Published in final edited form as:

Exp Eye Res. 2011 August ; 93(2): 170–177. doi:10.1016/j.exer.2010.09.006.

Endothelin, Astrocytes and Glaucoma

Ganesh Prasanna^{1,3}, Raghu Krishnamoorthy^{1,2}, and Thomas Yorio¹

¹ Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, 3500 Camp Bowie Blvd, Fort Worth, TX 76107.

²Department of Cell Biology and Anatomy, University of North Texas Health Science Center, 3500 Camp Bowie Blvd, Fort Worth, TX 76107.

Abstract

It has become increasingly clear that astrocytes may play an important role in the genesis of glaucoma. Astrogliosis occurs in response to ocular stress or the presence of noxious stimuli. Agents that appear to stimulate reactive gliosis are becoming increasingly clear. One class of agents that is emerging is the endothelins (ETs; specifically, ET-1). In this review we examine the interactions of ET-1 with astrocytes and provide examples where ET-1 appears to contribute to activation of astrocytes and play a role in the neurodegenerative effects that accompany such reactivation resulting in astrogliosis. These actions are presented in the context of glaucoma although information is also presented with respect to ET-1's role in the central nervous system and brain. While much has been learned with respect to ET-1/astrocyte interactions, there are still a number of questions concerning the potential therapeutic implications of these findings. Hopefully this review will stimulate others to examine this potential.

I. Role of astrocytes in neural tissues

Astrocytes represent a major component of the glial cell type in the central nervous system. In addition to brain and spinal cord, astrocytes are also present both in the retina and optic nerve, each with distinct regional and functional differences. In addition to their role as neuronal supportive cells by regulating ionic balance, metabolic supply and structural support, astrocytes also perform other critical functions including regulation of neurotransmission, microvascular blood flow to the brain and synaptic plasticity (Halassa et al., 2007; Mulligan and MacVicar, 2004). Briefly, type I astrocytes express GFAP and connexin-43 whereas type II astrocytes express GFAP and A₂B₂ antibody recognizing protein but not connexin-43 (Miller et al., 1989; Radany et al., 1992; Belliveau et al., 1994). In the eye, astrocytes (mainly type I) are present both in the inner retina (at the ganglion cell layer and nerve fiber layer) as well as in the unmyelinated optic nerve head and characteristically express glial fibrillary acidic protein (GFAP). Type I astrocytes can be further subdivided into Type Ia and Ib based on location as well as GFAP and neural cell adhesion molecule (NCAM) expression (Hernandez, 2000). Type Ia are GFAP⁺/NCAM[−] and are interspersed in glial columns forming the edges around cribriform plates whereas Type Ib are GFAP⁺/NCAM⁺ and are closer to the vitreal surface of optic disc forming glial

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Corresponding Author: Thomas Yorio, PhD Professor of Pharmacology & Neuroscience UNT Health Science Center 3500 Camp Bowie Blvd. Fort Worth, Texas 76107.

³Current Affiliation: Alcon Research, Ltd. 6201 South Freeway, Fort Worth Texas 76134.

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columns and cribriform plate as well as surrounding blood vessels in the prelaminar region (Hernandez, 2000). Under conditions of neurotrauma or chronic disease, quiescent astrocytes transform into a state of reactivation.

Ocular astrocytes (retinal type Ib and optic nerve head type Ia) are thought to contribute to both homeostatic functions of the retinal ganglion cells (RGCs) and optic nerve as well directly contributing to the pathophysiology resulting in damage to both ganglion cells and optic nerve. The readers are referred to an excellent review on role of astrocytes and microglia in glaucoma by Johnson and Morrison (2009). Several factors are implicated in astroglial activation and optic nerve degeneration pertaining to glaucoma including but not limited to peptides like endothelin-1 (ET-1) (Prasanna et al., 2002; MacCumber et al., 1990; Baba, 1998), cytokines like TNF- α and the TGF- β superfamily (including bone morphogenic proteins) (Yuan and Neufeld, 2000; Pena et al., 1999; Zode et al., 2007; Lutjen-Drecoll, 2005), oxidative stress and advanced glycation end products (AGE) (Tezel et al., 2007), and trophic factors (including GDNF, NT4/5 and Trk receptors) (Wordinger et al., 2003).

