Experimental Eye Research 146 (2016) 259–268

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Research article

Glio-vascular modifications caused by Aquaporin-4 deletion in the mouse retina

Grazia Paola Nicchia [a, b, *, 1], Francesco Pisani [a, 1], Laura Simone [a, c], Antonio Cibelli [a], Maria Grazia Mola [a], Massimo Dal Monte [d], Antonio Frigeri [b, e], Paola Bagnoli [d], Maria Svelto [a, f, g]

[a] Department of Bioscience, Biotechnologies and Biopharmaceutics and Centre of Excellence in Comparative Genomics, University of Bari "Aldo Moro", 70126 Bari, Italy
[b] Department of Neuroscience, Albert Einstein College of Medicine, New York, Bronx, NY, USA
[c] IRCCS "Casa Sollievo della Sofferenza", Research Hospital, San Giovanni Rotondo, Italy
[d] Department of Biology, University of Pisa, 56127 Pisa, Italy
[e] School of Medicine, Basic Medical Sciences, Neuroscience and Sense Organs, University of Bari "Aldo Moro", 70126 Bari, Italy
[f] Institute of Biomembranes and Bioenergetics, National Research Council, Via Amendola 165/A, 70126 Bari, Italy
[g] National Institute of Biostructures and Biosystems (INBB), Viale Medaglie D'Oro 305, 00136 Rome, Italy

ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form
8 March 2016
Accepted in revised form 22 March 2016
Available online 24 March 2016

Keywords:
Aquaporin-4
Retina
Neuromyelitis optica
Gliovascular biology
Blood-retinal barrier
Intraretinal vasculature
Astrocytes
Müller cells

ABSTRACT

Aquaporin-4 (AQP4) is the Central Nervous System water channel highly expressed at the perivascular glial domain. In the retina, two types of AQP4 expressing glial cells take part in the blood-retinal barrier (BRB), astrocytes and Müller cells. The aim of the present study is to investigate the effect of AQP4 deletion on the retinal vasculature by looking at typical pathological hallmark such as BRB dysfunction and gliotic condition.

AQP4 dependent BRB properties were evaluated by measuring the number of extravasations in WT and AQP4 KO retinas by Evans blue injection assay. AQP4 deletion did not affect the retinal vasculature, as assessed by Isolectin B_4 staining, but caused BRB impairment to the deep plexus capillaries while the superficial and intermediate capillaries were not compromised. To investigate for gliotic responses caused by AQP4 deletion, Müller cells and astrocytes were analysed by immunofluorescence and western blot, using the Müller cell marker Glutamine Synthetase (GS) and the astrocyte marker GFAP. While GS expression was not altered in AQP4 KO retinas, a strong GFAP upregulation was found at the level of AQP4 KO astrocytes at the superficial plexus and not at Müller cells at the intermediate and deep plexi. These data, together with the upregulation of inflammatory markers (TNF-α, IL-6, IL-1β and ICAM-1) in AQP4 KO retinas indicated AQP4 deletion as responsible for a gliotic phenotype. Interestingly, no GFAP altered expression was found in AQP4 siRNA treated astrocyte primary cultures. All together these results indicate that AQP4 deletion is directly responsible for BRB dysfunction and gliotic condition in the mouse retina. The selective activation of glial cells at the primary plexus suggests that different regulatory elements control the reaction of astrocytes and Müller cells. Finally, GFAP upregulation is strictly linked to gliovascular crosstalk, as it is absent in astrocytes in culture. This study is useful to understand the role of AQP4 in the perivascular domain in the retina and its possible implications in the pathogenesis of retinal vascular diseases and of Neuromyelitis Optica, a human disease characterized by anti-AQP4 autoantibodies.

1. Introduction

Aquaporins are water channel proteins conferring a faster water flux to several cell plasma membranes in the presence of osmotic and hydrostatic gradients (Yang and Verkman, 1997). Aquaporin-4 (AQP4) is the Central Nervous System (CNS) water channel, which

* Corresponding author. Department of Bioscience, Biotechnologies and Biopharmaceutics and Centre of Excellence in Comparative Genomics, University of Bari "Aldo Moro", Bari, Italy.
E-mail address: graziapaola.nicchia@uniba.it (G.P. Nicchia).
[1] G.P. Nicchia and F. Pisani equally contributed to the study.

http://dx.doi.org/10.1016/j.exer.2016.03.019
0014-4835/

Abbreviations

AQP4	Aquaporin-4
KO	knockout
WT	Wild-type
BRB	blood-retinal barrier
KD	knockdown
GFAP	Glial Fibrillary Acid Protein
qPCR	quantitative polymerase chain reaction
TNF-α	Tumor necrosis factor-α
IL-6	Interleukin-6
IL-1β	Interleukin 1β
ICAM-1	Intercellular adhesion molecule1
siRNA	small interfering RNA
CNS	Central Nervous System
BBB	blood–brain barrier
NFL	Nerve Fibre Layer
RGC	Retinal Ganglion cell
C-terminal	carboxyl-terminal
NMO	Neuromyelitis Optica
IgG	Immunoglobulin G
NFL	Nerve Fibre Layer
CD-31	Cluster of Differentiation
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
HRP	horseradish peroxidase
GS	Glutamine Synthetase
VEGF	vascular endothelial growth factor
IPL	Inner Plexiform Layer
INL	Inner Nuclear Layer
OPL	Outer Plexiform Layer
ONL	Outer Nuclear Layer.

is highly concentrated at the perivascular glial end-feet and takes part in the blood–brain barrier functional unit (Amiry-Moghaddam et al., 2003; Frigeri et al., 1995; Nicchia et al., 2008).

