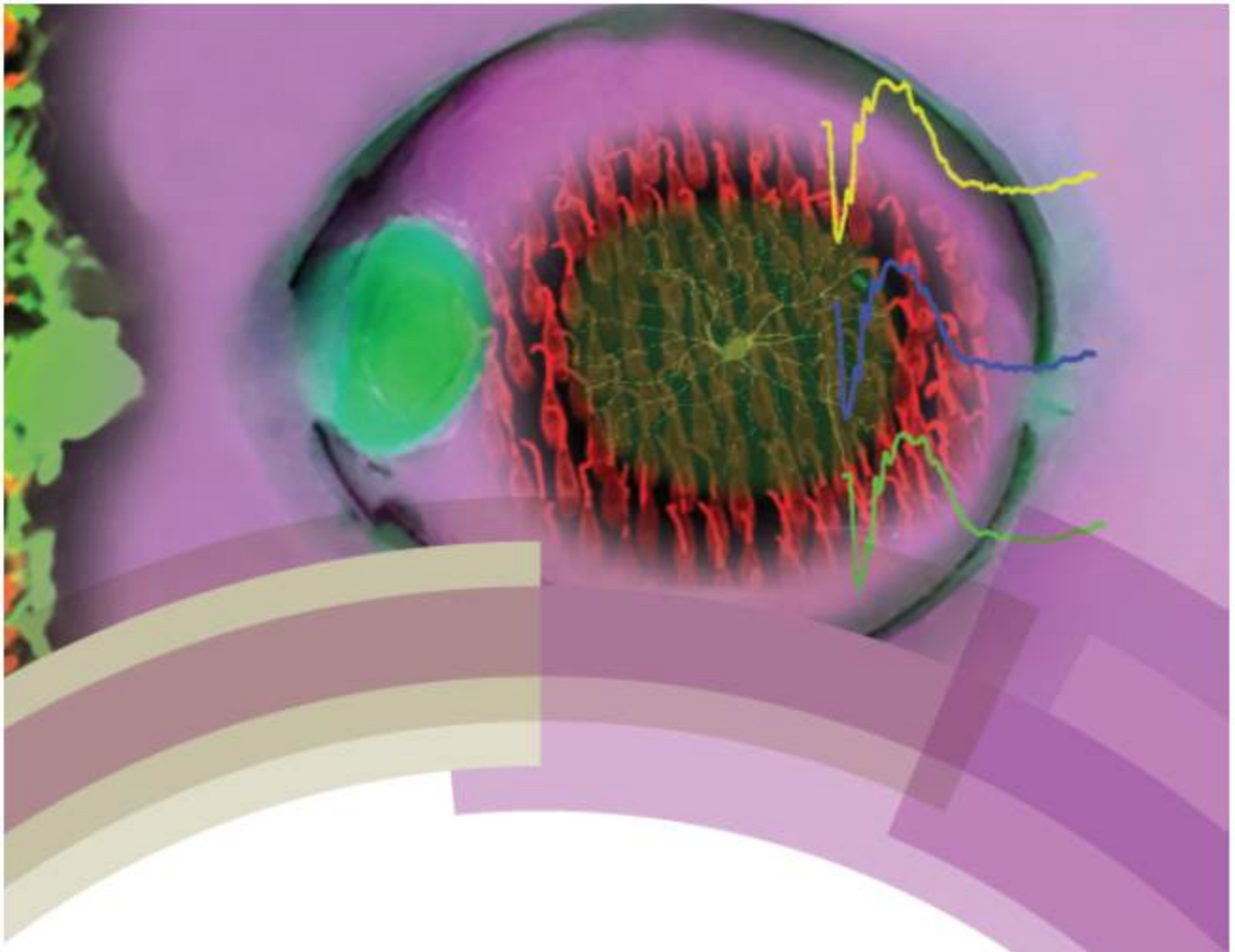

Drug Discovery

Therapies for Retinal Degeneration

Targeting Common Processes

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and Thomas G. Cotter

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Chapter 4

CNS Targets for the Treatment of Retinal Dystrophies: a Win-Win Strategy

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Abstract

As an extension of the central nervous system (CNS), the retina shares with the brain certain developmental, physiological, and pathological characteristics. However, the underlying mechanisms and pathological signs common to neurodegenerative conditions of both the retina and brain have been relatively overlooked. In animal models and in human patients, marked retinal alterations have been demonstrated in Alzheimer´s disease, Parkinson´s disease, and multiple sclerosis, among other pathologies. Furthermore, neurodegeneration of the retina and brain appears to be mediated by similar mechanisms, which include protein aggregation, neuroinflammation, and cell death. Analysis of the retina, which is easily accessible to objective techniques, may therefore constitute an effective tool for the screening and follow-up of CNS neurodegeneration. Moreover, patients with retinal neurodegeneration could potentially benefit from the broad array of pharmacological compounds that have been designed and tested for the treatment of the aforementioned CNS pathologies. Supporting this view, we have shown that GSK-3 inhibitors, which have already been tested in clinical trials to treat several neurodegenerative conditions of the brain, attenuate retinal damage and vision loss in a mouse model of retinitis pigmentosa. Furthermore, systemic proinsulin treatment preserves visual and cognitive function in mouse models of retinitis pigmentosa and precocious aging, respectively.

