

# A Minireview on Vasopressin-regulated Aquaporin-2 in Kidney Collecting Duct Cells

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The kidney collecting duct is an important renal tubular segment for the regulation of body water and salt homeostasis. Water reabsorption in the collecting duct cells is regulated by arginine vasopressin (AVP) via the vasopressin V2-receptor (V2R). AVP increases the osmotic water permeability of the collecting duct cells through aquaporin-2 (AQP2) and aquaporin-3 (AQP3). AVP induces the apical targeting of AQP2 and transcription of AQP2 gene in the kidney collecting duct principal cells. The signaling transduction pathways resulting in the AQP2 trafficking to the apical plasma membrane of the collecting duct principal cells, include AQP2 phosphorylation, RhoA phosphorylation, actin depolymerization and calcium mobilization, and the changes of AQP2 protein abundance in water balance disorders have been extensively studied. These studies elucidate the underlying cellular and molecular mechanisms of body water homeostasis and provide the basis for the treatment of body water balance disorders.

**Key Words:** Arginine vasopressin, Aquaporins, Body water balance

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## Body Water Balance and Water Channel Proteins in the Kidneys

The Kidneys regulate body water and sodium balance<sup>1)</sup>. Absorption of water in the kidney tubule depends on the driving force for water reabsorption and osmotic equilibration of water across the tubular epithelium. The majority of glomerular filtrate is constitutively reabsorbed by the proximal tubules and descending thin limbs of Henle's loop. The ascending thin limbs, thick ascending limbs, and distal convoluted tubules are relatively impermeable to water and they deliver the tubular fluid into the connecting tubules and collecting ducts. The collecting ducts are importantly involved in the regulation of body water balance, since vasopressin-regulated water reabsorption occurs in this tubular segment<sup>2,3)</sup>.

Body water balance is achieved and finely regulated by a number of cellular and molecular processes, including kidney tubular reabsorption/secretion of water and

sodium through water channels (aquaporins: AQPs) and sodium transporters<sup>1-5)</sup>. Since water can slowly diffuse through lipid bilayers, all biological membranes exhibit some degree of water permeability<sup>6)</sup>. Nevertheless, renal tubular epithelial cells have to have the plasma membranes which have distinctively high water permeability for water transport and urinary concentration. Consistent with this, an identification of aquaporin membrane water channel provided a novel insight into the physiology of water balance and the pathophysiology of water balance disorders<sup>7,8)</sup>.

Thirteen mammalian aquaporins have been identified. Three major subtypes of aquaporins are known: (1) the classical aquaporins (AQP1, -2, -4, and -5), which are water-selective channels transporting water molecules; (2) aquaglyceroporins (AQP3, -7, -9, and -10), which are permeated by small uncharged molecules in addition to water; and (3) unorthodox aquaporins (AQP6, -8, -11, and -12), whose function is currently being studied. Of the known aquaporins, eight aquaporins (AQP1, -2, -3, -4, -6,

-7, -8, and -11) are expressed in mammalian kidney<sup>1-3,5</sup>. These renal aquaporins are expressed in (1) the proximal tubules, (2) the descending thin limbs and vasa recta, and (3) the collecting ducts.

In the proximal tubule which has an extraordinary high water permeability<sup>9,10</sup>, AQP1 is abundantly expressed in the apical and basolateral plasma membranes<sup>11</sup>. The AQP1 expression contributes to the near-isosmotic fluid reabsorption that is driven by an active sodium transport *via* Na, K-ATPase. In the descending thin limbs and descending vasa recta, AQP1 expression is involved in the countercurrent exchange process of the renal medulla<sup>12</sup>. The high water permeability of the descending vasa recta lowers blood flow to the inner medulla by shunting water to ascending vasa recta and hence it allows perfusion of the renal medulla without washing out the salt and urea gradients necessary for urine concentration<sup>12</sup>.