Astrogliosis: Characterization and morphological changes

The reactive state of astrocytes or astrogliosis is usually defined based on morphological criteria including thickened astrocytic processes and enlarged soma. However, it is becoming evident that the orientation of astrocytic processes in response to a particular stimulus may dictate the reversibility of astrogliosis and is dependent on how acute or chronic the stimulus is and how focal or global the insult occurs (Escartin and Bonavent, 2008). Specifically, astrogliosis resulting from the local disruption of brain parenchyma would promote an extension of astroglial processes along a linear plane demarcating the lesioned area forming an impermeable and irreversible glial scar which can be defined as *anisomorphic gliosis* (Kalman, 2004). Whereas with a less focal injury as seen in chemical lesions and in chronic diseases, ensuing astrogliosis can be defined as *isomorphic gliosis*, which may be reversible (Escartin and Bonavent, 2008).

Consequently, depending on the type of insult, region of neural tissue affected and duration post-insult, reactive astrocytes can serve either in neuroprotective or neurodestructive roles. Typical changes during astrogliosis could involve an increase in astrocyte number (hyperplasia/proliferation), increase in number and length of astroglial processes and cell body size (hypertrophy) and migration of astroglia. Astroglial hypertrophy can typically be assessed by visualizing astrocyte morphology and assessing the number and length of astrocytic processes using Lucifer Yellow dye filling and also by immunolabeling neural tissues for determining the over-expression of intermediate filaments, GFAP and vimentin, which are hallmarks of astrogliosis that occurs following neurotrauma and neurodegenerative diseases including glaucoma (Li et al., 2008; Liu et al., 2006; Hernandez et al., 2008).

Astrocytes in glaucoma: Proliferation, hypertrophy or both?

Astrocytes in glaucomatous optic nerve heads also express increased levels of GFAP and vimentin in addition to possessing rounded cell bodies with thicker processes (Hernandez, 2000). Whether the glaucomatous optic nerve head exhibits a typical isomorphic or anisomorphic astrogliosis remains to be clearly defined. Astroglial proliferation, specifically in prelaminar and retrolaminar/pars scleralis regions has been observed in early and moderate stages of glaucoma (Minckler and Spaeth, 1981). Increased immunolabeling for synuclein, a protein associated with mitotic regulation has been detected in human glaucomatous optic nerve heads (Surgucheva et al., 2002), suggestive of astroglial proliferation in response to glaucomatous insults. Additionally, astrocytes in glaucomatous

optic nerve head appear to be migrating towards or present within nerve bundles compared to the orderly and stratified elongated astrocytic processes that appear to surround nerve bundles in normal optic nerve head tissues (Hernandez et al., 2008).

It is also unclear during various stages of glaucoma whether both retinal and optic nerve head astrocytes (ONAs) undergo proliferation and/or hypertrophy. In rodent models of glaucoma, Inman and Horner (2007) observe no astroglial proliferation in the retina of DBA/2 J murine model of glaucoma whereas Johnson et al., (2000) have demonstrated that during early stages of IOP elevation in Morrison rat model of glaucoma, ONAs undergo proliferation. However the following sections emphasize the interactions between astrocytes and endothelins (specifically, ET-1) from the perspectives of proliferation, hypertrophy and astrogliosis which can ultimately contribute to optic nerve damage as observed in glaucoma (Yorio et al., 2002).

II. Endothelin System in Astrocytes

Astrocytes, originally thought to be inert structural cells in the central nervous system, have now emerged as important players in neurodegeneration (Rossi and Volterra, 2009). Abnormal activation and/or proliferation of astrocytes termed astrogliosis has been observed in various neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, AIDS dementia, traumatic brain injury and glaucoma (Rossi and Volterra, 2009; Hirsch et al., 2003; Hernandez et al., 2008). During astrogliosis, there are changes in morphology and function of astrocytes in response to stress and noxious stimuli. Reactive astrocytes upregulate various intermediate filament proteins including, GFAP, nestin and vimentin (Eng and Ghirnikar, 1994; Frisen et al. 1995). At the site of injury, astrocytes undergo hypertrophy and proliferate forming a glial scar which limits further damage, but also acts as a barrier to regeneration. Reactive gliosis is also characterized by increased glutamate efflux from astrocytes which could potentiate excitotoxic cell death of neurons. Several endogenous agents have been implicated as possible mediators of astrogliosis, including endothelins. Endothelins play a key role in many neurodegenerative diseases in which reactive gliosis occurs including Alzheimer's disease, traumatic brain injury, and glaucoma (Zhang et al., 1994; Jiang et al., 1995; Yorio et al., 2002).

