BRAIN RESEARCH 1432 (2012) 46–55

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

# Aquaporin-4 immunoreactivity in Müller and amacrine cells of marine teleost fish retina

José R. Hombrebueno[a], Eun-Jin Lee[b], Noemí Martínez-Ruiz[a], Alicia García-Alcázar[c], Norberto M. Grzywacz[b], Joaquín De Juan[a,*]

[a]Departamento de Biotecnología, Universidad de Alicante, Ctra. San Vicente-Alicante S/N, 03080 Alicante, Spain
[b]Department of Biomedical Engineering, University of Southern California, Denney Research Building 140, Los Angeles, CA 90089-1111, USA
[c]Instituto Español de Oceanografía, Centro Oceanográfico de Murcia, Ctra. de la Azohía S/N, 30860 Puerto de Mazarrón, Murcia, Spain

ARTICLE INFO

*Article history:*
Accepted 3 November 2011
Available online 11 November 2011

*Keywords:*
Photoreceptor
Retinal cell
Aquaporin
Immunocytochemistry
Darkness adaptation

ABSTRACT

Aquaporins (AQPs) are membrane proteins that facilitate water transport across biological membranes and are essential for the proper function of neural tissue. Although AQPs have been extensively studied in mammalian retina, their presence in lower vertebrate retina is less frequently characterized. AQP4 expressed in mammalian and chick Müller cells plays a major part in maintaining retinal homeostasis. In this study, we examined the immunoreactivity of AQP4 in the adult retina of gilthead sea bream (*Sparus aurata*–teleost fish), during light and dark adaptation. The AQP4 expression was detected in Müller cell somas at the inner nuclear layer and in the end-feet processes near the vitreoretinal border. Moreover, AQP4 was also evident in cone photoreceptor cells and in a GABAergic subpopulation of amacrine cells (AQP4-ACs). Four different types of AQP4-ACs were characterized based on their morphology and dendrite stratification. Interestingly, a stronger AQP4 immunoreactivity was observed in the inner nuclear layer during dark adaptation, accompanied by a significant increment in AQP4-ACs cell size. Hence, AQP4 may play an important role in water distribution in the teleost fish retina.



## 1. Introduction

Aquaporin water channels (AQPs) are a family of integral membrane proteins that facilitate the bidirectional transport of water across plasma membranes (Agre et al., 2002; Preston et al., 1992; Zeidel et al., 1992). In the central nervous system, AQPs are critically involved in maintaining the ionic and osmotic balance necessary for correct neuronal function (Verkman, 2003), where water redistribution between intra- and extracellular compartments becomes essential. To date, 13 different types of AQPs have been identified (Goodyear et al., 2009; King et al., 2004; Takata et al., 2004). Among these, only the immunoreactivity of AQP0, AQP1, AQP4, AQP6 and AQP9 have been observed in mammalian retina. For example, in murine retina, AQP0 is present in protein kinase α- and β-expressing bipolar and amacrine cells (Iandiev et al., 2007); AQP1 in glycinergic (Kim et al., 2002) and GABAergic (Kang et al., 2005) populations of amacrine cells as well as in

* *Corresponding author at: Departamento de Biotecnología, Universidad de Alicante, Apdo.* Correos 99, E-03080 Alicante, Spain. Fax: +34 96 590 3965.
E-mail address: jdj@ua.es (J. De Juan).

*Abbreviations:* AQPs, Aquaporins; AQP4-ACs, Aquaporin-4 immunoreactive amacrine cell; Cy3, Carboxymethylindocyanine; GABA, γ-aminobutyric acid; GFAP, Glial fibrillary acidic protein; GS, Glutamine synthetase; ILM, Inner limiting membrane; INL, Inner nuclear layer; IPL, Inner plexiform layer; NDS, normal donkey serum; OPL, Outer plexiform layer; PB, Phosphate buffer; PBS, Phosphate buffered saline

0006-8993/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2011.11.014

photoreceptor cells (Iandiev et al., 2005); AQP4 in glial Müller cells (Hamann et al., 1998; Nagelhus et al., 1998; Patil et al., 1997); AQP6 in glial membranes surrounding ribbon synapses at the outer plexiform layer (OPL) (Iandiev et al., 2011) and AQP9 in tyrosine hydroxylase expressing amacrine cells (Iandiev et al., 2006). However, little is known about AQPs expression in lower vertebrate retina. Recently, AQP4 has been identified in the photoreceptor cell layer of adult zebrafish (Zichichi et al., 2011).

