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Localization of aquaporin-0 immunoreactivity in the rat retina

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Abstract

Previous RT-PCR experiments revealed the expression of gene transcripts for a variety of aquaporins in the neural retina, including aquaporin-0. We investigated by immunohistochemistry and Western blotting whether the aquaporin-0 protein is expressed in the retina of the rat. In addition to the lens, immunoreactivity for aquaporin-0 was expressed in the neural retina, but was absent in the pigment epithelium, choroidea, and sclera. In the neural retina, aquaporin-0 immunoreactivity was expressed by the nuclei and the synaptic terminals of protein kinase α- and β-expressing bipolar and amacrine cells, and by the nuclei of neuronal cells in the ganglion cell layer. The immunoreactivity for aquaporin-0 did not co-localize with calbindin, a marker of horizontal cells, or with aquaporin-4, the glial water channel. Transient retinal ischemia caused a slight decrease in the retinal content of aquaporin-0, likely by degeneration of protein kinase α-expressing bipolar cells. It is concluded that aquaporin-0 may be involved in the regulation of the activity of retinal second order neurons.

Keywords: Aquaporin; Bipolar cell; Amacrine cell; Ischemia; Retina; Rat

Intense neuronal activation causes rapid ion shifts between intra- and extracellular spaces which are – for osmotical reasons – associated with water movements. Aquaporin water channels are critically involved in the maintenance of the ionic and osmotic balance in the central nervous system [19]. There are at least 13 different members of the aquaporin protein family that mediate bidirectional water transport across membranes in response to osmotic gradients and differences in hydrostatic pressure [20]. It has been shown that the neural retinas of rat and man express gene transcripts for numerous aquaporins, including mRNAs for aquaporins 0, 1, 4, and 9 [15]. However, it is not known whether the retina expresses the proteins of all the aquaporins detected at mRNA level. The immunoreactivities of three aquaporins have hitherto been demonstrated in the retina. Immunoreactivity for aquaporin-1 is expressed by pigment epithelial cells [14], by a subpopulation of glycinergic amacrine cells [9], and by photoreceptor cells [8]. Aquaporin-4 is expressed by glial cells; the co-localization of aquaporin-4 and potassium channels in distinct membrane domains of retinal glial Müller cells has led to the suggestion that the spatial buffering potassium currents and water transport through glial cells are coupled [11]. Immunoreactivity for aquaporin-9 has been detected in tyrosine hydroxylase-expressing, putatively dopaminergic, amacrine cells in the rat retina [7].

In the present study we investigated the expression of aquaporin-0 protein in the retina of the rat. Aquaporin-0, also known as major intrinsic protein, is the most abundant membrane protein in lens fiber cells, and is also expressed in trace amounts in liver and testis [4–6]. A deficiency in aquaporin-0 results in the development of cataract [12]. Aquaporin-0 facilitates the transport of water and small neutral solutes, such as glycerol and urea, across the plasma membrane [10,18]. In addition, aquaporin-0 may have functional roles in the adhesion, structure and organization of lens fiber cells [13]. We investigated by immunohistochemistry and Western blotting whether the aquaporin-0 protein is expressed in the retina of the rat, and whether its expression is altered after retinal ischemia.

All experiments were done in accordance with the European Communities Council Directive 86/609/EEC, and were approved by the local authorities. Adult female Long-Evans rats weighing 250–350 g were held under 12 h:12 h light:dark (day–night) room conditions, with free access to food and water.

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Transient retinal ischemia was induced in one eye of three animals while the other eye remained untreated and served as control. Anesthesia was induced by intramuscular ketamine (100 mg/kg body weight) and xylazine (5 mg/kg). The anterior chamber of the treated eye was cannulated from the pars plana with a 30-gauge infusion needle, connected to a bag containing normal saline. The intraocular pressure was increased to 160 mm Hg for 60 min by elevating the saline bag. The animals were killed by carbon dioxide 3 days after reperfusion, and the eyes were removed.