4.1 Introduction

The embryonic retina is a classical model for the study of central nervous system (CNS) development. Both the neuroretina and the retinal pigment epithelium are derived from the anterior medial neural plate of the embryo[1]. During embryonic development, the retinal field develops in parallel with the rest of the CNS. This process involves a series of complex morphogenetic movements, during which the developing retina field passes through throughout the stages of the neural tube, cephalic vesicle, optic vesicle, and optic cup. The isolation of an already distinctive retina from the rest of the brain first becomes evident in the optic cup stage (Figure 4.1). Subsequently, the retinal cytoarchitecture follows molecular and cellular patterns that closely resemble those seen in other parts of the CNS[2]. Analyses of these developmental and anatomical similarities between the retina and the CNS, aided by the retina's accessibility to observation and experimental manipulation, have revealed numerous physiological and functional parallels between the retina and the brain[3].

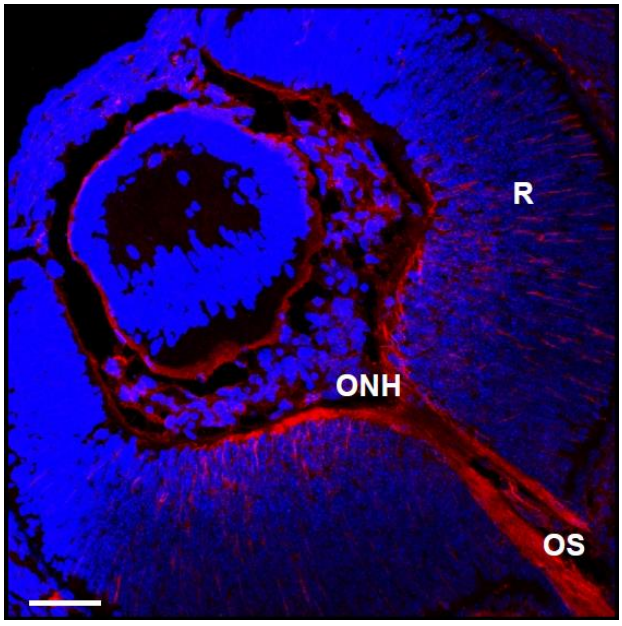

Figure 4.1 The developing retina. The relative isolation of the retina from the rest of the brain is first evident in an optic cup section from a mouse on embryonic day 12.5. The retina remains connected to the brain via the optic stalk and optic nerve. Early fibers of the optic nerve are immunostained for the detection of βIII-tubulin (red). Nuclei are labeled with DAPI (blue). ONH, optic nerve head; OS, optic stalk; R, neuroretina. Scale bar, 100 μm.