Endothelins are a family of vasoactive peptides which exist as three isoforms, endothelin-1 (ET-1) endothelin-2 (ET-2) and endothelin-3 (ET-3) each of which is encoded by distinct genes. Originally isolated from porcine endothelial cells (Yanagisawa et al., 1988), ET-1 has been shown to be expressed in a wide variety of cell types including kidney, lung, gastrointestinal tract, lung, liver and brain (Rubanyi and Polokoff, 1994). The precise role endothelins play in the central nervous system is not clear; it is however clear that ET-1 is expressed in various parts of the brain including paraventricular and supraoptic nuclei in the hypothalamus, cerebellum, motor neurons in the spinal cord and in peripheral ganglia. Expression in these key areas of the brain suggests that ET-1 has a neuromodulatory role in the central nervous system. In the rat eye, ET-1 expression was first observed by MacCumber et al. (1989) in various tissues including, iris, ciliary body, and choroid. Using *in situ* hybridization and immunohistochemical techniques, ET-1 expression was found in many regions of the human retina including the inner plexiform layer, RPE, photoreceptor inner segment, nerve fiber layer, ganglion layer and astrocytes (Ripodas et al., 2001). The expression pattern suggests the possibility that endothelins could function as a neuropeptide in the eye to modulate neurotransmitter function in the vertical pathway of the retina.

ET-1 acts by binding to two main classes of receptors, ET_A and ET_B receptors which are G protein coupled receptors belonging to the rhodopsin superfamily. In many instances, endothelin receptors are found in tissues that are in close proximity to the site of ET-1

synthesis and release, suggesting autocrine or paracrine effects of the peptide. While the ET_A receptor has been associated with many of the physiological actions of endothelins, the ET_B receptor appears to serve as a clearance receptor to regulate the concentrations of ETs in the extracellular compartments (Bohm et al., 2003). However, activation and over expression of the ET_B receptor can also lead to cell signaling, and when this action is persistent it can lead to either beneficial or detrimental effects in the associated tissue, depending on the nature and duration of stimuli.

While the role of endothelins in 'normal' retinal and brain physiology is not completely understood, a number of observations suggest that endothelins may be involved in astrocyte activation in disease states. Brain astrocytes express several various endothelins including ET-1, ET-3, as well as endothelin converting enzyme (ECE) and the endothelin receptors (ET_A and ET_B receptors) (Ehrenreich et al., 1991; MacCumber et al., 1990, Koyama et al., 1993, Lazarini et al., 1996). In the "resting state" astrocytes express low levels of endothelin components, however, following ischemia, neurotrauma or pathological conditions endothelins and their receptor expression are greatly enhanced (Jiang et al., 1993; Nie and Olsson, 1996). Transient global ischemia for 1 to 2 weeks in the rat hippocampus produced a significant increase in endothelin immunoreactivity along with increased astrocytic proliferation (Gajkowska, 1997). A number of observations link ET_B receptor activation to proliferative effects of ET-1 in brain astrocytes (Baba 1998; Ishikawa et al., 1997, Koyama et al., 1993; Lazarini et al., 1996). Apart from brain astrocytes, ET-1 has also been shown to promote proliferation of optic nerve head astrocytes, but this is mediated through the involvement of both ET_A and ET_B receptors (Prasanna et al. 2002, Murphy et al., 2010). In a rodent model of optic neuropathy using ET-1 perfusion to retrobulbar optic nerve, ET_B expression was higher in the ET-1 treated eye, compared to the untreated control eye. The authors observed strong colocalization of ET_B and GFAP in the ET-1 treated optic nerve, compared to control nerves (Wang et al., 2009), suggestive of reactive astrocytes. The authors reported stronger ET_B expression in the astrocyte soma and proximal processes, while GFAP was expressed more strongly in the distal processes. Lastly, ECE-like activity has been observed in bovine optic nerve, indicating that local ET-1 production can possibly affect neural, glial and microvascular tissues as well as their interactions (Dibas et al., 2005).

III. Factors influencing ET-1 synthesis and release

IOP

Intraocular pressure (IOP) is the most well characterized risk factor in primary open angle glaucoma and has been tightly correlated to optic nerve damage and optic disc cupping. ET-1 concentrations have been shown to be elevated both in patients and in animal models of ocular hypertension. For instance, ET-1 concentrations in aqueous humor of primary open angle glaucoma patients were found to be higher than those of normal age-matched control subjects (Lepple-Wienhues et al., 1992). While the clinical relevance of this finding is not completely clear it appears plausible that ET-1 in aqueous humor could be secreted in response to an increase in IOP. For instance, ET-1 levels were found to be 4-fold higher in the aqueous humor of the congenital elevated IOP canine model of glaucoma (Kallberg et al., 2002). In the Morrison's elevated IOP model, Prasanna et al. (2005) reported a 2- to 2.5-fold increase in aqueous humor ET-1 concentrations in rats subjected to elevated IOP, compared to their respective controls. Thanos and Naskar (2004) reported an increase in ET-1 mRNA expression in retinas of rats with elevated IOP. Apart from this, in clinical studies, patients with normal tension glaucoma were shown to have significantly higher (35 to 70%) concentrations of ET-1 in their circulation in comparison with normal subjects (Sugiyama et al., 1995; Cellini et al., 1997).

