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REVIEW

A role for aquaporin-4 in fluid regulation in the inner retina

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Abstract

Many diverse retinal disorders are characterized by retinal edema; yet, little experimental attention has been given to understanding the fundamental mechanisms underlying and contributing to these fluid-based disorders. Water transport in and out of cells is achieved by specialized membrane channels, with most rapid water transport regulated by transmembrane water channels known as aquaporins (AQPs). The predominant AQP in the mammalian retina is AQP4, which is expressed on the Müller glial cells. Müller cells have previously been shown to modulate neuronal activity by modifying the concentrations of ions, neurotransmitters, and other neuroactive substances within the extracellular space between the inner and the outer limiting membrane. In doing so, Müller cells maintain extracellular homeostasis, especially with regard to the spatial buffering of extracellular potassium (K^+) *via* inward rectifying K^+ channels (Kir channels). Recent studies of water transport and the spatial buffering of K^+ through glial cells have highlighted the involvement of both AQP4 and Kir channels in regulating the extracellular environment in the brain and retina. As both glial functions are associated with neuronal activation, controversy exists in the literature as to whether the relationship is functionally dependent. It is argued in this review that as AQP4 channels are likely to be the conduit for facilitating fluid homeostasis in the inner retina during light activation, AQP4 channels are also likely to play a consequent role in the regulation of ocular volume and growth. Recent research has already shown that the level of AQP4 expression is associated with environmentally driven manipulations of light activity on the retina and the development of myopia.

Keywords: AQP4, Kir4.1, Müller cells, Fluid homeostasis, Retina

Overview of ocular fluid homeostasis

Exquisite regulation of ocular fluids is necessary for optimization of visual function in vertebrates. Such functional optimization requires continuous movement of fluid between ocular compartments, as well as between these same compartments and the systemic circulation. In turn, such fluid movement facilitates removal of the many metabolic products of retinal, ciliary body, lens, and corneal metabolism (Moseley et al., 1984) while maintaining transparency in the optical compartments. However, the underlying mechanisms of ocular fluid regulation are still the focus of debate, with the roles of some components remaining to be clarified (Bishop et al., 2002; To et al., 2002; Candia & Alvarez, 2008).

What is known is that ocular fluid homeostasis is largely mediated *via* epithelia as is fluid regulation in other parts of the body (Hamann, 2002; Civan & Macknight, 2004). Fluid regulation and associated fluid movement across epithelia and cell membranes are achieved passively through diffusion or actively by the coupling of water movement to ion and metabolite transporters such as the sodium, potassium, and chloride cotransporter (NKCC1) (Adorante & Miller, 1990; Hamann et al., 2005) and monocarboxylate transporters (Zeuthen et al., 1996; Hamann et al., 2003) (also see Hamann, 2002, and MacAulay et al., 2004) or through specialized channels known as aquaporins (AQPs) that facilitate direct water transport across cell membranes. AQPs have been found to assist large transcellular water fluxes across cell membranes in response to osmolarity changes (Zeidel et al., 1992; Agre et al., 1993), throughout most of the body including the eye (Patil et al., 1997; Hamann et al., 1998; Verkman, 2003).

Ocular fluid is primarily derived from the secretion of aqueous by the ciliary processes (To et al., 2002). A lesser volume is sourced from the significant amounts of water produced in the retina as a consequence of the large metabolic turnover and the coupling of water to the uptake of metabolic glucose and lactate uptake from blood (Lehninger, 1970; To et al., 2002; Amiry-Moghaddam & Ottersen, 2003; Bringmann et al., 2005; Strauss, 2005). It is important that rapid production of metabolically derived fluid is continuously and rapidly cleared from all compartments of the eye in order to stabilize intraocular pressure in the eye and to maintain the local balance of ions for effective signal transduction in the retina (Stein, 2002; Amiry-Moghaddam & Ottersen, 2003; Dmitriev & Mangel, 2004; Strauss, 2005). Indeed, in all species studied from reptile to bird to mammal (Hughes et al., 1998), there is a net transfer of fluid across the retinal pigment epithelium (RPE) from