Surprisingly, the pathophysiological similarities between the retina and the rest of the CNS have been somewhat overlooked until recently, and drug development strategies for neurodegenerative conditions have primarily focused on those affecting the brain and spinal cord, and less on prevalent retinal diseases such as glaucoma and diabetic retinopathy. Recent findings have underscored the need for a new approach to the treatment of these two retinal pathologies. Because glaucoma was long considered a consequence of elevated intraocular pressure (IOP), therapeutic approaches traditionally focused on the management of IOP. However, the fact that the vision impairment persists even when IOP is controlled underscores the need for alternative neuroprotective therapies[4]. In the case of diabetic retinopathy, studies have shown that microvascular lesions, previously considered the primary lesion in this disease, are in fact preceded by morphological and functional changes[5]. Accordingly, the search for pharmacological therapies for these two diseases has shifted towards targets implicated in neurodegeneration.

Research attention has only recently turned to the presence of retinal alterations in patients with neurodegenerative disorders affecting the brain, such as Alzheimer´s disease (AD) and Parkinson´s disease (PD), among others. Retinal screening using non-invasive imaging, electrophysiological, and behavioral techniques could potentially detect these putative early markers of disease in animal models and in patients with CNS neurodegeneration[3, 6]. Retinal screening techniques provide a quantitative, objective output and could prove invaluable for the recruitment and follow-up of patients involved in clinical trials of drug candidates for the treatment of CNS neurodegenerative conditions, as well as providing a much less costly alternative to direct brain examination for population-wide screening[7-10].

In this chapter, we review the evidence of pathophysiological parallels between neurodegenerative conditions of the retina and the brain. We also discuss the experimental approaches we use to assess the potential of GSK-3 inhibitors and the insulin precursor proinsulin as drug candidates for the treatment of these pathologies.

4.2 Retinal Alterations in Neurodegenerative Conditions of the Brain and Spinal Cord

We have created a list of several neurodegenerative diseases (Table 4.1) that collectively affect a considerable proportion of the population, and in which retinal alterations have

been demonstrated. Preliminary studies suggest that several other conditions not included here, including Huntington´s disease, stroke, and psychiatric disorders, have manifestations in the retina[3,11-14]. The fact that these alterations are common to so many neurodegenerative conditions underscores the need for further studies to better understand the impact of CNS neurodegeneration on the retina.

Table 4.1 Retinal alterations observed in neurodegenerative conditions of the brain and spinal cord.

Neurodegenerative conditions of the brain and spinal cord	Retinal alterations	References
Alzheimer´s disease	Aβ deposition. Imbalanced phosphorylation of Tau. Decreased retinal perfusion. Microglial activation and reactive gliosis. Complement activation. Neuroinflammation. Thinning of the optic fiber layer and macula. Reduced electroretinographic response in retinal ganglion cells.	8, 20-25
Parkinson´s disease	α-synuclein deposition. Dopamine deficiency and deterioration of the perifoveal dopaminergic plexus.Microglial activation and reactive gliosis. Thinning of the optic fiber layer. Altered electroretinographic response.	8, 11, 25
Multiple sclerosis	Optic neuritis and neuroinflammation. Blood-retinal barrier dysfunction Retinal ganglion cell degeneration. Thinning of the optic fiber layer and optic nerve.	10

Alzheimer´s disease (AD) is a devastating condition characterized primarily by memory loss and a profound cognitive deficit. As demonstrated in both animal models and AD patients, a key hallmark of this disease is neuronal loss accompanied by the deposition of amyloid beta (Aβ, which forms senile plaques) and hyperphosphorylated Tau (pTau, which forms neurofibrillary tangles) in the cerebral cortex. For many years, diagnosis of AD has been primarily based on subjective evaluation of the patient's behavior. The search for objective and quantitative methods for AD diagnosis and the evaluation of disease

