총 설

Minireview on Renal Aquaporins

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The discovery of aquaporin membrane water channels (AQPs) by Agre and coworkers answered a long-standing biophysical question of how water crosses biological membranes specifically, and provided insight, at the cellular and molecular level, into the fundamental physiology of water balance and the pathophysiology of water balance disorders. The Agre lab, Johns Hopkins University, worked on identifying the function of Rhesus polypeptides and in the process of isolating a 32-kDa bilayer-spanning polypeptide component of the red cell Rh blood group antigen a 28-kDa polypeptide was partially copurified which displayed a number of biochemical characteristics. It was comprised of hydrophobic amino acids and exhibited an unusual detergent solubility allowing purification and biochemical characterization. The NH_2-terminal amino acid sequence was identified that subsequently allowed cDNA cloning. This protein was first known as "CHIP28" (channel-like integral protein of 28 kDa), but is now called aquaporin-1 or AQP1.

Expression of AQP1 in Xenopus laevis oocytes demonstrated that AQP1-expressing oocytes exhibited remarkably high osmotic water permeability (P_f = 150 to 200 x 10^{-4} cm/s), causing the cells to swell rapidly and explode in hypotonic buffer. The osmotically induced swelling of oocytes expressing AQP1 occurs with a low activation energy and is reversibly inhibited by $HgCl_2$ or other mercurials. Only inward water flow (swelling) was examined, but it was predicted that the direction of water flow through AQP1 is determined by the orientation of the osmotic gradient. Consistent with this, it was later demonstrated that AQP1-expressing oocytes swell in hyposmolar buffer but shrink in hyperosmolar buffer. Highly purified AQP1 protein from human red blood cells was reconstituted into proteoliposomes and were compared with liposomes without AQP1. AQP1 proteoliposomes exhibited P_f which is up to 50-fold above that of control liposomes, but permeability of urea and protons is not increased, indicating that AQP1 is water selective. As demonstrated in oocytes, AQP1 reconstituted in liposomes also displayed sensitivity to $HgCl_2$, which dramatically and reversibly reduced the osmotic water permeability. The AQP1-containing liposomes also exhibited a low activation energy for water permeation. Together, these studies indicated that AQP1 is both necessary and sufficient to explain the well-recognized membrane water permeability of the red blood cell and strongly suggests that AQP1 water channels are of fundamental importance for transmembrane or transcellular water transport in tissues where it is expressed.

Out of at least 10 aquaporin isoforms, at least 7 (AQP1, -2, -3, -4, -6, -7, and –8) are known to be present in the kidney at distinct sites along the nephron and collecting duct. Aquaporin-1 (AQP1) is extremely abundant in the proximal tubule and descending thin limb where it appears to be the main site for proximal nephron water reabsorption. It is also present in the descending vasa recta. AQP2 is abundant in the collecting duct principal cells and is the chief target for the regulation of collecting duct water reabsorption by vasopressin. Acute regulation involves vasopressin-induced trafficking of

AQP2 between an intracellular reservoir in subapical vesicles and the apical plasma membrane. In addition, AQP2 is involved in chronic/adaptational control of body water balance, which is achieved through regulation of AQP2 abundance. AQP3 and AQP4 are basolateral water channels located in the kidney collecting duct and represent exit pathways for water reabsorbed via AQP2. Additional aquaporins have been identified in kidney, including AQP6 that is expressed at the collecting duct intercalated cells in an entirely intracellular distribution. Two additional aquaporins (AQP-7, and –8) are also expressed in kidney, but less is known about their pathophysiological function.