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the subretinal space (SRS) to the choroid. This ''slowly percolating'' retinal fluid is also believed to aid in maintaining retinal adhesion (Marmor, 1999). It is well accepted that in normal circumstances, the RPE responds to changes in hydration of the outer retina, while glial cells respond to fluid changes within the inner retina (Bringmann et al., 2004, 2005, 2006). While solute-linked cotransport of water is accepted as the major factor in drawing fluid out of the retina for deposition across the RPE and into the choroid (Hughes et al., 1998), such water redistribution within the inner retina is carried out primarily by the major glial support cells of the retina, the Müller cells (Moseley et al., 1984; Marmor, 1999; Nagelhus et al., 1999; Bringmann et al., 2004). Müller cells are presumed to conduct water redistribution within the inner retina in response to the continuous forcing of water into the retina from the vitreous due to intraocular pressure (Marmor, 1999; Bringmann et al., 2004). Furthermore, the high impedance of water flow across the plexiform layers of the retina (Antcliff et al., 2001) highlights the importance of Müller cells in water transport across the retina. The understanding of fluid dynamics within the retina has also been expanded by the discovery of AQPs on Müller cells, and the focus of this review was to explore the involvement of AQPs in ocular fluid regulation, underlying the movement of fluid to and from the vitreous to regulate inner retinal hydration and during rapid ocular growth.

Fluid regulation by AQPs

AQPs are transmembrane water channels first identified by Preston et al. (1992) in the early 1990s. AQPs have since been shown to effectively and rapidly conduct water bidirectionally by mediating transcellular osmotically driven fluid movement (Preston et al., 1992; Zeidel et al., 1992; Meinild et al., 1998; Agre et al., 2002; Amiry-Moghaddam et al., 2003). AQPs have now been found in most species and tissues, with at least 13 mammalian types identified (for reviews, see Borgnia et al., 1999; Takata et al., 2004; and Hara-Chikuma & Verkman, 2006). Although the functional roles of the different types of channels remain to be elucidated, the sites of expression of many of these AQPs have been shown to support a likely physiological role in fluid transport. AQPs have been implicated in the functioning of the kidneys, the gastrointestinal system, anterior chamber of the eye, skin hydration, and more recently cell migration (see Verkman, 2002, and Verkman et al., 2008, for a review). A number of AQPs have also been found to cotransport glycerol and other small solutes, but AQPs 1, 2, 4, 5, and 8 are known to specifically facilitate the transport of water molecules (Agre et al., 1998; Takata et al., 2004).

Distribution and function of AQPs in the brain

AQP1, AQP4, and AQP9 have been identified in the brain, but it is AQP4 with a postulated role in brain edema that has become the focus of increased medical interest (Amiry-Moghaddam & Ottersen, 2003). The interest is derived from the fact that swelling of glial cells and extracellular volume changes are major hallmarks of ischemia-induced brain edema (Kimelberg, 1995). AQP4 is almost predominantly found on astroglia in the brain (Nielsen et al., 1997). Expression of AQP4 on the perivascular and subpial endfeet of astrocytes and the basolateral membrane of ependymal cells of the ventricles of the brain supports the hypothesis that AQP4 plays a role in regulating fluid movement between the extracellular space and the circulatory system (Nielsen et al., 1997; Nagelhus et al., 2004). In addition, it has also been suggested that AQP4 expression on brain astrocytes mediates fluid uptake from the extracellular space around synapses (Nielsen et al., 1997; Amiry-Moghaddam & Ottersen, 2003; Nagelhus et al., 2004). In doing so, AQP4 has been suggested (Amiry-Moghaddam & Ottersen, 2003; Nagelhus et al., 2004) to play an important role in maintaining homeostasis in the brain during neuronal activity among areas of high osmoregulatory demand.

AQP4 has also been implicated in the osmoregulation of the brain during pathological states (Amiry-Moghaddam & Ottersen, 2003; Bringmann et al., 2005), and expression of AQP4 on astrocytes has been found to be altered following cerebral infarction (Aoki et al., 2003). Temporal changes of perivascular AQP4 expression have been observed during the reperfusion phase of edema formation (Frydenlund et al., 2006). New evidence also suggests that gene variation of AQP4 expression may underlie severe brain edema formation following artery occlusion (Kleffner et al., 2008).