progression has led to the characterization of putative biomarkers, such as Aβ and hyperphosphorylated Tau in cerebrospinal fluid, as well as the development of new brain imaging techniques. However, the effects of AD are not confined to the brain, and also affect other CNS regions, including the retina (Table 4.1). Alterations in visual perception in AD were initially attributed to defects in visual image processing by the brain, but studies subsequently revealed structural alterations in the retinas of AD patients[15-19] and in animal models (Figure 4.2). The retinas of AD patients not only exhibit the same molecular hallmarks seen in the brain (deposition of Aβ and pTau)[20-24], but also display neurodegenerative alterations in retinal cytoarchitecture, including loss of ganglion cells and thinning of the inner plexiform and retinal nerve fiber layers[8,25] (Table 4.1). Based on these findings, a growing number of studies have suggested that retinal examination could constitute a useful tool for the diagnosis and follow up of AD[8].

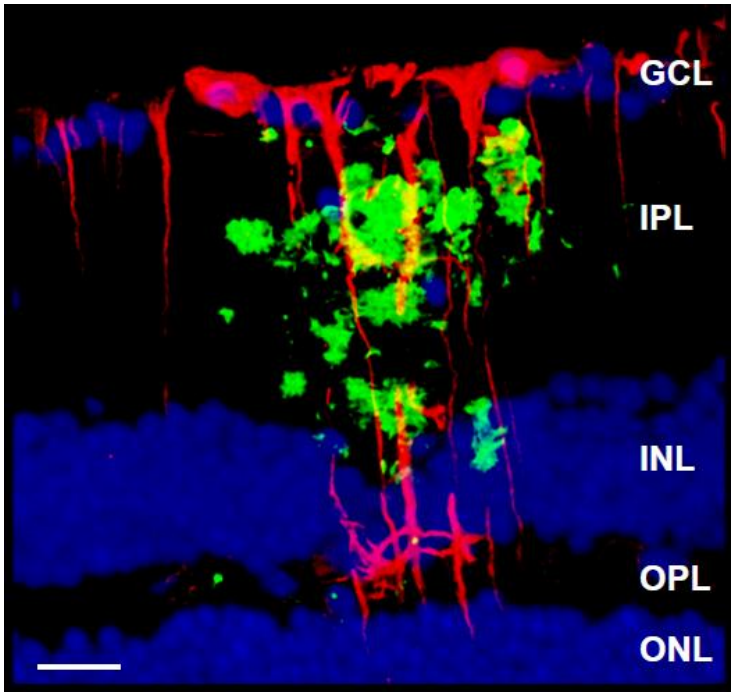

Figure 4.2 Retina from a mouse model of Alzheimer´s disease. Immunostaining for abnormally processed amyloid (green) reveals the presence of Aβ plaques in a retinal section from a mouse carrying human familial AD mutations in presenilin and amyloid precursor protein. The plaques are surrounded by reactive Müller glial cells and astrocytes, which are visualized by immunostaining for GFAP (red). Nuclei are labeled with DAPI (blue). GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer; OPL, outer plexiform layer; Scale bar, 25 μm.

Parkinson´s disease (PD) is widely considered a motor dysfunction disease caused by degeneration of dopamine-producing neurons in the *substantia nigra*. However, in addition to tremor and slowness of movement, the most evident clinical signs, PD patients also exhibit biochemical, neurochemical, cellular, and functional changes in the retina (Table 4.1). These include deposition of α-synuclein, a PD-specific hallmark[11], and changes in retinal structure, including a decrease in the thickness of the retinal nerve fiber layer and deterioration of the macula and fovea[8,25].

In contrast to the primary neuronal damage and loss associated with AD and PD, oligodendrocytes are the primary targets of the abnormal immune response that underlies multiple sclerosis (MS). The inflammation associated with MS leads to progressive demyelination of the central nerves[3,10]. Ocular inflammation and vision impairment are other recognized early signs of MS[3]. Visual impairment results from demyelination of the optic nerve, which is part of the CNS (Table 4.1), and consequent thinning of the retinal nerve fiber layer.

Despite the distinct etiologies of the neurodegenerative diseases described above, retinal involvement appears to be common to all. As such, retinal analysis could constitute a useful strategy for the screening, diagnosis, and follow-up of a variety of neurodegenerative conditions, and for evaluation of potential therapies in clinical trials.