Urinary concentration and dilution depend on the presence of a discrete segmental distribution of transport properties along the renal tubule, and urinary concentration depends on 1) the hypertonic medullary interstitium, which is generated by active NaCl reabsorption as a consequence of countercurrent multiplication in water-impermeable nephron segments and 2) the high water permeability (constitutive or vasopressin regulated) in other renal tubular segments for osmotic equilibration, which chiefly depends on aquaporins (AQPs). Thus defects in any of these mechanisms would be predicted to be associated with urinary concentrating defects. In particular, vasopressin is a peptide hormone that controls systemic osmolality through regulation of renal water excretion/reabsorption. Its main site of action is the renal collecting duct, where it regulates water transport, urea transport, and Na^+ transport. In collecting duct principal cells, vasopressin binds to a Gs-coupled receptor, the V_2 receptor, which stimulates an increase in intracellular cyclic AMP content via adenylyl cyclase. Binding of vasopressin to the V_2 receptor is also associated with intracellular calcium mobilization mediated by calcium release from ryanodine-sensitive intracellular stores via the type I ryanodine receptor, which triggers calmodulin-dependent regulatory processes within the cell. Many of the actions of vasopressin in the collecting duct are mediated by short-term responses that do not involve activation of gene transcription, such as stimulation of aquaporin-2 trafficking to the apical plasma membrane and activation of the urea transporter UT-A1 through phosphorylation. However, vasopressin has clearly long-term actions to alter the abundance of aquaporin-2, aquaporin-3, Na-K-2Cl cotransporter (NKCC2) and the epithelial Na channel (ENaC) subunits. Importantly, multiple studies have now emphasized a central role of AQP2 in several inherited and acquired water balance disorders. This includes inherited forms of nephrogenic diabetes insipidus, acquired states of nephrogenic diabetes insipidus (ex, hypercalcemia, hypokalemia, lithium treatment), and other diseases associated with urinary concentrating defects where AQP2 abundance and/or targeting is affected (ex, acute and chronic renal failure, ureteral obstruction). Conversely, AQP2 abundance and targeting appears to be increased in some conditions with water retention such as pregnancy and congestive heart failure. These long-term actions are thought to be associated with regulatory processes at a transcriptional level, involving either the transporter genes themselves or regulatory molecules that indirectly alter transporter protein abundance. Consistent with this, there is known to be a cAMP-responsive element in the 5`-flanking region of the AQP2 gene. Activation of PKA causes phosphorylation of a cAMP-response element binding protein (CREB protein), which binds to the DNA and increases transcription of the gene. It is worth emphasizing that CREB can be activated through phosphorylation by kinase other than PKA. In particular, both calmodulin-activated kinase II and calmodulin-activated kinase IV can phosphorylate CREB

at Ser-133, the same site phosphorylated by PKA. Therefore, regulated increases in intracellular calcium concentration could potentially increase AQP2 gene transcription through activation of calmodulin. Agents known to increase intracellular calcium in the IMCD include carbachol, ATP, and PGE2. Interestingly, the ATP-mediated increase in intracellular calcium was inhibited by the nonsteroidal anti-inflammatory agent indomethacin, which also decreases AQP2 abundance in the inner medulla. Studies using a fragment of the AQP2 promoter linked to a reporter gene expressed in cultured cells indicate that this cAMP response element operates synergistically with an AP1 binding site, which is activated by the binding of c-fos/c-jun. Production of c-fos itself was also stimulated via activation of PKA. However, this study also found that the time course of activation process was more rapid, and of shorter duration, than the increase in AQP2 expression seen in vivo, suggesting that other factors modify and prolong the response. Others have suggested that an AP2 site may also mediate the response to cAMP, although no similar effect in different cell lines was seen. A number of other transcription factor binding sites, including two GATA boxes, have also been identified, although their functional significance remains to be determined. Some may act as repressors of transcription, explaining how AQP2 expression is restricted to the collecting duct, and why collecting duct cells rapidly lose their AQP2 expression in culture.

The availability of antibodies and cDNA probes for the aquaporins expressed in the kidney has now permitted extensive study of the role of aquaporins in the physiology and pathophysiology of water balance. Further studies will concentrate more on defining the physiological and pathophysiological roles at the integrated level of each aquaporin, including identification of novel aquaporins and their regulation.

References

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