Gene deletion studies have been used to establish the phenotypic variation in function of AQP4 and also to indicate changes in brain and retinal integrity that follow when fluid homeostasis is experimentally manipulated (see Verkman, 2005, for a detailed overview of AQP knockout studies). Early studies using animals with AQP4 deletion (AQP4$-/-$) indicated that these animals were phenotypically normal in development, survival, and neuromuscular function (Ma et al., 1997). However, continuing work in Verkman's laboratory has shown that when homeostatic functioning is perturbed by either by ischemic stroke or hypoosmotic stress, AQP4$-/-$ animals show reduced water accumulation within the astrocyte endfoot processes of the brain (Manley et al., 2000). Such reduction in water accumulation within astrocytic endfeet suggests a role for AQP4 in the normal accumulation of fluid during the induction of edema. In fact, in the presence of cerebral edema, AQP4$-/-$ knockout mice exhibited reduced intracranial pressure, lower mortality rates, and significant improvement on neurological deficit scores compared to wild-type mice (Manley et al., 2000; Papadopoulos & Verkman, 2005), suggesting that AQP4 antagonists may potentially be useful therapeutic agents during the formation of edema.

AQP4 expression has also been studied in α-syntrophin (α-syn) knockout mice. The protein α-syntrophin forms part of the dystrophin complex, and AQP4 is anchored by α-syntrophin on the endfeet membranes of astrocytes (Frigeri et al., 2001). Thus, α-syn$-/-$ mice show selective reduction of perivascular and subpial AQP4 expression, while AQP4 on membranes facing neuropil remain intact (Neely et al., 2001; Amiry-Moghaddam et al., 2004a, 2004b). Such a selective reduction in AQP4 expression around blood vessels and subpial regions is accompanied by reduced vulnerability to develop edema postischemia, mainly as a consequence of reduced water entry into astrocyte endfeet during the formation of edema (Amiry-Moghaddam et al., 2003).

The bidirectional capability of AQP4 channels was also noted as mice lacking AQP4 exhibit increased astroglial endfeet volume in basal conditions (Amiry-Moghaddam et al., 2003). This increase in endfeet volume is presumably due to a lack or reduction in water efflux *via* AQP4 channels into brain capillaries. Thus, AQP4 expression not only plays a critical role in the development of edema but also in the facilitation of the normal efflux of metabolically generated water from the brain parenchyma into brain capillaries (Amiry-Moghaddam et al., 2003). Such a function must have important currently unexplored implications for the use of antagonists during the postischemic state.

Distribution of AQP4 on Müller cells in the retina

Given that the eye is an outgrowth of the brain, the retina would also be expected to demonstrate the same mechanisms for the regulation of fluid homeostasis. Similarly the retina, like the brain,

has extremely high energy demands and metabolic turnover, generating a need to prevent excess water accumulation during neuronal activity. Again this function is mediated by Müller cells (Nagelhus et al., 1998; Bringmann et al., 2004). Immunocytochemistry, immunoblotting, and electron microscopy techniques have localized AQP0, AQP1, AQP3, AQP4, AQP5, and AQP9 proteins to the human and rat eye (Nielsen et al., 1993; Patil et al., 1997; Funaki et al., 1998; Hamann et al., 1998; Nagelhus et al., 1998; Tenckhoff et al., 2005; Iandiev et al., 2006*a*, 2007), but the reported expression of AQP4 in the retina is restricted to Müller cells (Hamann et al., 1998; Goodyear et al., 2008).

Müller cells have long been known to be involved in many retinal homeostatic functions including volume regulation (Newman & Reichenbach, 1996; Nagelhus et al., 1998; Bringmann et al., 2004, 2006). Müller cells span the width of the retina from the inner limiting membrane to the outer limiting membrane, with the cell soma lying within the inner nuclear layer (INL) (Dreher et al., 1994; Newman & Reichenbach, 1996; Bringmann et al., 2006). AQP4 channels are found expressed on Müller cell membranes facing the retinal capillaries, abutting the vitreoretinal border and on membranes facing synapses in the plexiform layers in vascular mammalian retinae (Nagelhus et al., 1998, 1999) and also in similar areas in the avascular chick retina (Goodyear et al., 2008) (Fig. 1).

