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Mootaz Salman

Sheffield Hallam University/ Biomedical research Center, UK

The mechanism of Aquaporin expression and translocation in cerebral pathologies

Water is the principal constituent of cells and tissues in the animal kingdoms, and water exchange is essential for life. Water can pass through cell membranes by diffusion, but the rapid control of water flow into and out of cells in continually changing osmotic environments is mediated by a family of membrane proteins called aquaporins (AQPs), which are required to ensure appropriate membrane permeability to water molecules. At least 13 members of this family (named from AQP0 to AQP12) occur in mammals and are subdivided according to their Permeability characteristics into three major functional groups: 1) water-channels, 2) aquaglyceroporins, and 3) AQPs of unknown specificity. The wide distribution of AQPs throughout the body and their involvement in many physiologies and pathologies makes them a valuable and important target for drug therapies. Water homeostasis in the brain is crucial for maintaining the normal function of the central nervous system (CNS), which is considered to be very sensitive to any raise in intra-cranial pressure. Because of the rigid brain encasement, brain oedema could rapidly turn into a serious, life-threatening condition. It has been suggested that AQPs play a key role in maintaining brain homeostasis. At least six AQPs have been identified and characterized in the rodent brain: 1, 3, 4, 5, 8, and 9; 1, 4 and 9 are the best-studied examples. This project aimed to identify and study the molecular tools that could manipulate the translocation of brain AQPs as promising drug targets. Plasmid DNA encoding AQP4-, AQP1-, or AQP9-GFP fusion protein-was transfected into an immortalised HEK293 cell line; secondly into a more physiologically relevant cell line of U373 MG astrocytes and primary rat astrocytes. The responses of these AQPs were visualised following hypotonicity/hypertonicity-mediated translocation using confocal microscopy. The transfection protocol and reagents were optimized for each AQP in the different cell lines. Microarray on primary human astrocytes has been used to investigate the possible mechanisms involved in the neuroprotection effect. RT^2 Profiler PCR Arrays were used to confirm the transcriptional capacity along with quantitative real-time RT-qPCR for AQP1, 3, 4, 5, 8, 9 and calmodulin. Sandwich ELISA has been used to investigate the protein levels of AQP4. Cell surface biotinylation (CSB) has been to validate AQP4 translocational profile. In this work, successful optimisation of transfection of the investigated brain AQPs (AQP4, AQP1, and AQP9) in both HEK293 and U373 MG astrocytes, was achieved. The study also showed rapid and reversible, hypotonicity-mediated AQP translocation to the cell membrane in both HEK and U373 MG cells and may indicate the role of hypertonicity-mediated internalization of AQPs back to the cell for AQP4 and AQP1 in HEK293 cells. CSB data confirm; for the first time, the translocational profile of endogenous AQP4 in primary astrocytes. The microarray data and KEGG pathway analysis suggested the involvement of MAPK and the want signalling pathways which is confirmed by the data from 184 different genes from the RT^2 Profiler PCR Arrays. All the investigated cerebral AQPs genes were expressed and RT-qPCR data show significant upregulation AQP4 accompanied by a significant down-regulation of AQP1,5,9 and calmodulin. ELISA results confirm those findings for the investigated AQP4 and proteins. In conclusion, AQPs are important drugs targets and have a role in many CNS pathologies. Understanding the molecular mechanism of their regulation could lead to novel drugs that target the AQPs in astrocytes rather than the neurons which will have a far less adverse drug reactions.

Biography

Mootaz Salman is a pharmacist and researcher PhD student at the Biomolecular Sciences Research Centre (BMRC) at Sheffield Hallam University, working with Prof. Nicola Woodroofe and Dr. Matthew Conner's research group and a member of the multi-institute Aquaporin research collaboration. His research interest focus on the identification of new drug targets for brain oedema and epilepsy through his work on the special water channels called "Aquaporins". Mootaz graduated with Outstanding Distinction in his MSc winning the Sheffield Hallam University prize for the most scientific contribution and ranked 1st in year. His PhD research requires the skilled use of numerous techniques ranging from standard biochemical and molecular biology to cutting edge micro-array and laser confocal microscopy. Mootaz is an international ambassador at Sheffield Hallam University, ambassador for British Society of Experimental Biology (SEB); and STEM ambassador since 2014. He has given four invited oral presentations and talks at major international conferences in Canada, Netherlands, Romania and Japan along with three talks at a national level. He is also an active member in a number of scientific societies nationally and internationally including USA, Canada and Japan. He has been selected to be an abstract reviewer at two major international conferences; Brain 2015 in Canada and also for the upcoming Brain 2017 in Berlin, along with being an abstract reviewer and a member of poster judgement panel during the North of England Postgraduate Conference (NEPG) which is the UK's largest annual postgraduate conference for medical biosciences. Mootaz has successfully participated in organising a number of national and international high profile conferences and also he has been selected to chair scientific sessions at two national events.

mootaz_84@yahoo.com