It is possible that mechanical transduction could play a role in ET-1 induction, although the detailed mechanisms are still unclear (Ostrow and Sachs, 2005). For example in porcine aortic endothelial cells, ET-1 synthesis was up-regulated nearly eightfold upon being subjected to cyclic stretch (Cattaruzza et al., 2000). On the other hand, in stretched rat aortic smooth muscle both ET-1 and ET_A receptor mRNA expression cells declined, whereas ET_B mRNA levels were increased nearly 10-fold. ET-1 (10 nM) treatment produced apoptotic changes in the stretched rat aortic smooth muscle cells, through its actions on the ET_B receptor.

Hypoxia

Even though endothelins have been shown to be upregulated in the brain following ischemia and brain trauma, the mechanisms underlying these effects are not completely understood. Hypoxia promotes ET-1 expression through hypoxia-inducible factor-1 (HIF-1) which has a binding site in the upstream promoter region of the ET-1 gene. Yamashita et al (2001) demonstrated that AP-1, GATA-2 and NF-1 play a role in stabilizing the binding of HIF-1 and recruitment of p300/CBP to the regulatory elements of the ET-1 gene. Tezel and Wax (2004) found increased immunostaining for HIF-1 alpha in the retina and optic nerve head of glaucomatous donor eyes compared with the control eyes. It is possible that activation of HIF-1 could mediate ET-1 expression in glaucomatous retina and optic nerve head. Apart from ischemia, other factors including thrombin, TGF- β 2 and TNF- α have been shown to regulate ET-1 secretion. Interestingly however, hypoxic condition alone does not cause an increase in ET-1 synthesis and release from optic nerve head astrocytes however, it significantly elevates TNF- α -mediated ET-1 release (Desai et al., 2004).

Tumor necrosis factor- α

Tumor necrosis factor- α (TNF- α) is a cytokine produced mainly by macrophages, but also by a variety of cell types including glial cells. In recent years, TNF- α has gained attention for its role in various neurodegenerative disorders including Alzheimer's disease, Parkinson's disease and glaucoma (Tansy et al., 2008; Tezel, 2008; Rossi and Volterra, 2009). By immunohistochemical techniques, Tezel et al., (2001) found increased immunostaining for TNF- α or its receptor in retina sections of glaucomatous eyes than in control eyes of age-matched donors. Using immunofluorescence the authors demonstrated that retinal immunostaining for TNF- α was mainly in glial cells (colocalization with GFAP-positive Muller cells and astrocytes), whereas immunostaining for TNF- α receptor-1 was observed mainly in the retinal ganglion cells. This suggests a direct effect of glial TNF- α on retinal ganglion cells (RGCs).

TNF- α treatment has been shown to produce ET-1 release from human non-pigmented ciliary epithelial cells and optic nerve head astrocytes (Prasanna et al., 1998; Prasanna et al., 2002; Desai et al., 2004). Narayan et al. (2004) demonstrated increased ET-1 mRNA expression and peptide secretion after treatment of cultured retinal pigmented epithelium with 10nM TNF- α . These observations suggests that TNF- α could have indirect effects on neurodegeneration which are mediated through ET-1. Neurodegeneration and astrogliosis could be mediated through a variety of stimuli, including TNF α and ET-1. Additionally, ET-1 has been demonstrated to cause RGC death and disruption of axonal transport in the optic nerve supportive of a direct role for ET-1 and glaucomatous neurodegeneration (Chauhan et al., 2004; Stokely et al., 2002; Wang et al., 2008; Krishnamoorthy et al., 2008).