AQP4 is a pure water transporter (Agre et al., 1998), preferentially expressed in astrocytes of the brain (Jung et al., 1994; Nielsen et al., 1997). In vascular mammalian retinas, AQP4 is distributed on Müller cell membranes facing capillaries, near the vitreoretinal border and on membranes facing synapses on the plexiform layers (Nagelhus et al., 1998, 1999). A similar distribution is found in avascular chick retinas (Goodyear et al., 2008, 2009). The enrichment of AQP4 at vitreal and perivascular end-feet regions means that it plays a central role in retinal fluid homeostasis, by contributing to water outflow from the inner retina into the vitreous body and retinal capillaries (Nagelhus et al., 1998). Furthermore, AQP4 is involved in the extracellular potassium homeostasis around active neuropil (Kofuji and Newman, 2004; Newman, 1993). All the aforementioned facts suggest the importance of AQP4 in maintaining efficient retinal signal transduction. In turn, teleost fish retinas show dramatic changes during the light/dark cycle, such as retinomotor movements (Ali, 1971; Burnside, 2001) or spinule formation/dissolution (De Juan and García, 2001; Downing and Djamgoz, 1989; Wagner, 1980), which may involve volume changes and aqueous redistribution through the retina. Hence, the study of AQP4 in light- and dark-adapted retinas in teleosts will provide better knowledge of its influence on retinal homeostasis. The aim of this study was to analyze the AQP4 expression in the adult retina of the commercial marine teleost fish, the gilthead sea bream (*Sparus aurata* L.) by immunocytochemistry, and whether its expression is altered during light and dark adaptation.

## 2. Results

### 2.1. AQP4 immunoreactivity in the *S. aurata* retina during light and dark adaptation

Our first goal was to determine the AQP4 expression pattern and whether it is affected by dark adaptation. For this purpose, we performed immunocytochemistry in vertical sections (Fig. 1A) of light-adapted (Fig. 1B) and dark-adapted (Figs. 1C–F) retinas. In light- and dark-adapted retinas, AQP4 immunoreactivity was found in cone photoreceptor cells (Fig. 1D), cells in the inner nuclear layer (INL) (Fig. 1E) and in the inner limiting membrane (ILM) (Fig. 1F). In cone photoreceptors, immunoreactivity was exhibited by the outer and inner segments, cell somata and axon terminals. In the INL, AQP4 was present in a putative population of amacrine cells (AQP4-ACs) located at the proximal margin and in their processes extending through the inner plexiform layer (IPL). In addition, the middle region of the INL also showed AQP4 immunoreactivity. No specific labeling was observed at the ganglion cell layer. Since AQP4 expression is restricted to Müller cells in mammals and chicks (Goodyear et al., 2008, 2009; Hamann et al., 1998; Nagelhus et al., 1998, 1999), and in the photoreceptor cell layer of adult zebrafish (Zichichi et al., 2011), its presence in certain populations of amacrine cells could be a novel finding in vertebrate retinas.

Dark-adapted retinas revealed a stronger AQP4 immunoreactivity than light-adapted retinas. A quantitative method was carried out in order to analyze the AQP4 immunostaining intensity of light- and dark-adapted retinas. The graph in Fig. 1 shows the intensity of AQP4 immunostaining in cone photoreceptor segments, in three different levels of the INL (distal, middle and proximal), and in the ILM of light- and dark-adapted retinas. Dark-adapted retinas presented significantly higher values in proximal and middle levels of the INL, with an increment in the AQP4 immunostaining intensity of 42% and 47% respectively ($p<0.001$). However, no significant differences were observed in cone photoreceptor segments or distal areas of the INL and ILM. This result suggests that the AQP4 expression in proximal and middle levels of the INL is regulated during the light/dark cycle.

### 2.2. AQP4 immunoreactivity in Müller cells

Teleosts Müller cells express glial fibrillary acidic protein (GFAP) at the inner retina and glutamine synthetase (GS) in cell soma and radial fibers extending to both limiting membranes (Bejarano-Escobar et al., 2009; Jones and Schechter, 1987; Lillo et al., 2002; Yazulla and Studholme, 2001). In the *S. aurata* retina, GFAP immunostaining was positive for Müller cell processes near the ILM (Fig. 2B). Double immunostaining showed that AQP4 is closely associated with the plasma membrane of Müller cell end-feet (Fig. 2C), with a prominent expression in vitreal membrane domains. Moreover, AQP4-immunoreactive cells in the middle of the INL were also colocalized with GS immunoreactivity (Fig. 2F). This indicates that AQP4 is expressed in the Müller cells of *S. aurata*.

### 2.3. Double immunofluorescence for AQP4 with GABA or glycine

In the retina, most amacrine cells contain either γ-aminobutyric acid (GABA — Marc et al., 1995; Vaney and Young, 1988; Vardi and Auerbach, 1995) or glycine (Goebel and Pourcho, 1997; Grünert and Wässle, 1993; Marc and Liu, 1984; Pourcho and Goebel, 1985; Rice and Curran, 2000). Therefore, we performed double-labeling with antisera against AQP4 with GABA and glycine. Fig. 3 shows vertical sections double-labeled with antibodies against AQP4 (Figs. 3A, D), GABA (Fig. 3B), and glycine (Fig. 3E) in the *S. aurata* retina. All AQP4-ACs in the INL adjacent to IPL showed GABA immunoreactivity (Fig. 3C). In addition, these neurons did not show glycine immunoreactivity (Fig. 3F). These results suggest that AQP4-ACs in the inner part of the INL may use GABA as their neurotransmitter.

### 2.4. Morphological characterization of AQP4-ACs

We morphologically characterized the population of AQP4-ACs. Only those cells that exhibited good AQP4 immunostaining and clear processes were chosen for this study. AQP4-ACs were