AQP2 Shuttling and its Binding Protein Complex*

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Introduction

Regulation of the water permeability of the apical plasma membrane of kidney collecting duct principal cells is critical to the regulation of renal water excretion and body water balance. Aquaporin-2 (AQP2) is the predominant vasopressin-regulated water channel protein of the renal collecting duct principal cells where it constitutes the major route of water movement across the apical plasma membrane. Vasopressin rapidly increases the osmotic water permeability of the collecting duct epithelium by binding to V2-receptors in the basolateral plasma membrane and inducing the cAMP-dependent translocation of AQP2-containing intracellular vesicles in the cytoplasm to the apical plasma membrane of the collecting duct principal cells. Although this fundamental mechanism is established, specific intracellular protein targeting pathways involved and protein-protein interactions of proteins in AQP2-expressing vesicles or plasma membrane are not clearly understood.

Vasopressin regulation of AQP2 trafficking

The final concentration of the urine depends on the medullary tonicity built up by the loop of Henle, and the high osmotic water permeability of the collecting ducts. The osmotic water permeability in the collecting duct is regulated by vasopressin. The identification of the aquaporins (AQPs) and subsequently AQP2, the predominant vasopressin-regulated water channel in the collecting duct, has allowed detailed insight into the fundamental physiology of renal water metabolism and pathophysiology of altered renal water handling in the body.

AQP2 is localized in the apical and subapical parts of collecting duct principal cells, and immunoelectron microscopy revealed that AQP2 is abundant both in the apical plasma membrane and in the subapical vesicles. As illustrated in Fig. 1, a marked redistribution of AQP2 from intracellular vesicles to the apical plasma membrane occurs in response to vasopressin stimulation. The collecting duct represents the final site for the control of water excretion into the urine. Water permeability of the collecting duct is tightly regulated under the control of the vasopressin which allows reabsorption of water from the tubular fluid down an osmotic gradient. Vasopressin binds to the V2-receptors present in the basolateral membrane of collecting duct principal cells. Acting through the GTP-binding protein GS, the interaction of vasopressin with the V2 receptor activates adenylyl cyclase, which accelerates the production of cAMP from ATP. Subsequently, cAMP binds to the regulatory subunit of protein kinase A, resulting in dissociation of the regulatory subunit from the catalytic subunit. This activates the catalytic subunit, which phosphorylates

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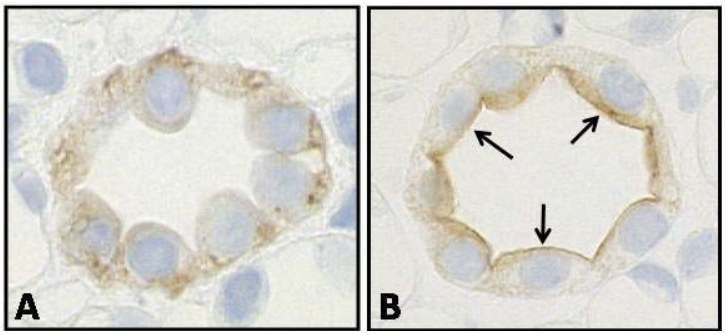

Fig. 1. Effects of short-term dDAVP (2 h) on AQP2 trafficking in vasopressin-deficient Brattleboro rats.

various proteins including AQP2. AQP2 is then translocated from intracellular vesicles to the plasma membrane thereby increasing the water permeability of the apical plasma membrane. When vasopressin is removed, water permeability returns to basal levels, reflecting endocytic retrieval of AQP2 water channels, which may subsequently be available for re-use. Consistent with this, vasopressin treatment of rats in vivo was found to be associated with redistribution of AQP2 to the apical plasma membrane of collecting duct cells, whereas treatment with vasopressin V2-receptor antagonist produced an internalization of AQP2 from the apical plasma membrane to intracellular vesicles and multivesicular bodies.