IV. Proliferation of astrocytes – role for ET-1

There have been several studies dating back to the 1990s right after the discovery of ETs indicating that ET-1 in addition to being a potent vasoregulator (i.e. both vasoconstriction

and vasorelaxation) was also a potent mitogenic agent for different cell types including smooth muscle and astrocytes (MacCumber et al., 1990; Baba 1998). Several papers have also described a putative role for ETs in exacerbation of various neuropathologies and neurotrauma via direct actions on neuronal tissues or by altering the blood-brain barrier (e.g. hypoxia, vasospasms, etc.) as well as those actions involving astrogliosis (Nie and Olsson, 1996; Rogers et al., 1997; Baba, 1998; Schinelli, 2006; Yorio et al., 2002). Specifically, ET-1 being a potent vasoconstrictor and mitogen is considered to play a key role in regulating vascular, neuronal and astroglial interactions. Therefore in glaucomatous optic neuropathy, the establishment of a true cause and effect relationship remains unclear with regards to whether ET-1's actions are mediated via astrogliosis. ET-1 can also promote neurodegeneration via direct actions on RGC apoptosis and disruption of axonal transport in optic nerve (Krishnamoorthy et al., 2008; Chauhan et al., 2004; Stokely et al., 2002).

Endothelin and astrocyte proliferation

There have been several seminal reports describing proliferation of astrocytes following exposure to ET-1 either via autocrine actions or exposure to exogenous ET-1 (Lazarini et al., 1996; Rogers et al., 1997; Teixeira et al., 2000; Prasanna et al., 2002; Desai et al., 2004; Gadea et al., 2008; Herrero-Gonzalez et al., 2009; Murphy et al., 2010). These mitogenic effects of ET-1 on astrocytes are observed in murine, rat and human astrocytes isolated from cortical and forebrain tissues, and from optic nerve head. The mitogenic response to ET-1 treatment for astrocytes isolated from different locations has been measured using various end points including expression of Ki-67, a protein which becomes expressed in all active phases of the cell cycle (G1, S, G2, or M) but not in resting cells (Gadea et al., 2008; Herrero-Gonzalez et al., 2009), incorporation of bromodeoxyuridine (BrdU), formazan MTT assay (Gadea et al., 2008; Prasanna et al., 2002; Desai et al., 2004; Murphy et al., 2010), and [³H] thymidine incorporation (Prasanna et al., 2002). In these *in-vitro* studies, depending on the dose and type of astrocytes, ET-1-induced mitogenic response ranged from 25-100% over a negative control (vehicle-treatment) over a period of 24-96 hours post-treatment.

ET-1 induced proliferation of human optic nerve head astrocytes (hONAs) can be further increased in the presence of hypoxia when compared to that observed under normoxic conditions (Desai et al., 2004). This finding suggests that astrocytes, including hONAs, are exposed to multiple proliferative stimuli including ET-1, hypoxia and TNF- α , which can converge upon signaling pathways including ERK1/2, JNK, and p38 MAPK that ultimately activate cyclin dependent kinases (CDKs) and promote mitogenesis (Gadea et al., 2008; Desai et al., 2004). Recently, Wang et al., (2008) have demonstrated that following middle cerebral artery occlusion (MCAO) in rats, astrocyte hypertrophy and proliferation as well as distal occurrence of hippocampal neuronal death occurred in a sequential manner which was inhibited by a CDK inhibitor, olomoucine. Whether ET-1 or other mitogens for ONAs are contributing to astroglial proliferation in an *in vivo* glaucoma model or in human optic nerves needs further examination

Role of connexin-43 in astroglial proliferation and effect of ET-1

Connexin-43 is a major gap junction protein that in addition to regulating ionic balance by forming a syncytium also plays a key role in cell proliferation in astrocytes (Herrero-Gonzalez et al., 2009). There is evidence to indicate that ET-1-induced astrocyte proliferation is directly dependent on down-regulation of connexin-43, which appears to be mediated via ET_B receptor activation (Rozyczka et al., 2005; Blomstrand and Giaume, 2006; Herrero-Gonzalez et al., 2009). It is of interest to note that hONAs have a reduction in gap junctional intercellular communication (GJIC) and redistribution of connexin-43 under conditions of increased hydrostatic pressure to mimic elevated IOP in glaucoma (Malone et al., 2007). Such mechanosensation-mediated alteration in ion channel activation and

connexin re-distribution can cause changes in astrocyte shape due to cytoskeletal remodeling (Ostrow and Sachs, 2005). In glaucomatous monkey optic nerve head tissues, connexin-43 in astrocytes is mainly localized intracellularly whereas in normal optic nerve head tissues connexin-43 is present on astrocyte cell membranes (Hernandez et al., 2008). In the Morrison rat model of glaucoma, there is a gradual reduction in connexin-43 expression in optic nerve head tissue with increase exposure to IOP elevation from 3 days to 7 days, indicative of disruption of the astrocytic syncytium (Johnson et al., 2000). Interestingly, in the same glaucoma model (Morrison), there was an increased expression of immunoreactive ET-1 and ET_B both of which colocalized with GFAP in the rat optic nerve head exposed to elevated IOP (Prasanna et al., 2005). The overall consequence of reduced or altered GJIC in astrocytes as observed following exposure to elevated IOP in glaucoma could result in the disruption of homeostatic function of astrocyte-neuron interactions, which may lead to axon degeneration.