In the collecting ducts cells, vasopressin-regulated water channel AQP2 is highly abundant in the apical plasma membrane and subapical vesicles<sup>13,14</sup>. Water reabsorption in the collecting duct is regulated by both short-term regulation and long-term adaptational mechanisms, both of which are mainly dependent on AQP2 expression<sup>1-3,5</sup>. Because the collecting duct is the final site for regulation of renal water excretion, the changes in both AQP2 trafficking and protein abundance, and hence the changes in the water permeability of the collecting duct could be involved in a variety of disease conditions demonstrating altered capacity to concentrate urine<sup>1-3,5</sup>. In contrast to the apical water transport through AQP2, water transport across the basolateral plasma membrane of the collecting duct principal cells are mediated by AQP3 and AQP4<sup>15,16</sup>. Rats with lithium-induced nephrogenic diabetes insipidus have dramatically reduced apical AQP2 and basolateral AQP3 expression levels, along with a marked polyuria and urinary concentrating defect<sup>17,18</sup>. Moreover, transgenic mice lacking AQP3 are severely polyuric<sup>19</sup> and the inner medullary collecting ducts from AQP4 deficient mice have a significant reduction in vasopressin-stimulated water permeability<sup>20</sup>. Therefore, these findings demonstrate that basolateral membrane water

transport can also play a critical role in water reabsorption.

## Regulation of Body Water Homeostasis by Vasopressin

Vasopressin is a peptide hormone that controls plasma osmolality through the regulation of renal water excretion/reabsorption<sup>21</sup>. The feedback mechanism involving the hypothalamus, the posterior pituitary gland, and the kidneys play a key role in the whole body osmotic regulation<sup>1</sup>. The Verney receptor in the hypothalamus senses the changes of plasma osmolality and peptide hormone vasopressin is released when plasma osmolality rises to a level above a physiological threshold (290-295 mOsm/KgH<sub>2</sub>O for most individuals). The main action site of vasopressin is the kidney collecting duct, where it regulates water, urea, and sodium transport<sup>1-3,5,21</sup>. In the kidney collecting duct principal cells, vasopressin binds to the basolateral G-protein coupled vasopressin V2 receptors that, through a complex regulatory mechanism, results in increased osmotic water transport across the epithelium of the collecting duct, returning filtered water back to the blood. The ability of vasopressin to decrease water excretion occurs largely through actions on the renal collecting duct cells that result in the regulation of two molecular water channels, AQP2 and AQP3.

## Regulation of Renal Aquaporin-2 by Vasopressin

The signaling transduction pathways involved in the apical trafficking and endocytosis of AQP2, and the changes of AQP2 protein abundance, have been extensively studied (Table 1). AQP2 plays a key role in both short-term regulation and long-term adaptation of collecting duct water permeability<sup>1-3,5,21</sup>. Short-term regulation is the process by which vasopressin rapidly increases water permeability of collecting duct principal cells by stimulating vasopressin V2-receptor (V2R) in the basolateral plasma membrane and translocation of AQP2 from intracellular vesicles to the apical plasma membrane. This response was seen with-

**Table 1.** Intracellular signaling pathways for AQP2 trafficking or endocytosis

Trafficking
cAMP/PKA signaling pathway
Intracellular calcium ( $\text{Ca}^{2+}$ ) mobilization (calcium-calmodulin-mediated myosin activation)
PI3K-dependent activation of AKT
AS160 Phosphorylation
RhoA-dependent cytoskeletal dynamics
Endocytosis
Clathrin-mediated endocytosis
Ubiquitination of AQP2 C-terminus at K270
PGE2
Dopamine

in 5 to 30 minutes after increasing the peritubular vasopressin concentration. Long-term adaptation of collecting duct water permeability is seen when circulating vasopressin levels are increased over a period of hours to days, resulting in an increase of the AQP2 abundance per cell in the collecting ducts. This process allows urine concentration and is essential for water balance homeostasis.

### Cellular and Molecular Mechanisms for the Regulation of AQP2

Vasopressin V2 receptor is expressed not only in the principal cells of the collecting duct, but also in the cells of the thick ascending limb and the cells of the distal convoluted tubule<sup>22</sup>. In the thick ascending limb, vasopressin regulates active sodium chloride reabsorption, thereby playing a role in both countercurrent multiplication and luminal dilution. Vasopressin also accelerates active sodium chloride reabsorption in the distal convoluted tubule, the site of action of the thiazide diuretics. Despite these actions on sodium chloride transport, the most important action from the perspective of body water balance is on the collecting duct *via* the regulation of two water channels, AQP2 and AQP3.

