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Research article

Changes in aquaporin-4 and Kir4.1 expression in rats with inherited retinal dystrophy

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ABSTRACT

Muller glial cells (MGC) are essential for normal functioning of retina. They are especially involved in potassium (K+) and water homeostasis, via inwardly rectifying K+ (Kir 4.1) and aquaporin-4 (AQP4) channels respectively. Because MGC appear morphologically and functionally altered in most retinal pathologies, we studied the expression of AQP 4 and Kir 4.1 during the time course of progressive retinal degeneration in Royal College of Surgeons (RCS) rats, an animal model for the hereditary human retinal degenerative disease *Retinitis pigmentosa*. Simultaneous detection of AQP4 and Kir 4.1 was performed by quantitative real-time polymerase chain reaction (QRT-PCR), Western blot and immunohistochemistry at birth and during progression of the pathology. Although small quantities of AQP4 and Kir 4.1 mRNA were detected at birth (postnatal day (PNd) 0) in both control and dystrophic rat retinas, proteins could not be detected at this age. Detectable proteins appeared in the second week of postnatal life. From PNd15 onwards, the time course in the expression of both AQP4 and Kir 4.1 mRNAs and protein was similar in dystrophic and control rats, with a progressive increase peaking at PNd60 and a subsequent decrease by one year. AQP4 protein and mRNA content were significantly lowered in dystrophic compared to control rats. Kir 4.1 protein levels were also lower in dystrophic retinas, while mRNA concentrations were unchanged and/or slightly higher in dystrophic rats. The discrepancies between Kir4.1 mRNA and protein suggest perturbation in protein translation due to the pathology. AQP4 and Kir 4.1/vimentin co-immunolabeling showed that: 1) apical radial processes of some MGC invaded the subretinal zone, and 2) MGC morphology was distorted in advanced pathology. MGC became hypertrophic both during the pathology and also with age in control rats. In conclusion, our results confirm that this inherited photoreceptor degeneration also leads to progressive alterations in physiological and morphological parameters of MGC which may aggravate retinal impairment.

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1. Introduction

Muller cells (MGC) are the major glial cells of the retina. Besides their nutritional function for neurons, they play protective roles against retinal damage (García and Vecino, 2003). Alterations in their physiological and morphological characteristics are observed in various retinal diseases, often leading to their proliferation and activation (Bringmann and Reichenbach, 2001; Bringmann et al., 2006; Vecino et al., 2015) with accompanying changes in retinal ion and water homeostasis (Liu et al., 2007; Iandiev et al., 2008; Rehak et al., 2009; Zhao et al., 2011). These parameters are mostly controlled by ion channels and transporter mechanisms including aquaporins (AQPs) and inwardly rectifying potassium channels (Kir). AQPs are a family of channel proteins required for

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Table 1
RT PCR primers.

Gene	Species	SYBR green primers
18S	Rat	Sense 5′-AAG TCC CTG CCG TTT GTA CAC A-3′ Antisense 5′-GAT CCG AGG GCC TCA CTA AAC-3′
Kir 4.1	Rat	Sense 5′-CAA AGA AGA GGG CTG AGA CG-3′ Antisense 5′-TTG AGC CGA ATA TCC TCA CC-3′
AQP4	Rat	Sense 5′-CGG TTC ATG GAA ACC TCA CT-3′ Antisense 5′-CAT GCT GGC TCC GGT ATA AT-3′

the bidirectional regulation of water transport through plasma membranes (Verkman and Mitra, 2000). Among the thirteen identified AQPs (AQP0 – AQP12) (Verkman, 2005), at least seven (AQP0, AQP1, AQP3, AQP4, AQP5, AQP6 and AQP9) are found in the eye but only AQP0, AQP1, AQP4, AQP6 and AQP9 are expressed in the neural retina (Iandiev et al., 2006a; Verkman et al., 2008; Schey et al., 2014). AQP4 is of particular interest, being expressed in retinal astrocytes and MGC (Nagelhus et al., 1998) where it functions to maintain water and ion homeostasis, probably in association with the Kir channel, Kir 4.l. Kir 4.1 is one of several subtypes of potassium (K+) channels expressed in retina (Bringmann et al., 1997; Kofuji et al., 2002; Pinto and Klumpp, 1998). Kir 4.1 is found predominantly on the endfeet of MGC and in astrocytes wrapping the retinal blood vessels in the superficial nerve fiber layer (NFL), and is the major channel involved in the spatial buffering of K+ concentrations (Kofuji et al., 2002). The co-localization of AQP4 and Kir4.1 in MGC membrane end feet suggests that water and K+ flux in these cells may be functionally coupled (Nagelhus et al., 1999; Iandiev et al., 2008).