The expression of AQP4 on endfeet membranes of the Müller cell supports the notion that Müller cells contribute to transretinal fluid movements, particularly following the ion and osmotic changes induced by neuronal activity. Removal of excess water from the inner retina is achieved by facilitating water fluxes into the vitreous body and retinal capillaries (Nagelhus et al., 1998). Expression of AQP4 channels may thus facilitate retinal signal transduction in both vascular (Nagelhus et al., 1998) and avascular (Goodyear et al., 2008) retinae (Fig. 1).

As noted earlier, AQP4 channels are not the only water transport mechanism implicated in fluid homeostasis of the retina. AQP1 has been found on photoreceptors and a subset of amacrine cells, predominantly in the outer retina. The authors consider this to reflect astrocytic expression rather than Muller cell expression (Iandiev et al., 2006*b*). However, the altered expression of AQP1 was attributed to astrocytic expression rather than due to the expression of AQP1 on Müller cells.

Cotransport proteins known to facilitate transcellular ion movement elsewhere in the body also function as molecular water pumps in retina and in the absence of an osmotic gradient (Hamann & La Cour, 2005). Such mechanisms would appear to predominate in the outer retina where the microvilli of the Müller cell facing the SRS are reported to be devoid of AQP4 channels (Nagelhus et al., 1998). Furthermore, if AQP4 contributes to the development and efflux of edema from the brain and eye, surviving AQP4 knockout animals must rely on other mechanisms of fluid homeostasis such as cotransporter proteins. However, no changes in the expression of several other AQPs have been reported in AQP4 knockout animals (Ma et al., 1997). On the other hand, a downregulation of expression of the glutamate transporter 1 is seen in cultured astrocytes in AQP−/− mice. This could possibly lead to previously unforeseen alterations in ion and water homeostasis (Zeng et al., 2007).

AQP4 is yet to be localized to the RPE, and there is contradictory evidence whether other AQPs are present (Ruiz & Bok, 1996; Hamann et al., 1998; Stamer et al., 2003). It seems more likely that AQP4 in the inner retina will influence rapid fluid flow across the retina and in combination with the activity of ion cotransporters at the RPE will alter transport out of the retina and into the choroid (Hughes et al., 1998).

A physiological role for AQP4 in retinal fluid homeostasis

Given that neuronal activity is accompanied by the metabolic production of water and efflux of K^+ out of cells, it is not surprising that Müller cells play a central role in the regulation of extracellular fluid homeostasis and tissue volume regulation (Newman et al., 1984; Newman, 1985a, 1987; Brew et al., 1986; Uckermann et al., 2004) (Fig. 2). Neuronal activity accompanying light onset results in a transient increase in potassium ($[K^+]$) in the plexiform layers of the retina and a decrease in extracellular K^+ ($[K^+]_o$) in the SRS, creating a need for the spatial buffering of the $[K^+]_o$ (Karowski & Proenza, 1977; Steinberg et al., 1980). Such light activity–dependent increases in $[K^+]$ in inner retina require siphoning away