In the retina, two types of AQP4 expressing glial cells take part in the blood-retinal barrier (BRB), astrocytes and Müller cells (Newman, 2001). Astrocyte end-feet control the barrier properties only at the level of the superficial plexus whereas Müller cell end-feet do so also at the level of intermediate and deep plexus. Müller cells generated inside the retina span into its entire thickness providing structural and functional support to neurons (Newman and Reichenbach, 1996). AQP4 would here control the osmotic imbalance caused by the high neuronal activity (Goodyear et al., 2009). The relationship between AQP4 and the Kir4.1 potassium channel in Müller cells in the control of potassium homeostasis (Bosco et al., 2005; Nagelhus et al., 1999) is considered the reason for the impaired light-neuronal signal transduction reported for AQP4 KO mouse retina (Li et al., 2002). Astrocytes are stellate, without orientation, enter the retina from the brain along the developing optic nerve (Stone and Dreher, 1987) and form a homogeneous plexus on the Nerve Fibre Layer (NFL) and Retinal Ganglion cell (RGC) layer. Astrocytes here play a key role in maintaining either the BRB properties or the survival of RGC, whose axons form the optic nerve. Though the role of AQP4 in maintaining water homeostasis in Müller cells has been extensively studied (Goodyear et al., 2009; Verkman et al., 2008), less attention has been paid to addressing its function under normal conditions in the perivascular glial domain. Both Müller cells and astrocytes are enriched in intermediate filaments, however, Glial Fibrillary Acid Protein (GFAP) is the major component of the filaments in astrocytes (Dixon and Eng, 1981; Eng, 1985) but is found at very low levels in Müller cells (Bignami and Dahl, 1979). GFAP is thought to provide astrocytes with mechanical force as well as a particular shape, especially at the level of the terminal end-feet (Hyder et al., 2011). Several GFAP isoforms exist (Kamphuis et al., 2012), the canonical GFAP isoform is α, which is able to assemble in filaments based on a C-terminal motif that is instead absent in GFAPδ and k, destabilizing the filaments (Blechingberg et al., 2007a; Hol and Pekny, 2015; Kamphuis et al., 2012).

BRB dysfunction and glial activation are key pathological features in a number of retinal vascular diseases, such as retinopathy of prematurity, diabetic retinopathy, and branch retinal vein occlusion, considered a primary cause of visual impairment and blindness (Coorey et al., 2012). Interestingly, altered AQP4 expression is also associated with some of these pathologies, such as branch retinal vein occlusion (Koferl et al., 2014) and diabetic retinopathy (Kumar et al., 2014; Qin et al., 2012). In diabetic rats, AQP4 knockdown (KD) leads to exacerbation of retinopathy with BRB dysfunction and inflammatory response (Cui et al., 2012). In Neuromyelitis Optica (NMO), a CNS autoimmune inflammatory "aquaporinopathy" affecting the optic nerve and spinal cord (Bergamaschi and Ghezzi, 2004; Jarius and Wildemann, 2010; Lennon et al., 2004, 2005) and leading to paralysis and blindness, AQP4 is the molecular target of the autoantibody NMO IgG, the pathological hallmark of the disease. Interestingly, optic neuritis in NMO patients is characterized by retinal modifications at the vascular level and on the NFL (Camicione et al., 2010; Green and Cree, 2009; Merle et al., 2008), most likely caused by NMO IgG binding to AQP4 expressing cells in the retina and optic nerve.

Understanding the molecular basis of the role played by AQP4 at the level of retinal vasculature is fundamental for clarifying the pathogenesis of the diseases involving AQP4 dependent alterations in the retinal vasculature. Moreover, it will help in elucidating the role that glial cells play in the pathogenesis of such diseases, therefore supporting the developing of novel therapies. The aim of the present study is to investigate the effect of AQP4 deletion on the retinal vasculature by looking at typical pathological hallmark such as BRB dysfunction and gliotic condition.

2. Materials and methods

2.1. Animals

5–10 month old AQP4 KO mice with a CD1 genetic background (Basco et al., 2013) and age matched controls were used. CD1 mice and Wistar rats were used for astrocyte primary cultures. Experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and in compliance with the Italian law on animal care (Italian Health Department Approved Project n 100/2014-B). All experiments were designed to minimize the number of animals used and their suffering. The mice used here were bred in the approved facility at the University of Bari.

2.2. Antibodies

The following primary antibodies were used: goat polyclonal anti-AQP4 (Santa Cruz, CA, USA); goat polyclonal anti-CD31 (Santa Cruz, CA, USA); rabbit polyclonal anti-GFAP (Sigma–Aldrich, Milan,