-112]. In every case, rod cell death is caused by apoptosis and peaks 3 days post-injury. Yang and colleagues have suggested that Bax-mediated apoptotic signaling pathways play an important role in retinal detachment-associated photoreceptor cell death [113]. This group established experimental retinal detachment in wild-type and Bax-deficient mice by subretinal injection of 1.4% sodium hyaluronate. While wild-type animals exhibited photoreceptor cell loss and TUNEL positive staining, TUNEL positive staining and ONL thinning were not observed in Bax deficient retinas.

Zacks and colleagues demonstrated the time dependent activation of caspase -3, -7, -9 and poly-ADP ribose polymerase (PARP) following retinal detachment in Brown Norway rats [114]. More recently these researchers demonstrated that retinal degeneration activates the FAS-mediated apoptosis pathway in this animal model [109]. FAS-receptor, FAS-ligand, caspase-8 and BID message were transcriptionally upregulated, and a FAS-receptor/FAS-ligand complex formed. In addition, subretinal delivery of the caspase-9 inhibitor zLEHD.fmk, or an anti-FAS receptor antibody, decreased caspase-9 activity in the rats.

Hisatomi and colleagues have suggested that apoptosis occurs mainly by a mitochondria-dependent pathway rather than Fas/Fas-L or downstream caspase dependent pathway [115]. They administered a Fas/Fc chimera recombinant protein or the universal caspase inhibitor Z-VAD.fmk subretinally to Brown Norway rats with retinal detachment. Neither treatment prevented photoreceptor apoptosis or improved retinal function. Moreover, they noted that apoptosis-inducing factor (AIF), a novel caspase-independent apoptotic factor, relocalized from the mitochondria to the nucleus of apoptotic cells following detachment [115, 116].

While the mechanism of apoptosis in retinal detachment has not been clearly defined, some therapies exist and novel therapies are being developed. If reattachment surgery is performed within 10 days of the occurrence of an off-macula detachment, there is a 70% chance of recovery of vision to 20/40 or better. The success of reattachment surgery is dependent on the time
of treatment. For example, if an off-macula detachment is treated 10 days-6 weeks post-injury, the chance of recovery drops to 20-35% [117]. Later treatment times or repeated surgeries often cause membranes to form on the vitreal surface of the retina. This condition is called proliferative vitreoretinopathy (PVR). PVR causes the retina to pucker and prevents it from reattaching.

In addition, some ophthalmologists have treated their retinal detachment patients with 70% oxygen until surgery could be performed. Studies in cats and ground squirrels revealed that treatment with 70% oxygen preserved some photoreceptor structure and function following retinal detachment [118, 119].

Additional experimental therapies have been tested in different animal models. Studies in a rat model of retinal detachment revealed brain derived neurotrophic factor (BDNF) and bFGF significantly impeded apoptosis and retinal dysfunction; however, BDNF treatment did not reduce overall cell death and apoptosis in a cat model of retinal detachment [112, 115]. Another growth factor that has been tested for treatment of retinal detachment is GDNF. AAV-mediated delivery of GDNF proved efficacious in Lewis rats. rAAV-GDNF-treated eyes retained longer outer segments, possessed fewer apoptotic cells and exhibited less photoreceptor degeneration than controls [120]. Finally, Nour and colleagues tested a P2Y2 receptor agonist (INS37217) in normal and rds+/- mice with retinal detachment to see if enhancing reabsorption of extraneous subretinal fluid could minimize some of the adverse effects associated with retinal detachment [121]. P2Y2 receptors are found on the apical membrane of RPE cells and their activation has been shown to stimulate ion transport and fluid reabsorption [122, 123]. Subretinal administration of INS37217 significantly increased rod and cone ERGs, reduced the number of TUNEL positive photoreceptors and enhanced the rate of retinal reattachment in both the normal and rds+/- animals [121].

**Lead toxicity**

Lead, a heavy metal that is highly toxic to the central nervous system, causes a broad range of neurological symptoms ranging from deficits in cognitive function and encephalopathy to auditory and visual deficits. Toxicity is most often found in children following ingestion of lead-based paint chips; however, neonates and adults exposed to toxic levels of this compound are also affected.