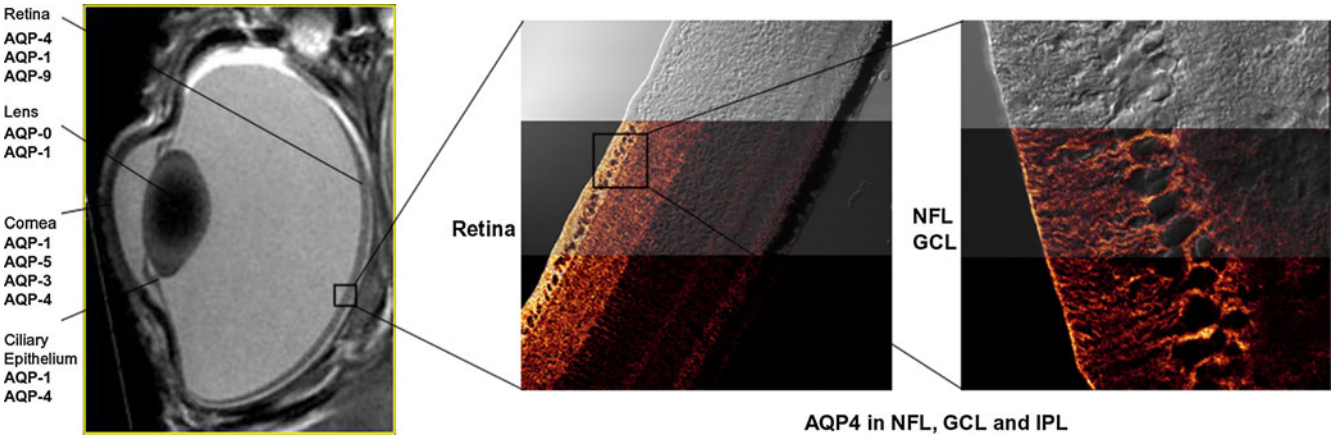

Fig. 1. (Color online) AQP4 in the retina. Image on the left is a magnetic resonance imaging photograph showing the known distribution of AQPs in the eye (photograph produced with the assistance of the Howard Florey Institute). The confocal micrographs (middle and right images) show localization of AQP4 in the avascularized chick retina during form-deprivation myopia. Expression of AQP4 has been localized to the endfeet of the Müller membrane at the vitreal interface, NFL, GCL, and the IPL, and shows similar localization to vascularized retinae, with the exception of endfeet abutting retinal capillaries. Upregulation of AQP4 during form deprivation occurs primarily in the NFL abutting the vitreal interface. This is thought to alter fluid flow into the vitreous cavity, leading to ocular enlargement. IPL, inner plexiform layer; NFL, nerve fiber layer; GCL, ganglion cell layer.

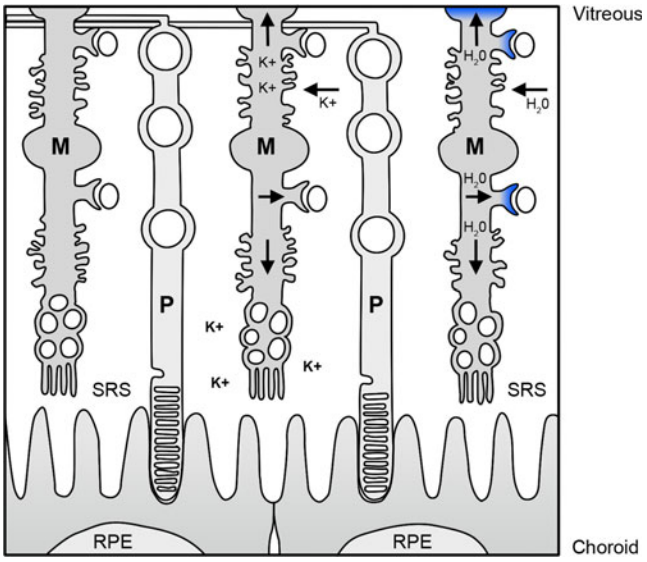

Fig. 2. (Color online) Retinal K^+ and fluid regulation by Müller cells. The high permeability of K^+ into the Müller cell allows the light-induced excess K^+ to be easily siphoned away from the extracellular space of the IPL and deposited in low-concentration areas such as the SRS and the vitreal interface and in vascular animals, into intraretinal blood vessels. Extracellular volume regulation is achieved in part by Müller cells using AQP4 channels located at the vitreoretinal border and on endfeet processes abutting retinal capillaries (blue shaded area on the Müller cell). This physical similarity has led to suggestions of a functional-dependent relationship between K^+ siphoning and water transport by Müller cells. Figure adapted from Newman and Reichenbach (1996). IPL, inner plexiform layer; M, Müller cell; P, photoreceptor cell.

from areas of high concentration by the Müller cells to help maintain the responsiveness of retinal cells to prolonged light stimulation (Newman, 1984, 1987; Newman et al., 1984; Newman & Reichenbach, 1996; Nagelhus et al., 1998). The excess K^+ is deposited into the vitreous (or where available into retinal blood vessels) or alternatively deposited back into the SRS to replenish the depletion of K^+ there (Mori et al., 1976; Newman & Odette, 1984; Newman et al., 1984; Newman, 1985b, 1987; Karwoski et al., 1989; Frishman et al., 1992). The predominant K^+ channel in Müller cells primarily responsible for the spatial buffering of $[K^+]_o$, is the inward rectifying channel from the 4.1 subfamily (Kir4.1 channel) (Newman, 1993; Ishii et al., 1997).