MGC display significant morphological, cellular and molecular changes in various retinal pathologies and these changes seem to particularly affect AQP4 and Kir4.1. In the present work, we decided to determine the temporal evolution of these changes in order to understand how they could be related to the inherited degenerative retinal pathology developed by Royal College of Surgeon rats (*rdy/rdy* RCS rats). These rats are considered as a relevant model for human *Retinitis pigmentosa* (Bourne et al., 1938; Gal et al., 2000). *Retinitis pigmentosa* is actually a family of human hereditary retinal photoreceptor dystrophies leading to blindness. It is genetically heterogeneous with mutations found in more than 50 genes identified in the retinal pigment epithelium (RPE) and photoreceptor cells (Daiger et al., 2013). This pathology leads to

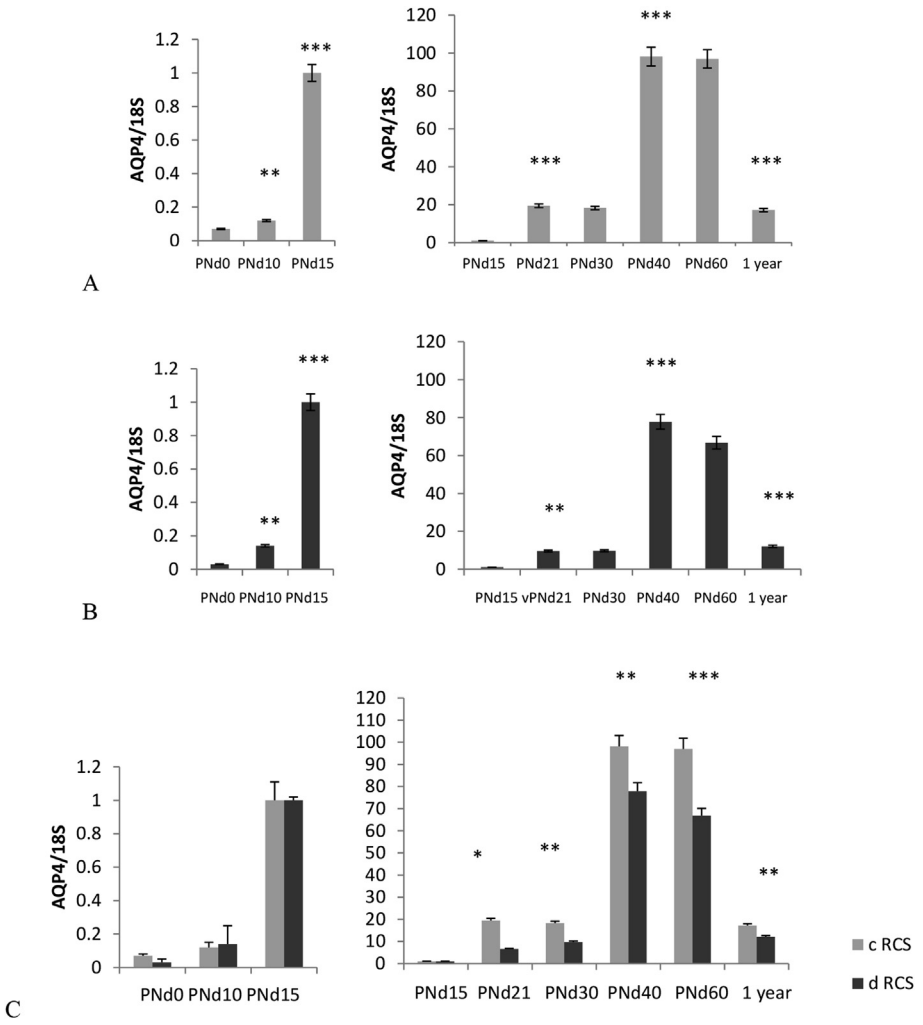

Fig. 1. Time course of AQP4 mRNA expression from birth to 1 year in retinas of control (cRCS) and dystrophic RCS (dRCS) rats. The mRNA contents were compared between each age for cRCS (Fig. 1A) and for dRCS (Fig. 1B). In Fig. 1C, the mRNA contents were compared between cRCS and dRCS rats at each age. **A**. In cRCS rat, AQP4 was detected at very low level between birth and eye opening (PNd15). Thereafter the levels increased significantly from PNd 15, through PNd21 and especially at PNd40 to decrease by one year. **B**. In dRCS rats, AQP4 mRNA levels were also low at birth, increasing up to PNd40 and subsequently decreasing. **C**. Direct comparison between cRCS and dRCS rats showed similar temporal profiles of AQP4 mRNA expression up to 1 year. Whereas no significant differences appeared at early stages of postnatal development, at PNd21 and beyond, levels were lower in dystrophic compared to control RCS rats. *, $p < 0.05$; **, $p < 0.01$; ***$p < 0.001$.

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