The short-term regulation of AQP2 occurs as a result of membrane trafficking<sup>14,23</sup>. In the absence of vasopressin stimulation, AQP2 water channels were found predominantly in the recycling endosomes<sup>2,14</sup>. This was demon-

strated by the findings of colocalization of AQP2 and Rab11 protein, a marker of apical recycling endosomes<sup>24</sup>. In contrast, when vasopressin was added to isolated collecting ducts, AQP2 water channels were seen predominantly in the apical plasma membrane. These actions of vasopressin are associated with changes in the phosphorylation of the AQP2 protein at four serine sites (S256, S261, S264, and S269) near the C-terminus<sup>25-27</sup>. Exocytosis of AQP2 is associated with phosphorylation at S256<sup>28</sup>. Vasopressin also markedly increases phosphorylation at S269<sup>26</sup>. This phosphorylation event, which is increased by vasopressin, inhibits AQP2 endocytosis<sup>29,30</sup>. Vasopressin decreases phosphorylation of S261 by reducing the activity of one or more MAP kinases<sup>31,32</sup>. Phosphorylation at this site has been found to be associated with decreased stability of the AQP2 protein<sup>31</sup>. Moreover, phosphorylation of AQP2 could influence an interaction between AQP2-containing vesicles and the cell cytoskeleton, microtubules, or accessory cross-linking proteins. A recent study demonstrated that AQP2 phosphorylation could be affected by extracellular pH changes<sup>33</sup>.

The long-term regulation of AQP2 occurs as a result of a vasopressin-induced increase in the total abundance of the AQP2 protein in collecting duct cells. These long-term actions are thought to be associated with regulatory processes at the transcriptional or post-transcriptional level. The half-life of the AQP2 protein could be increased by vasopressin. In cultured mpkCCD cells, the half-life increased from 9 to 14 hours<sup>34</sup>. Vasopressin enhances AQP2 protein abundance by altering its proteasomal degradation through a PKA- and p38-MAP kinase dependent pathway<sup>31</sup>. AQP2 is degraded in the proteasome and lysosome<sup>35,36</sup>. The process of endocytosis and subsequent targeting to the proteasome and lysosome is thought to be regulated or ubiquitylation of the C-terminal tail of the AQP2 protein at lysine 270<sup>35</sup>. Identification of E3 ubiquitin-protein ligases specific to AQP2 degradation is currently under investigation<sup>36</sup>. The relationship between S269 phosphorylation induced by vasopressin and ubiquitylation at lysine 270 is a matter of current investigation. Moreover, transcription of the AQP2 gene is markedly

increased by vasopressin, resulting in increased cellular mRNA levels and increased AQP2 translation<sup>37</sup>). Transcriptional regulation of AQP2 is thought to be a result of vasopressin-induced increase in intracellular cAMP levels with concomitant increases in activation of protein kinase A.

In addition to the transcriptional regulation of AQP2, microRNA (miRNA) appears to play a role in post-transcriptional regulation through inhibition of translation of target mRNA *via* translational repression of the RNA-induced silencing complex (RISC)<sup>38</sup>). A recent study specifically predicted miRNAs targeting AQP2 expression by *in silico* analysis, and two predicted AQP2-targeting miRNAs (miR-32 and miR-137) were further investigated to understand a novel molecular mechanism of AQP2 protein regulation<sup>39</sup>). This study provides a novel insight on the regulation of AQP2 protein expression, at least in part, *via* RNA interference, i.e., AQP2-targeting miRNAs<sup>39</sup>). Other signal transduction pathways including prostaglandins, angiotensin II, aldosterone, PI3K/Akt pathways, cytoskeleton, intracellular Ca<sup>2+</sup> concentration, and vesicle-targeting receptors have been described in previous studies and reviews<sup>2,3,5,23,33,40-43</sup>).

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### Conflicts of Interest

Authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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