Isolated retinas were fixed in 4% paraformaldehyde for 2 h. After several washing steps in buffered saline, the tissues were embedded in saline containing 3% agarose (w/v), and 70 μm thick slices were cut with a vibratome. For cryosections of whole eyes, the eyes were fixed in 4% paraformaldehyde overnight, embedded in sucrose (30%), and immersed in optimal cutting temperature compound (Tissue-Tek; Sakura Finetek, Zoeterwoude, The Netherlands) overnight. Sections (12 μm) were cut with a cryostat and were mounted on gelatin-coated slides. For double-labeling, the slices were incubated in 5% normal goat or donkey serum plus 0.3% Triton X-100 in saline for 2 h at room temperature and, subsequently, in a mixture of primary antibodies overnight at 4 °C. After washing in 1% bovine serum albumin in saline, secondary antibodies were applied for 2 h at room temperature. Control slices were stained without primary antibodies; no unspecific labelling was observed following incubation with secondary antibodies (not shown). Images were taken with a confocal laser scanning microscope LSM 510 Meta (Zeiss, Oberkochen, Germany). The pinhole was set at 172 μm; the thickness of the optical section was adjusted to <0.8 μm. The following antibodies were used: rabbit anti-aquaporin-0 (1:200; Alpha Diagnostic), goat anti-aquaporin-4 (1:200; Santa Cruz), goat anti-γ-crystallins (1:100; Santa Cruz), mouse anti-protein kinase α (PKCα; 1:100; Santa Cruz), mouse anti-protein kinase βI (PKCβ; 1:100; Santa Cruz), guinea pig anti-vesicular glutamate transporter-1 (vGlut1; 1:500; Synaptic Systems), mouse anti-synaptobrevin (1:200; Synaptic Systems), mouse anti-calbindin (1:200; Swant), Cy3-conjugated goat anti-rabbit IgG (1:400; Dianova), Cy2-coupled goat anti-mouse IgG (1:400; Dianova), Cy3-coupled donkey anti-goat IgG (1:400; Dianova), Cy2-coupled donkey anti-rabbit IgG (1:200; Dianova), and Cy2-coupled donkey anti-guinea pig IgG (1:100; Dianova).

To purify cell nuclei, retinal tissues were lysated and homogenized within 2 ml of a homogenization buffer containing 0.23 M sucrose, 20 mM MgCl2, 20 mM Tris–HCl, 1 mM dithiothreitol, and 0.1% (v/v) Triton X-100 (pH 7.4), by using a Potter S homogenization device. After addition of 3.6 ml homogenization buffer containing 1.8 M sucrose (without dithiothreitol and Triton X-100), the homogenates were placed onto a 1.8 M sucrose solution, and centrifuged at 38,000 × *g* for 45 min. The

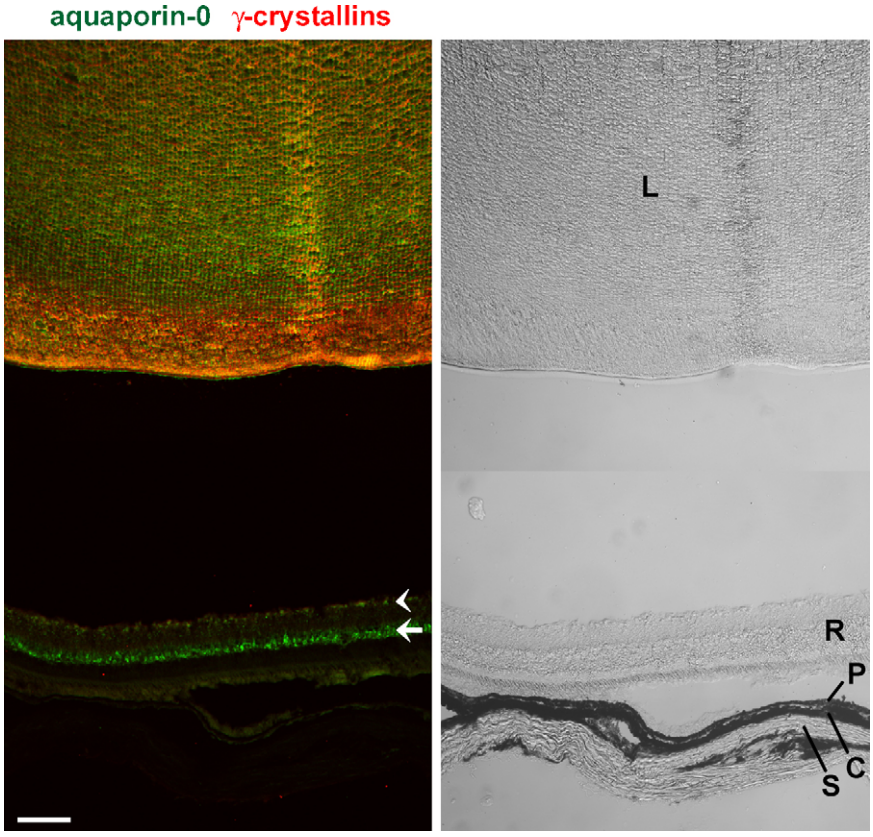

Fig. 1. Localization of the immunoreactivities for aquaporin-0 and γ-crystallins in the rat eye. A cryosection was immunostained against both proteins (left). Co-labeling yields a yellow-orange merge signal. Right: Differential interference contrast image. While the lens (L) is immunopositive for both proteins, the neural retina displays immunoreactivity for aquaporin-0 in the inner nuclear layer (arrow) and at the border between the inner plexiform layer and the ganglion cell layer (arrowhead). C, choroidea; P, pigment epithelium; R, neural retina; S, sclera. Bar, 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)