Interestingly, Kir4.1 and AQP4 channels spatially colocalize on the endfeet processes of the Müller cell facing the vitreous and blood vessels of the mammalian retina. This has led to suggestions of a functional-dependent relationship (Nagelhus et al., 1999). Typically, these retinal regions of colocalization of Kir4.1 and AQP4 channels play a role in creating K^+ sinks for regulating high concentrations of $[K^+]_o$ around active neuropil as described above (Newman, 1993; Kofuji & Newman, 2004).

A reduction in the electroretinogram (ERG) b-wave (the retinal response to light onset) in AQP null mice compared to controls suggests that the light onset–induced K^+ changes are impaired in the absence of AQP4 (Li et al., 2002). Li et al. (2002) argued that the reduced water permeability in AQP4 null mice could lead to changes in retinal hydration and consequently K^+ levels, which in turn could influence K^+ siphoning by Müller cells. However, it must be noted that the b-wave of the ERG has primarily a bipolar cell origin (Robson & Frishman, 1998; Sharma et al., 2005), and hence, a reduction in the amplitude and latency of the b-wave in AQP4 null mice more than likely reflects a dampening of the depolarizing response of bipolar cell activation.

Investigations of ischemic insults targeting the retina of AQP4 knockout mice have also highlighted the involvement of AQP4 in regulating retinal neuronal activity. Da and Verkman (2004) found that elevated intraocular pressure induced significantly less impairment of retinal function as demonstrated by preservation of the b-wave of the ERG in ischemic retinae from AQP4−/− mice. Further, significantly less cell loss and better maintenance of the thicknesses of the INL and inner plexiform layers was seen in AQP4−/− mice compared to wild-type controls. However, no differences were seen in Müller cell swelling or extracellular space expansion. It should also be noted though as above that lower baseline b-wave amplitudes were found in one strain of AQP4−/− mice compared to control, thus confirming the earlier findings of Li et al. (2002) and raising the possibility that the presence of an already impaired b-wave amplitude may have masked any measurable impairment in AQP4−/− mice following the ischemic insult.

Pannicke et al. (2004) noted that ischemia induces a downregulation of Kir4.1 channels that is accompanied by decreased outward K^+ currents from Müller cells. This downregulation led to glial cell swelling in the face of hypotonic stress (Pannicke et al., 2004). Thus, given that AQP4 channel expression was preserved, the authors believe glial swelling results from the accumulation of intracellular K^+ ions leading to a change in the osmotic gradient between the Müller cell and the extracellular space. Such glial cell swelling follows the reduced K^+ efflux, generation of a reverse osmotic gradient, and drawing water into the glial cell through AQP4 channels.

The above-mentioned studies are suggestive of a functional association between the water transport and the spatial buffering of K^+. The physical colocalization of AQP4 and Kir4.1 on endfeet of Müller cells in highly K^+-permeable regions in the mammalian retina lends support for an association. However, an association does not necessarily imply a dependency in function, and indeed more recent evidence indicates that a functional relationship between Kir4.1 and AQP4 seems unlikely (Ruiz-Ederra et al., 2007). Ruiz-Ederra et al. (2007) found no changes in Kir4.1 expression in

AQP4−/− mice, nor did they observe any significant differences in K^+ currents between wild-type and AQP4−/− mice in isolated Müller cells. Thus, further clarification of the relationship is needed both in the normal intact environment and after physiological stress is imposed. The functional coupling of Kir4.1 and AQP4 is further complicated as Na^+ and bicarbonate transport have also been hypothesized to be implicated in the K^+-mediated water influx through AQPs, presumably by creating an osmotic gradient for water to enter the Müller cell (Nagelhus et al., 2004). Furthermore, the coenrichment of Kir4.1 and AQP4 is absent at the microvilli of the Müller cell where only Kir4.1 expression has been reported (Nagelhus et al., 1999). As alluded to earlier, Müller cells are also known to clear excess K^+ from the inner retina by deposition back into the SRS (Newman & Reichenbach, 1996; Kofuji & Newman, 2004). Presumably, other mechanisms including other ion channels and cotransporters must work in concert with the apical membrane of the RPE for osmoregulation of the SRS.