4.3 Neurodegenerative Conditions of the Brain and Retina Share Common Pathological Mechanisms

In contrast to the well documented retinal manifestations of CNS neurodegeneration (outlined in 4.2), retinal diseases do not appear to affect the brain or other regions of the CNS to the same extent. However, in line with the common origin and physiological similarities of both structures, neurodegenerative diseases of the brain and retina share common pathophysiological mechanisms, particularly at the molecular and cellular levels (Table 4.2). To illustrate these shared characteristics, we have selected two retinal dystrophies, glaucoma and age-related macular degeneration (AMD), which differ etiologically and in terms of the retinal cells affected.

Table 4.2 Pathological signs common to neurodegenerative diseases of the retina and brain.

	Alzheimer´s Disease	**Parkinson´s Disease**
Glaucoma	Aβ deposition[6,28] pTau deposition[27] Neuroinflammation + microglial activation[8,25] Axonal atrophy and deficits in axonal transport [8,25] Neuronal cell death[8,25]	Synuclein deposition[32] Neuroinflammation + microglial activation[8,25] Axonal atrophy and deficits in axonal transport [8,25] Neuronal cell death[8,25]
Age-related macular degeneration	Aβ deposition[29,30,31] Complement activation[6] Neuroinflammation + microglial activation[6] Neuronal cell death[6]	Neuroinflammation + microglial activation[6] Neuronal cell death[6]

As mentioned before, elevated IOP is common feature and the greatest risk factor for glaucoma. However, a link between glaucoma and AD has been proposed[26], and the presence of protein deposits characteristic of AD (Aβ and pTau) has been described in the retinas of glaucoma patients[6,27,28]. Moreover, in both diseases comparable Aβ and pTau profiles are observed in the compartments closest to the primary affected tissue. Thus, compared with unaffected individuals, lower levels of Aβ and higher levels of pTau are found both in the cerebrospinal fluid of AD patients and in the vitreous humor of glaucoma patients[6]. Similarly, the aggregation of pathological proteins is observed in both AD and AMD. The primary risk factor for AMD is aging, followed by other behavioral and genetic risk factors. Genetic risk factors include the presence of the *APOE* allele, which is associated with an increased risk of both AMD and AD[7]. Aβ aggregates have also been detected inside drusen deposits in the retinas of AMD patients[29-31]. Protein aggregates are also an important feature of PD, and aberrant deposits of synuclein are found in both PD and glaucoma patients[11,32].

Protein aggregation, a key pathological feature of AD, is a likely trigger of neuroinflammation[33,34], which is perhaps the most common trait of degenerative pathologies of the CNS (Table 4.2). Neuroinflammation has been well documented in CNS neurodegeneration including retinal dystrophies[35,36] (Table 4.2), although whether this type of immune response contributes to the initiation and progression of neurodegeneration remains unclear[33,34,37]. Studies of the retina and the brain have shown that the inflammatory

response to local neurodegeneration involves common cellular and molecular players. This response can be endogenous or peripheral in origin. Here we will focus on the endogenous inflammatory response, which is common to neurodegenerative pathologies of the retina and the brain. Macroglia and microglia/perivascular monocytes are the two main endogenous cell types that drive CNS inflammation. In the brain, the reactive macroglial cells are astrocytes, while in the retina this role is shared by Müller glial cells and astrocytes[35]. Homeostatic macroglial cells play essential roles in the development, normal function, and wellbeing of neural tissue. Astrocytes and Müller cells provide trophic support for neurons, regulate the formation and activity of synapses, and are responsible for the extracellular clearance of ions and neurotransmitters[38,39]. Macroglia are also responsible for the maintenance of the blood-brain and blood-retinal barriers. Many of these "housekeeping" functions are also carried out by microglial cell populations consisting of resident macrophages of the CNS[40]. Retinal and brain glial cell populations react to neural tissue damage or dysfunction by altering their morphology and molecular signature. In these conditions, reactive glial cells produce proinflammatory cytokines, chemokines, and complement proteins that can either exacerbate or attenuate the pathology depending on the intensity and duration of the response[34,35,37]. However, interventions that impinge upon the inflammatory response may result in contrasting outcomes depending on the intervention period, most likely due to the complex interrelated and inter-dependent pathways that mediate inflammation[41]. Moreover, the efficacy of anti-inflammatory therapies depends on the degree to which neurodegeneration has progressed, since different mechanisms predominate depending on the disease stage[37]. Further research is therefore required to elucidate the specific roles of the molecules and cells involved and to identify the most suitable therapeutic targets and windows.