Whether or not ET-1-mediated ONA proliferation also involves reduction in connexin-43 expression and consequently results in axon loss in glaucoma remains to be determined. Connexin-43 immunoreactivity has been recently detected in human retinal (mainly nerve fiber layer and ganglion cell layer) and optic nerve tissues predominantly associated with GFAP-positive astrocytes (Kerr et al., 2010). Lastly, there appears to be a greater association of connexin-43 loss with astrocyte proliferation than with astrocyte hypertrophy. If ONA proliferation is occurring in glaucoma, it is perhaps an early event in the disease continuum which may also participate and/or support astroglial migration that also occurs in response to elevated IOP. In support of this concept are data from the Morrison rat model of glaucoma wherein, immunolabeling for PCNA, a specific marker of cell proliferation appears to co-localize with GFAP (indicative of ONAs) that follows a temporal response following 3 days and 13 days of IOP elevation but not later (Johnson et al., 2000). These findings indicate that proliferation is an early event followed by migration, extracellular matrix remodeling, etc. in the optic nerve head (Johnson et al., 2000). Therefore, do the mitogenic factors like TNF- α and ET-1 contribute to the early proliferation of ONA in glaucoma either in response to IOP elevation or directly in parallel with IOP elevation? It is of interest to note that modulation of connexin-43 levels via various methods have resulted in decreased neuronal cell death following ischemia, neurotrauma, etc. (Kerr et al., 2010; Cronin et al., 2008; Frantseva et al., 2002a; 2002b).

Another speculation regarding astroglial proliferation comes from a recent finding by Buffo et al (2008) that some adult astrocytes in mice cerebral cortex can serve as a source for proliferating reactive astrocytes in response to neurotrauma. Additionally, Castañeda et al., (2010) have determined that ET-1 and ET_B receptors might be associated with regulation of adult neural stem cells and their migration through neurogenic and gliogenic pathways since endothelinergic components are highly expressed in following neurotrauma in adult murine subependymal zone. These adult stem cells express elevated levels of GFAP, incorporate BrdU and are also positive for elevated levels of immunoreactive ET-1 and ET_B receptor expression (Castañeda et al., 2010). Whether a similar population could exist in the optic nerve and respond similarly to glaucomatous insults and mitogen exposure is not known. Thus one could hypothesize that the dual actions of neuroprotective versus neurodegenerative actions of astroglial proliferation could also depend on two different situations; one involving reactive ONAs experiencing glaucomatous insults (IOP elevation, mitogens like ET-1, hypoxia, cytokines, etc.) that cause neurodegeneration while similar glaucomatous insults initiates a different pool of precursor or stem cells causing them to differentiate and proliferate into adult neural or astroglial cells.