AQP2 contains a consensus site for PKA phosphorylation in the cytoplasmic COOH terminus (Serine 256). Using AQP2-transfected LLCPK1 cells it was shown that PKA mediated phosphorylation of Ser-256 is critical for vasopressin-induced trafficking of AQP2 from intracellular vesicles to the plasma membrane. Consistent with this, immunohistochemistry revealed that phosphorylated AQP2 (Serine 256) is localized at both the apical plasma membrane and intracellular vesicles, and immunoblotting demonstrated that phosphorylation of this serine was regulated via vasopressin-V2-receptors in rat kidneys. Thus, it appears likely that PKA phosphorylation of AQP2 is likely to be involved in the regulated trafficking of AQP2 to and from the plasma membrane. It still remains unclear whether phosphorylation of other serines is involved in the regulated exocytic or endocytic events, although a recent study revealed large increase in Ser269 phosphorylation following dDAVP in cultured mpkCCD cells.

Vesicle targetting receptors, the so called SNARE proteins, are believed to play a key role in synaptic vesicle targetting, docking and fusion. VAMP2, which is a vesicle-SNARE, has been found associated with AQP2-bearing vesicles, and recently, target SNAREs such as syntaxin-4 and SNAP23 has been identified in collecting duct principal cells using RT-PCR and immunoctychemistry where they are localized in the apical plasma membrane of collecting duct principal cells. This supports the view that SNARE vesicle targeting receptors may play a role in vasopressin regulation of AQP2-trafficking. However, functional data to support this view is awaited.

The cytoskeleton has been known to be involved in the AQP2 trafficking in kidney collecting duct. In particular, the microtubular network has been implicated in this process, since chemical disruption of microtubules inhibits the increase in permeability both in the toad bladder and in the mammalian collecting duct. Thus, AQP2 vesicles may be transported along microtubules on their ways to the apical plasma membrane. Consistent with this, the microtubule-based motor protein dynein and the associated protein Arp1, which is part of the protein complex dynactin, was found by immunoblotting to be among the proteins associated with AQP2 vesicles from rat inner medulla. Actin filaments are also involved in the hydrosmotic response. Recently evidence was provided that the myosin light chain kinase (MLCK) pathway, through calmodulin-

mediated calcium activation of MLCK, leads to phosphorylation of myosin regulatory light chain and non-muscle myosin 2 motor activity. Studies in isolated perfused rat inner medullary collecting ducts showed the MLCK-inhibitors ML-7 and ML-9 reduce the vasopressin-induced increase in water permeability, indicating that MLCK may be a downstream target for the vasopressin-induced Ca2+ signal.

In addition, we utilized a simple multi-layer fluidic device and demonstrated that the luminal fluid shear stress (FSS) *per se* increased AQP2 translocation to the plasma membrane in the primary cultured inner medullary collecting duct cells, which was associated with F-actin depolymerization despite the absence of AVP stimulation. This suggests that the reorganization of actin network is essential for promoting AQP2 translocation to the membrane. Recently, small GTPase Rho is known to regulate AQP2 trafficking through regulation of actin cytoskeleton. Inactivation of RhoA by phosphorylation and increased formation of RhoA-RhoGDI complexes seem to control the dissociation of actin fibers seen after vasopressin stimulation. Consistent with this, forskolin-induced inhibition of RhoA leads to a partial depolymerization of actin cytoskeleton, promoting AQP2 translocation. Moreover, FSS modulates the activity of small GTPases in the endothelial cells and further studies are needed to understand the mechanistic details of how small GTPases regulate the response of collecting duct cells to FSS.

Summary and perspectives

Aquaporin-2 (AQP2) is the predominant vasopressin-regulated water channel protein of the renal collecting duct principal cells where it constitutes the major route of water movement across the apical plasma membrane. A number of signaling pathways is involved in the regulation of AQP2 trafficking in response to vasopressin stimulation including cAMP-dependent AQP2 phosphorylation, microtubular network, actin filaments, calcium, and so on. Recent studies exploiting LC-MS/MS proteomics and in vitro phage display technique are focusing on the identification of regulatory proteins associated with AQP2 trafficking and/or endocytosis.

References

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