Investigating hAQP4 Inhibition and its Protein-Protein Interactions

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The human body contains 13 protein water channels called aquaporins (AQPs). Among these, AQP4 is the most abundant isoform found in the human brain [1]. Specifically, human AQP4 (hAQP4) exhibits a polarized localization at the endfeets of astrocytes, which is essential for a healthy brain. Bidirectional water flow across the blood-brain barrier facilitates brain water homeostasis, regulation of intracranial pressure and waste clearance [2]. Multiple neurological diseases, such as Alzheimer's disease and brain oedema [3], but also the most severe form of brain tumour, glioblastoma, have been associated with dysfunction in the molecular mechanisms of hAQP4 [4]. To find new therapies for treatment and strategies for diagnosis, it is therefore of major interest to understand the regulation of hAQP4, focusing on affecting the channel directly where it sits in the membrane and/or influencing the polarized localization of the protein in the cell. Specific inhibition of hAQP4 has proven to be very difficult where potent regulators of the function are rare and various systems for evaluation commonly give non conclusive results [5]. Therefore, we screened several established and putative AQP4 inhibitors, together with other therapeutics targeting neurological disorders, for inhibition using a water permeability assay. Recombinant hAQP4 was reconstituted into liposomes followed by incubation with selected compounds. The proteoliposomes were then rapidly mixed with a hyperosmolar solution in a stopped-flow mixing system to cause vesicle shrinkage, which was monitored by 90° light scattering. Our findings suggest that established AQP4 inhibitors might not have a direct effect on AQP4 while drugs for neurological disorders may have a putative effect on the water channel.

The second approach is to elucidate the translocation pathway of AQP4 by identifying specific interaction partners and membrane protein complexes [6]. A cDNA library representing fragments of protein expressed in the brain and recombinant hAQP4 are cloned with complementary YFP fragments and evaluated using bimolecular fluorescence complementation (BiFC) after transformation into *Sacharomyces cerevisiae*. Cells exhibiting high fluorescence frequency were isolated using fluorescence-activated cell sorting (FACS) and the corresponding DNA fragments were extracted and identified by sequencing after amplification by PCR. Among our initial hits from the screen we found the established regulatory protein calmodulin as well as nicastrin; an interesting putative interaction partner of AQP4, being a subunit of the gamma secretase complex.

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