Lead accumulates in many ocular tissues, but highest concentrations are localized in the retina [124]. ERG studies in lead-exposed workers have revealed long-term scotopic retinal deficits including selective rod-mediated decreases in sensitivity, amplitude and temporal resolution and increases in implicit times and dark-adaptation [125, 126]. Surprisingly, children (ages 7-10) that were exposed to a lifetime of lead (through gestation and postnatal development) exhibited
dose-dependent increases in both scotopic a- and b-wave amplitudes and had normal visual acuity [127]. Analysis of the levels of lead in the mothers’ blood throughout the course of pregnancy, and the patients’ blood subsequently thereafter, indicated that there was a significant dose-dependent relationship between the scotopic ERG response and the lead concentration at 12 weeks gestation [127]. This finding correlates with a lead developmental non-human primate study [128, 129]. Monkeys exhibited persistent dose-dependent increases in scotopic b-waves with no changes in ERG implicit times. The opposite effect was observed in rats [130-132]. Rats exposed to lead during neonatal development (from birth to weaning) exhibited dose-dependent decreases in rod cell function. These findings, in conjunction with the human and non-human primate studies, suggest that the time at which one is exposed to lead during development may determine the effect on the scotopic ERG response.

Lead exposure results in retinal degeneration. Studies in neonatal and adult rats reveal that lead causes selective apoptosis of rods and bipolar cells, exhibiting high molecular weight DNA fragmentation and condensed nuclei [125, 130]. Low level developmental lead exposure results in the onset of rod cell loss at P10, with 75% of rod cells remaining by P90 [10]. Decreases in rod numbers, correlate with decreases in rhodopsin levels. In addition, age and dose-dependent decreases in rod a- and b-wave sensitivity can be observed [125].

Biochemical studies in neonatal rats demonstrated that lead exposure inhibits cGMP phosphodiesterase and increases calcium concentrations in the rod outer segments [125, 132, 133]. The increased intracellular calcium levels activate a cascade of signaling events causing mitochondrial changes and ultimately apoptosis. In vitro studies demonstrated that lead and/or calcium decrease retinal mitochondrial ATP synthesis and causes the release of cytochrome c and an increase in caspase-9 and caspase-3 activity [134, 135]. Moreover, mitochondrial membrane potential is decreased, and mitochondrial structure is altered [135]. Cyclosporin A, a mitochondrial permeability transition pore (PTP) inhibitor, blocked the effects of calcium and lead in vitro [135]. These findings suggest that rod mitochondria are the target site of calcium and lead and that binding causes the PTP to open and triggers the cytochrome c/caspase cascade in rods [135].

A recent rodent study revealed that overexpression of Bcl-XL, an anti-apoptotic factor, blocked lead-induced apoptosis in mice [10]. Although calcium levels remained high, normal mitochondrial function was maintained throughout adulthood, and there was no evidence of rod loss or changes in rhodopsin content. In addition, Bcl-XL prevented an increase in rod mitochondrial contact sites, blocked the translocation of Bax, from the cytosol to the mitochondria and inhibited the cytochrome c/caspase cascade. These
exciting findings offer insights into developing possible therapeutics for a number of retinal degenerations as well as neuronal degenerations caused by calcium overload, lead exposure or mitochondrial dysfunction.

Summary

In this chapter we have presented information regarding rod cell apoptosis in the context of inherited, disease-induced, injury-induced, and chemical-induced retinal degenerations. In each case, the cause of rod cell death is due to apoptosis; however, the pathways causing apoptosis are still being elucidated. Interestingly, there appear to be some commonalities in the apoptotic pathways between the different retinal degenerations. In two models of RP, as well as in CAR and in lead toxicity, a rise in rod photoreceptor intracellular calcium levels has been detected. Additionally, the mitochondria seems to play an important role in the progression of the apoptotic cascade in all the cases discussed. Finally, in every example, with the exception of the rd(rd) model of RP, there is induction of caspase activity. While more work is still needed to further clarify the rod photoreceptor apoptotic pathways for retinal degenerations, this research should lead to the development of promising therapeutics for these blinding pathologies in the future.

Acknowledgements

The authors would like to thank Jean Bennett and Ottrina S. Bond for reviewing this manuscript.

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