V. Astroglial hypertrophy and endothelin receptors: CNS disorders and glaucoma

Astroglial hypertrophy and role of intermediate filaments

Very little is known about ET-1's role in astroglial hypertrophy and reactive gliosis in the glaucomatous optic nerve. However, information from different CNS models may be suggestive of conditions in glaucomatous optic neuropathy. Therefore, from the perspective of astroglial hypertrophy and activation in CNS, Wilhelmsson et al (2004) have observed that an entorhinal cortex lesion (ECL) in wild-type (WT) mice resulted in considerable neuronal degeneration accompanied by astroglial activation and ET_B receptor upregulation in the hippocampus, which typically receives axon projections from the entorhinal cortex. In contrast, in mice with intermediate filament knockout i.e. GFAP^{-/-}/vimentin^{-/-} (GFAP^{-/-}/Vim^{-/-}), ECL resulted in hippocampal astroglia having shorter processes, reduced ET_B receptor expression on astrocytes and a biphasic response to synaptic regeneration. Acute effects (at 4 days post-lesion) including loss of synapses in GFAP^{-/-}/Vim^{-/-} mice when compared to WT mice and 14 days post-ECL, a significant and complete restoration of neuronal synapses and reduced hypertrophy of astrocytes was observed in GFAP^{-/-}/Vim^{-/-} mice. These findings imply that ET_B receptor expression and distribution may be controlled by expression of GFAP and vimentin and that astrogliosis may fundamentally govern inhibition of neuroregeneration (Wilhelmsson et al., 2004). In a more recent report, Li et al., (2008) performed distal middle cerebral artery transection in WT and GFAP^{-/-}/Vim^{-/-} mice which resulted in *larger* infarct size in GFAP^{-/-}/Vim^{-/-} mice after 7 days post-ischemia compared to that seen in WT mice and that ET_B receptor distribution was restricted only to the nucleus in GFAP^{-/-}/Vim^{-/-} mice. It therefore appears that intermediate filaments like GFAP and vimentin are responsible for production, stability and distribution of ET_B receptors in reactive astrocytes (Li et al., 2008). These 2 examples provide evidence for both neurodegenerative and neuroprotective roles for reactive gliosis likely involving ET-1 and ET_B receptors. The presence of short astrocyte processes in GFAP^{-/-}/Vim^{-/-} mice is in contrast to the long hypertrophied astrocytes processes present in WT mice that usually control several neuro-astroglial homeostatic functions including spatial buffering, removal of neurotransmitters, repairing blood-brain barrier, glial scar formation etc. (Wilhelmsson et al., 2004).

In the context of glaucoma, it would be quite interesting to determine the impact of elevated IOP in GFAP^{-/-}/Vim^{-/-} mice from the perspectives of retinal ganglion cell (RGC) damage, astrogliosis, optic nerve damage and effect on the ET-1 system. Additionally since both ET-1 and ET_B receptors appear to be elevated in glaucomatous optic nerve tissues in addition to GFAP expression (Wang et al., 2009; Wang et al., 2006; Prasanna et al., 2005), it is possible that temporal changes in ONA function (i.e. neuroprotective to neurodestructive or vice-versa) could occur similar to above CNS findings. There is a report by Inman and Horner (2007) to indicate that *retinal* astrocytes only undergo hypertrophy without exhibiting proliferation in a DBA/2J mouse model of pigmentary dispersion glaucoma. These authors however did not evaluate astroglial changes in the optic nerve head in the DBA/2J mice. Whether *retinal* astrocytes behave differently (i.e. hypertrophy only) from their optic nerve head counterparts in that only ONAs exhibit both proliferation and hypertrophy in response to glaucoma stimuli remains to be clearly defined in humans, although findings in the Morrison rat model of glaucoma do indicate ONA proliferation occurring early following IOP elevation (Johnson et al., 2000). Additionally in hONAs, ET-1 has also been demonstrated to cause extensive changes to ECM including expression of fibronectin and changes to matrix metalloproteinases (MMP-2) and inhibitors of MMPs (TIMP-1 and -2) (He et al., 2007). Changes to ECM are another hallmark of hypertrophied astrocytes in glaucoma (Hernandez et al., 2008).

Recently Lam et al., (2009) have demonstrated in non-human primates subjected to unilateral ocular hypertension (OHT) that robust astroglial activation is observed in lateral geniculate nucleus (LGN) and V1 region of visual cortex following OHT for 2-8 weeks and these changes were accompanied by significant loss of metabolic activity. Therefore, another area worth investigating is whether glial changes occurring in glaucomatous brain tissue (specifically LGN and visual cortex) in addition to those occurring in retina and ONH are also regulated by ET-1/ET_B systems and whether these changes result in functional neural deficits.