The next stage of the pathological process is characterized by the damage of neurons (or other cell types) at the axonal and/or synaptic levels, potentially resulting in neuronal death. This is a common feature of neurodegeneration of both the brain and retina, and is most likely the result of protein aggregation and neuroinflammation. In summary, despite the distinct etiological bases and functional alterations of neurodegenerative conditions of the brain and retina, these diseases are underpinned by common pathological mechanisms.

Further studies of these common mechanisms are essential to better understand the underlying pathology and to aid the development of effective therapies.

4.4 GSK-3 as an Example of a Common Therapeutic Target for Neurodegenerative Conditions of the Brain and Retina

Glycogen synthase kinase 3 (GSK-3) is highly evolutionary conserved intracellular serine-threonine kinase that is expressed by almost all cells in the body. Initially identified as a glycogen synthesis enzyme (for which it is named), GSK-3 is currently considered a multitasking enzyme, for which over 100 confirmed and 500 predicted substrates have been identified. Owing to its near ubiquitous expression and broad array of substrates, GSK-3 is well positioned as a key regulator of multiple and diverse biological processes. Perhaps unsurprisingly, aberrant GSK-3 activity has been implicated in several human diseases, including neurodegenerative and psychiatric disorders[42,43]. Despite their etiological differences, inflammation is a common feature of the onset and/or progression of these pathologies. GSK-3 plays a pivotal role in regulating the balance between pro-inflammatory and anti-inflammatory cellular responses. Because GSK-3 inhibitors shift the cellular response from pro-inflammatory to anti-inflammatory, GSK-3 is considered a potential therapeutic target for diseases with an inflammatory component[44,45]. Lithium, a weak inhibitor of GSK-3, was the first GSK-3 inhibitor identified, and has been used for the treatment of mood disorders[46,47]. Since then, researchers have sought to identify and design selective GSK-3 inhibitors, given their therapeutic potential in a variety of neurodegenerative diseases. Tideglusib, a highly specific GSK-3 inhibitor, has shown a broad therapeutic safety window for the treatment of both AD and progressive supranuclear palsy in clinical trials[48], and its efficacy for the treatment of autism spectrum disorders is currently being tested (https://clinicaltrials.gov/ct2/show/NCT02586935?term=tideglusib&rank=2).

Recent findings suggest that the therapeutic potential of GSK-3 inhibitors may extend to retinal dystrophies. Because neuroinflammation is common to neurodegenerative pathologies of both the brain and retina, attenuation of inflammatory signaling may be therapeutically beneficial in both cases. Recent studies by our group, in collaboration with Dr. Ana Martinez's group (https://www.cib.csic.es/research/structural-and-chemical-

biology/translational-medicinal-and-biological-chemistry), have shown that GSK-3 inhibition has neuroprotective effects in two different *ex vivo* models of retinal pathologies (glaucoma and retinitis pigmentosa [RP]). We found that treatment with structurally diverse GSK-3 inhibitors reduced ganglion cell death caused by N-methyl-D-aspartate (NMDA) excitotoxicity in retinal explants, a model of glaucoma-related damage of retinal ganglion cells (Figure 4.3)[49]. Moreover, we showed that photoreceptor cells were preserved in retinal explants from the rd10 mouse model of RP that were treated with different GSK-3 inhibitors (Figure 4.3). Remarkably, these *in vitro* findings have been corroborated *in vivo* in rd10 mice, in which loss visual function is attenuated by inhibiting GSK-3[50].

