Light- and electron-microscopic analysis of aquaporin 1-like immunoreactive amacrine cells in the rat retina

In-Beom Kim, Eun-Jin Lee, Su-Ja Oh, Jin-Woong Chung, Myung-Hoon Chun
Department of Anatomy, College of Medicine, The Catholic University of Korea

Background: The first stages in the neural processing of image motion take place within retina. Some types of ganglion cells, which are the output neurons of the retina, are stimulated by image movement in one direction, but are inhibited by movement in opposite direction (Barlow and Levick, 1965). This phenomenon is called "direction selectivity (DS)". DS ganglion cells receive many synaptic inputs with one type of amacrine cells termed starburst cholinergic amacrine cells (Famiglietti, 1992). Cholinergic amacrine cells form asymmetric relationship between inputs from bipolar cells and outputs onto DS ganglion cells (Famiglietti, 1991), and release both excitatory acetylcholine and inhibitory γ -aminobutyric acid (GABA) onto DS ganglion cells (Brecha et al., 1988; O'Malley et al., 1992). Thus, they have been assumed to act as an origin in DS mechanism. Recently, however, it has been reported that laser-irradiated disruption of rabbit cholinergic amacrine cells still maintains DS responses of ganglion cells (He and Masland, 1997). This finding has raised a question concerning the implication of cholinergic amacrine cells in DS mechanism, and the origin of DS remains obscure. Therefore, the origin, the cellular locus and the synaptic mechanisms of DS have needed to be elucidated.

Aquaporin 1 (AQP1, also known as CHIP, channel forming integral membrane proteinof 28 kDa) is the first identified protein to function as a water channel and has been recently shown to be present in the rat retina. Our previous study (Kim et al., 1998) has shown the AQP1-like immunoreactivity was present in a certain population of amacrine cells (Fig. 1).

Figure 1. Light photographs taken from 40-μm-thick vertical vibratome sections of the rat retina processed for AQP1-like immunoreactivity. Immunoreactivity is seen in two somata of amacrine cells located in the inner nuclear layer (*INL*). Their dendrites branch and ramify in stratum 2 and in the borders of strata 3 and 4 of the inner plexiform layer (*IPL*), and thus, two bands of labeled processes are visible in the IPL. The outer nuclear layer (*ONL*) and the outer plexiform layer (*OPL*) are also immunoreactive. Scale bar = 50 μm.

AQP1-like immunoreactive (IR) amacrine cells ramify their dendrites in stratum 2 and in the border of strata 3 and 4 of the IPL (Fig. 1), where the dendrites of cholinergic amacrine cells ramify. This study was conducted in order to further characterize these AQP1-like IR amacrine cells in the rat retina.

Methods: Single- and double-labeling immunocytochemistry, confocal microscopy, and immunoelectron microscopy were performed.

Results: With immunocytochemistry using specific antisera against AQP1, wholemount preparations and 50-μm-thick vibratome sections were examined at the light and electron microscopic level. AQP1-like IR amacrine cells had symmetric bistratified dendrites. Their dendritic field ranged from 90 to 230 μm. Double-labeling with antisera against AQP1 and GABA or glycine demonstrated that these AQP1-like IR amacrine cells exhibited glycine immunoreactivity. Double-labeling with antisera against AQP1 and choline acetyltransferase (ChAT) demonstrated that strata of the AQP1-like IR amacrine cell dendrites costratified with those of cholinergic amacrine cell dendrites (Fig. 2). These light microscopic features of AQP1-like IR amacrine cells in the rat retina are similar to those of DAPI-3 amacrine cells in the rabbit retina.