AQP4, a potential target in myopia?

Myopic refractive errors are typically characterized by excessive elongation of the axial dimensions of the eye such that visual images cannot focus on the neural retina. Excessive accumulation of fluid in the vitreous is accompanied by reduced choroidal blood flow and decreased choroidal thickness in both the development of clinical myopia and the experimentally induced myopic refractive errors (Shih et al., 1993; Crewther, 2000; Fitzgerald et al., 2002). Yet, few studies have addressed the question of the origin and disposition of the fluid that is responsible for the abnormal axial elongation and ocular volume in myopia, despite the fact that at least 30% of the adult world population is affected by myopia (Seet et al., 2001; Morgan & Rose, 2005). The public health and socioeconomic implications of such prevalence are enormous. One potential explanation for the disposition of fluid comes from evidence of changes to the normal pattern of ionic abundances from the retina to the choroid during recovery from experimentally induced myopia (Liang et al., 2004; Crewther et al., 2006). Liang et al. (2004) and Crewther et al. (2006) demonstrated that form-deprivation myopia in chicken is associated with a substantial increase in K^+, Na^+, and Cl^- abundances in the outer retina and also prolonged increases in Na^+ and Cl^- in the inner retina. These changes are associated with axial elongation of the vitreous and thinning of the retina and choroid and hence volume changes in the eye.

AQP4 has been shown to play an important role in regulating fluid flow both in the brain and across the retina, with AQP4 expression up- or downregulating in certain pathological conditions as outlined above (Amiry-Moghaddam & Ottersen, 2003; Bringmann et al., 2005). Thus, we suggest that AQPs could offer an important mechanism for the transport of water across the retina during normal physiological conditions and hence would be expected to be involved in pathophysiological situations when fluid movement is affected, as in ischemia or volume changes during refractive error development. Given the reported changes to the osmotic environment during the development of experimentally induced myopia, it is likely that AQP4 serve in the movement of fluid across the retina. The involvement of AQP4 in myopia has recently been confirmed by our laboratory and demonstrates a temporal upregulation of AQP4 during the early increase in the rate of ocular growth that is concomitant with the induction of myopia in chickens (Goodyear et al., 2008). AQP4 expression was found to significantly upregulate at the nerve fiber layer abutting the vitreoretinal border in chicks deprived of form vision (Fig. 1). The observation of a very early upregulation of AQP4 is not surprising, however, given the rapid elongation of the eye and enlargement of the vitreous chamber evident in form-deprived myopic eyes. Thus, the perturbation of AQP4 expression as a consequence of the changes to retinal signal transduction during reduced visual experience leads to excess fluid movement and deposition into the vitreous chamber (and possibly reduced fluid outflow into the choroid), resulting in ocular enlargement.

Conclusions

The eye is a unique encapsulated outgrowth of the brain where functioning requires exquisite fluid control. It is clear that AQP4 expression plays an important role in this maintenance of fluid homeostasis in both the eye and the brain. Experimentation that addresses the role of AQP4 in subtly altered physiological environments, such as during normal ocular growth or during the abnormal growth accompanying the development of refractive errors such as myopia and hyperopia, is likely to provide further understanding of ocular homeostasis and normal fluid movements. This will benefit not only our understanding of the role of AQP4 in central nervous system functioning and of the genesis and management of refractive error but also our understanding of the majority of acquired ocular pathologies (e.g., macular degeneration, diabetic retinopathy, and glaucoma) in which more permanently altered retinal fluid homeostasis leads to permanent retinal damage and ocular morbidity.

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