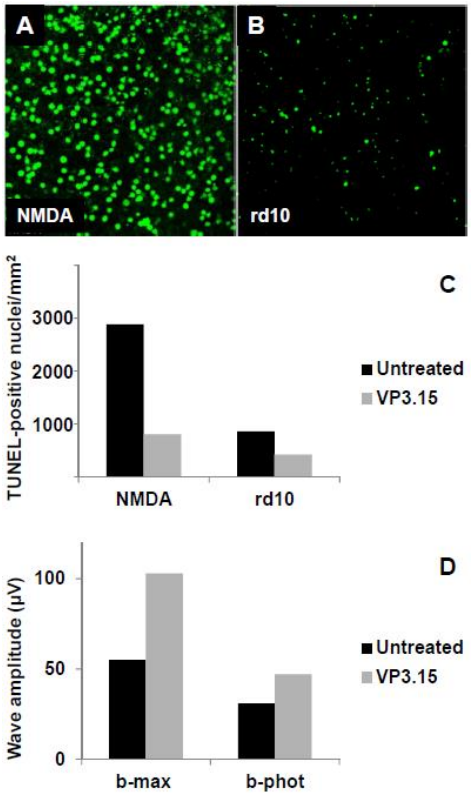

Figure 4.3 Effects of *in vitro* and *in vivo* GSK-3 inhibition on retinal neurodegeneration. A) Retinal explant from a wild-type mouse treated with NMDA (glaucoma model). B) Retinal explant from a rd10 mouse (RP model). TUNEL staining reveals ganglion and photoreceptor cell death (green nuclei in A and B, respectively). C) Treatment of retinal explants for 24 hours with the GSK-3 inhibitor VP3.15 attenuates cell death in both models. Experimental data taken from[49]. D) *In vivo* treatment with VP3.15 partially preserves visual function, as determined by electroretinography (amplitudes of two relevant waves at P32 are shown). Experimental data taken from[50].

Taken together with other published data, these findings support the clinical therapeutic potential of GSK-3 inhibitors for the treatment of retinal dystrophies. Intraperitoneal

lithium has been used to reduce intraocular pressure in a rat model of glaucoma[51], and the histone deacetylase valproic acid, a putative indirect inhibitor of GSK-3[52], provides short-term visual function benefits in RP patients[53,54]. Repositioning of drugs approved for the treatment of CNS neurodegeneration could provide therapeutic alternatives for the neurodegenerative component of retinal dystrophies. Moreover, non-invasive, quantitative analysis of retinal structure and function could facilitate the development of novel treatments with no clearly sustained effects in clinical trials directed to brain pathologies.

4.5. Proinsulin, a Candidate Drug for Neurodegenerative Conditions of the Brain and Retina

The CNS is a well-recognized site of action of insulin and insulin-like factors (IGF-I and -II)[55]. The insulin gene is transcriptionally active in tissues other than the pancreas, although at a much lower level, and local insulin gene expression has been demonstrated in the brain and retina[56-58]. Furthermore, potential autocrine/paracrine effects of insulin/proinsulin in the CNS have been suspected for some time, based on the local presence of moderate insulin/proinsulin levels that are independent of peripheral levels[56,59]. Specific functions of insulin in the brain include the regulation of food intake, body weight, reproduction, and glycemic control by the hypothalamus, and the promotion of hippocampus-dependent learning and memory[60,61]. The neuroprotective effects of insulin signaling are essential for healthy aging of the brain[62], and defective insulin signaling has been demonstrated in the AD brain[63]. These observations suggest that insulin may be a candidate therapeutic agent for AD[64]. Proinsulin was long considered a low-activity precursor of insulin owing to its poor metabolic potential (5–10% that of insulin). However, recent studies in mammals and previous studies in chicken have revealed non-metabolic actions of proinsulin, and identified a role for this factor as a bioactive signaling molecule during development[56,65,66].