ET_B receptor involvement in astrogliosis

Typically, hypertrophied astrocytes exhibit upregulated ET_B receptor expression in many *in vivo* models of neurodegeneration including glaucoma (Prasanna et al., 2005; Wang et al., 2009), spinal cord injury (Peters et al., 2003), CNS lesion (Wilhelmsson et al., 2004) and brain ischemia (Li et al., 2008). It has been previously suggested that blockade of ET_B receptor may be a useful strategy to blunt formation of glial scarring after neurotrauma (Peters et al., 2003) and optic nerve damage in response to glaucoma and IOP elevation (Prasanna et al., 2005). It is relevant to point out that glial scarring is minimized in GFAP^{-/-}/Vim^{-/-} mice subjected to neurotrauma (Pekny et al., 1999). ET_B receptor has also been implicated in regulating ET-1-induced cortical astrocyte proliferation since BQ-788, an ET_B receptor antagonist but not BQ-123, an ET_A receptor antagonist, blunted ET-1's effects (Gadea et al., 2008). In case of hONAs, both ET_A and ET_B receptors appear to participate in regulating cell proliferation (Prasanna et al., 2002). An interesting finding was made in a rat model of glaucoma (laser-induced unilateral IOP elevation) wherein a time-dependent increase in ET_B receptor (by 2-6 fold) and ET-2 (by 1-2 fold) mRNA expression was observed in retinal tissues along with TNF superfamily member 1A and genes associated with glial activation (Yang et al., 2007). Elevated expression of ET_B receptor mRNA was by far the highest among the 20 genes analyzed in detail by Yang et al. (2007) and furthermore in the same study, none of the aforementioned ET-related and TNF mRNA changes were detected in the rat optic nerve transection model of glaucoma. It is relevant to note that Rattner and Nathans (2005) have observed an upregulation of mRNA expression of ET-2 and ET_B receptor (>10-fold) in Muller glia following 24 hour light-induced retinal degeneration. In case of either ET-1-induced optic neuropathy or elevated IOP exposure in rats or primates and in human glaucomatous eyes, ET_B receptor is over expressed in the optic nerve head tissues often co-localizing with GFAP indicative of reactive astroglia (Wang et al., 2009; Wang et al., 2006; Prasanna et al., 2005).

Furthermore, in human optic nerve head tissues, ET_B/GFAP co-localization was observed only in the astrocytic processes but not in the cell body (Wang et al., 2009). This is interesting because ET_B present on astrocytic processes provides the possibility that ET_B-mediated signaling may lead to downregulation of connexin-43 thereby potentially perturbing astroglial-axon and intercellular astroglial communication, resulting in astroglial proliferation and/or hypertrophy in glaucoma. On the other hand, continuous intracerebroventricular injection of an ET_B agonist, Ala^{1,3,11,15}-ET-1 (500 pmol/day for 7 days) in rats caused an increase in the numbers of GFAP- and vimentin-positive astrocytes in the hippocampus, caudate putamen and cerebrum, which did not induce both neuronal degeneration and activation of microglia/macrophage in these brain regions (Koyama et al., 2003). From the perspective of retinopathies including rat models of retinal degeneration, diabetic retinopathy and proliferative vitreoretinopathy, treatment with bosentan or tezosentan, two mixed ET_{A/B} receptor antagonists, prevented neuronal apoptosis, reduced both retinal microvascular basement membrane thickening and retinal gliosis (Torbidoni et al., 2006; Evans et al., 2000; Iribarne et al., 2008).

In the context of glaucoma therefore, whether a) there is a neuroprotective role for ET_B receptor activation early in the process of astrogliosis remains to be determined and b) a putative early ET_B-induced neuroprotective role ultimately turns neurodestructive in response to continuous exposure to glaucomatous stimuli also needs to be determined. This again reiterates the need to better understand the relationship of ET-1, ET receptors, astrogliosis in the optic nerve head and axon degeneration in the disease continuum of glaucoma.

V. Conclusions

In summary, there are several interesting questions that need to be addressed to further determine the relationship between ET, astrogliosis, and glaucoma. These are: Do optic nerve head astrocytes (ONA) proliferate in human glaucoma? If so, when does this occur in the disease continuum? Can the astrogliosis in glaucomatous optic nerve heads be classified as isomorphic or anisomorphic gliosis? What is the relationship of ET-1 in ONA proliferation in the glaucomatous human optic nerve? Does ET_B receptor-mediated astrogliosis result in a biphasic effect on glaucomatous optic nerve, an early neuroprotective effect followed by a chronic neurodestructive effect? Is there a role for connexin-43 and ET-1 in causing and exacerbating the glaucomatous ONA proliferation and/or hypertrophy? What is the consequence of blocking ET_B receptors on glaucomatous ONA proliferation, astrogliosis and neurodegeneration? Can ET-receptor antagonists be effective in reducing the neurodegeneration that accompanies glaucoma? How early would we need to identify glaucoma patients for this approach to be effective? Do glial changes in LGN and visual cortex in glaucomatous brain tissue involve ET-1/ET_B system? While these questions certainly need to be addressed there is a building set of experimental data that suggests endothelins are candidate agents that could contribute to the detrimental neuronal effects seen in glaucoma and drugs that block their action should be considered as target agents for future drug development.

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Figure 1. Astrocytes and Endothelin Interactions

This figure depicts the effects of various inputs (increased IOP, ischemia, TNF α) on activation of astrocytes and the role ET-1 and ETA and ETB receptors might play in axonal loss and retinal ganglion cell apoptosis.