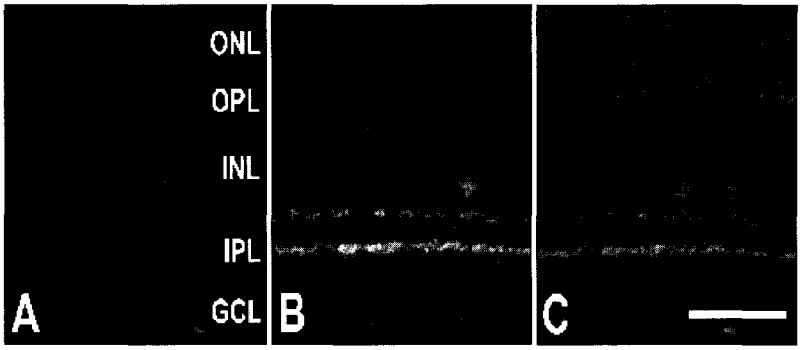

Figure 2. Confocal micrographs taken from a vertical vibratome section processed for AQP1 (A) and ChAT (B) immunoreactivities. AQP1 immunoreactivity was visualized using a Cy3-conjugated secondary antibody (red); ChAT immunoreactivity was visualized using a FITC-conjugated secondary antibody (green). A. One AQP1-like immunoreactive amacrine cell is visible. B. ChAT immunoreactive processes stratify in sublaminae a and b of the inner plexiform layer (IPL), forming a prominent band. C. Co-stratification of AQP1-labeled processes and ChAT-labeled processes is clearly noted. ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; GCL, ganglion cell layer. Scale bar = 50 μm.

Their synaptic features were also investigated by using immuno-electron microscopy. Their most frequent synaptic input is from other amacrine cell processes in both sublaminae a and b of the IPL (Fig. 3A), followed by a few cone bipolar cells (Fig. 3B). Their frequent output synapses are onto other amacrine cells and ganglion cells in both sublaminae a and b of the IPL (Fig. 4).

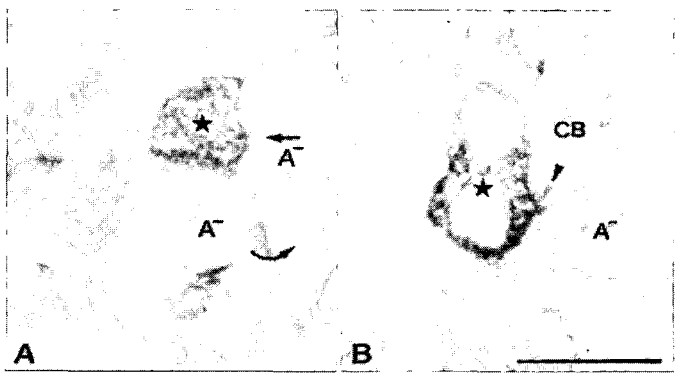

Figure 3. Electron micrographs showing input synapses of AQP1-like IR amacrine cells through the IPL of rat retina. A. A cell process (*star*) receives synaptic input (*arrow*) from an unlabeled amacrine cell process (*A-*), which is postsynaptic (*curved arrow*) to another unlabeled amacrine cell process (*A-*) in stratum 2 of the IPL. B. A cell process (*star*) forms a postsynaptic dyad with an unlabeled amacrine cell process (*A-*) at the ribbon synapse (*arrowhead*) of a cone bipolar axon terminal (*CB*) in stratum 3 of the IPL. Scale bar = 0.5 μm

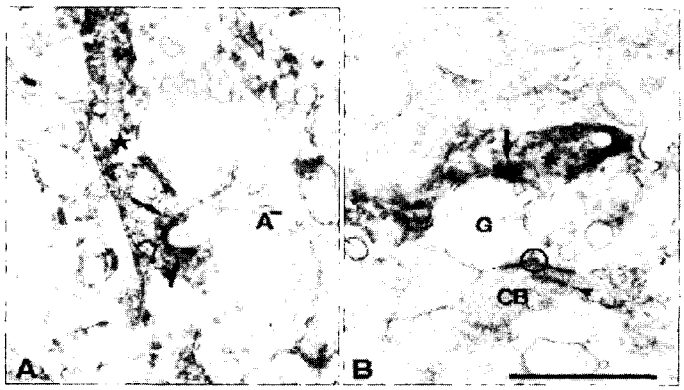

Figure 4. Electron micrographs showing output synapses of AQP1-like IR amacrine cells in sublamina a of the IPL. A. A labeled amacrine cell process (*star*) makes an output synapse (*arrows*) onto an unlabeled amacrine cell process (A-) in stratum 2 of the IPL. B. A labeled amacrine cell process (*star*) makes an output synapse (*arrow*) onto an unlabeled ganglion cell process (*G*) which comprises a postsynaptic dyad at the putative ribbon synapse (*circle*) of a cone bipolar axon terminal (*CB*) in stratum 2 of the IPL. Arrowhead indicates a synaptic ribbon. Scale bar = 0.5 μm

Conclusion: Although more detailed studies are clearly needed to elucidate the nature presynaptic sources and postsynaptic targets of AQP1-like IR amacrine cells in the rat retina, these findings suggest they play an important role in DS mechanism in the rat retina.