In addition to its developmental role, proinsulin exhibits neuroprotective effects in mammalian models of RP, a form of retinal neurodegeneration that causes photoreceptor cell death and vision loss[67-69]. The therapeutic potential of human proinsulin (hPI) has been demonstrated in studies in which hPI levels in rodents were systemically increased. In the rd10 mouse, low-level constitutive transgenic expression of hPI in muscle delayed

photoreceptor cell death and attenuated vision loss (Figure 4.4)[67]. The potential neuroprotective effect of proinsulin has also been reported in two more clinically valid settings. In the rd10 mouse model, local administration of hPI microbeads attenuated photoreceptor cell death[69], while in the P23H rat model of autosomal dominant RP, hPI administered by intramuscular injection of adeno-associated viral vector preserved the structure and function of photoreceptors and their contacts with postsynaptic neurons[68]. Given the aforementioned similarities between neurodegenerative conditions of the retina and brain, we extended our studies to a mouse model of AD-like cognitive impairment. In line its attenuation of vision loss in RP models, systemic proinsulin treatment attenuated cognitive deficits in the SamP8 mouse model of premature senescence (Figure 4.4), an effect that correlated with decreased brain inflammaging[70].

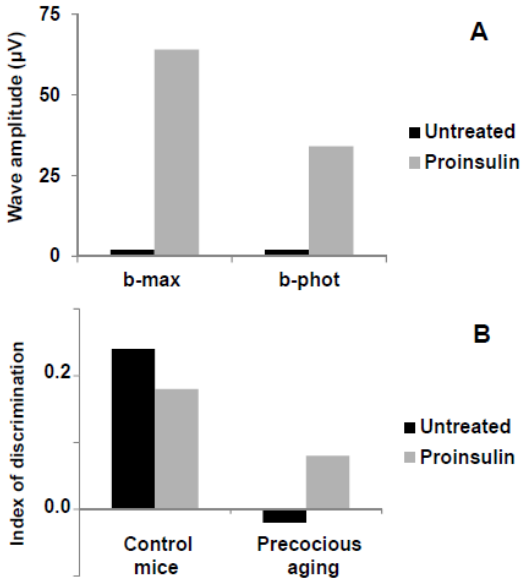

Figure 4.4 Neuroprotective effect of proinsulin in neurodegenerative conditions of the retina and brain. A) Transgenic proinsulin expression in the rd10 mouse partially attenuates vision loss, as determined by electroretinography (amplitudes of two relevant waves at P35 are shown). Experimental data taken from[67]. B) AAV-mediated proinsulin expression partially preserves cognitive function, evaluated using the discrimination of the novel object in the recognition test (0 = no discrimination), in a mouse model of precocious aging. Experimental data taken from[70].

Although the physiological relevance of proinsulin in adult mammals is yet to be determined, it constitutes a promising neuroprotective agent that warrants testing in diverse neurodegenerative conditions. Importantly, systemic proinsulin administration does not

alter body weight or blood glucose levels[67,70], two of the main adverse effects associated with systemic insulin treatment.

4.6 Conclusions

The molecular and cellular mechanisms underpinning degenerative conditions of the retina have received significantly less research attention than those of the brain, despite the striking developmental, anatomical, functional and pathophysiological parallels described in this chapter between the retina and other parts of the CNS. Further investigation of the mechanisms underlying retinal diseases will generate knowledge that could facilitate the development of new therapies and further our understanding of the shared pathological bases of brain neurodegeneration and retinal dystrophies. Unfortunately, the development and testing of a plethora of putative pharmacological treatments for AD and other neurodegenerative conditions of the brain has not rendered the expected results yet. These experimental treatments could be now applied to the treatment of retinal degeneration and, thus, the accessibility of the retina and the availability of non-invasive, quantitative techniques for retinal analysis may provide an alternative, excellent starting point for the development of putative treatment for neurodegenerative disorders that affect the CNS.

In summary, a unified view of CNS neurodegeneration that encompasses both the brain and the retina could further our understanding of neurodegeneration in general, and facilitate the development of new therapies for affected patients.

Acknowledgments

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