Molecular Physiology of Renal Aquaporins and Sodium Transporters: Exciting Approaches to Understand Regulation of Renal Water Handling

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The discovery of aquaporin membrane water channels by Agre and coworkers answered a long-standing biophysical question of how water crosses biologic membranes specifically, and provided insight, at the molecular level, into the fundamental physiology of water balance and the pathophysiology of water balance disorders. The importance of this achievement is underscored by awarding Peter Agre the Nobel Prize in chemistry in 2003. Of the known twelve aquaporin isoforms, at least eight are known to be present in the kidney at distinct sites along the nephron and collecting duct. 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. AQP2 is also present in the ascending 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 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 expression. Importantly, multiple studies have now underscored a critical 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, and other diseases associated with urinary concentrating defects where AQP2 expression and targeting is affected as recently reviewed (1).

Vasopressin also controls body fluid osmolality through the regulation of renal sodium (2) and urea reabsorption (3). Thus, the main vasopressin action sites in the kidney tubules are the collecting duct and the thick ascending limb (TAL) of the loop of Henle, where vasopressin binds to the vasopressin V2-receptor and stimulates an increase of intracellular cAMP content via adenylyl cyclase (4). Subsequently, cAMP activates protein kinase A (PKA), which phosphorylates various proteins including AQP2 and type 1 bumetanide-sensitive Na-K-2Cl cotransporter (NKCC2 or BSC-1) (5,6). Using so-called “antibody-based targeted proteomics,” long-term regulation of each of these renal transporters and channels can be examined in intact animals so as to understand the integrated response to various physiologic and pathophysiological conditions (7).

In this issue of JASN, Chen et al. (8) have shown that glucocorticoid-deficient rats challenged by 36 h of water deprivation exhibit impaired urine concentration with increased urine flow and decreased maximum urine osmolality. Using the targeted proteomics approach with semiquantitative immunoblotting to investigate whether there were changes in the abundance of primary renal sodium and water transport proteins, the study provides an understanding at the molecular level for the impaired urinary concentrating ability in glucocorticoid-deficient rats. It is well established that adrenal insufficiency impairs the renal capacity to maintain water balance, and in a previous study from the same group the role of mineralocorticoid deficiency in renal water balance regulation was highlighted (9). In that particular study, the authors demonstrated increased urine production and reduced urine osmolality in association with downregulation of NKCC2 and Na,K-ATPase in the outer medulla whereas AQP2 and AQP3 expression was increased in cortex and inner medulla (9). Hence, the present study (8), focusing exclusively on the effects of 10 d of glucocorticoid deficiency on urine concentration, extends the work in defining the changes in the expression of renal water and sodium transporting proteins during conditions with alterations in adrenal hormone levels. In a study by Schwartz and Kokko (10), in vitro–perfused cortical collecting ducts from adrenalectomized rabbits exhibited a markedly blunted hydro-osmotic response to vasopressin, and this was corrected by addition of either aldosterone or dexamethasone. However, the steroids themselves, in the absence of vasopressin, had no intrinsic effect on the water permeability of the collecting duct. With the results of the study by Chen et al. demonstrating that short-term glucocorticoid deficiency is associated with down-regulation of NKCC2 in the mTAL as well as AQP1 and urea transporter A1 (UT-A1) in the inner medulla and decreased AQP2 expression in both the outer and inner medullary collecting ducts, the authors provide an explanation at the molec-
ular level for the renal defects causing impaired urine concentration in response to glucocorticoid deficiency in rats. The result may seem somewhat surprising given that previous studies have addressed the effect of glucocorticoid deficiency on renal function and consistently found that glucocorticoid-deficient rats have impaired urinary dilution mainly due to nonosmotic stimulation of vasopressin secretion (11). The findings of reduced UT-A1 levels in the inner medulla may also seem somewhat surprising, as previous studies have demonstrated that glucocorticoid treatment is associated with reduced expression of UT-A1 (12). This may represent yet unknown or dose-dependent effects on transporter expression.

Chen et al. add another interesting dimension to the understanding of the role of glucocorticoids for regulation of renal water handling, because the changes appeared in the absence of changes in plasma vasopressin levels. Previously, it was suggested that the concentrating defect seen in glucocorticoid-deficiency was at least partly the result of the absence of the permissive effect that glucocorticoids exert on the vasopressin response. This may partly account for the observed changes in the study by Chen et al., because the decrease in the expression of Gsα protein in the outer medulla of the glucocorticoid-deficient rats may be associated with reduced cAMP generation and thereby reduced abundance of the protein. However, by the approach taken, the authors combine detailed examinations of renal function and analysis of cellular changes and nicely demonstrate complimentary molecular changes. The study incorporated detailed examinations of the circulatory parameters including measurement of mean arterial BP (MAP) and cardiac output. Importantly, the glucocorticoid-deficient rats had a decrease in cardiac output and MAP. Thus, the impairment in urine concentrating ability seen in glucocorticoid-deficient rats was associated with impaired countercurrent multiplication, diminished osmotic equilibration via reduced AQP2, and diminished urea equilibration via UT-A1. Thus, these molecular changes occurred primarily in the relative oxygen-deficient medulla and the results of the study by Chen et al. raise the possibility that the reduction in MAP, and hence in the perfusion of the kidney inner medulla, may play critical roles for the observed changes.

In this issue of *JASN*, another exciting paper demonstrates yet another important aspect of aquaporin function in renal disease. Mutations in the AQP2 gene cause autosomal recessive and dominant nephrogenic diabetes insipidus (NDI), and de Mattia et al. (13) demonstrate that lack of vasopressin-induced phosphorylation of the AQP2 mutant AQP2-R254L may explain dominant NDI. Normally, vasopressin binds to basolateral V2 receptors of the principal cells, activating adenylate cyclase through G proteins, leading to cAMP increase and thereby reduced abundance of the protein. However, it was not retained in the endoplasmic reticulum. From these observations, the authors hypothesized that the AQP2-R254L mutation interfered with proper phosphorylation of S256 and plasma membrane expression of the protein, as AQP-S256A, which mimics nonphosphorylated AQP2, is retained in vesicles in oocytes and in mammalian cells (12). Using the same technique to test whether the retention of AQP2-R254L in the MDCK cells is due to a lack of S256 phosphorylation, the authors subjected MDCK cells expressing AQP2-WT, AQP2-R254L or AQP2-S256A to orthophosphate labeling in the presence or absence of forskolin. The results of these studies indicated that the lack of phosphorylation at S256 by PKA may be the molecular reason for the impaired translocation of AQP2-R254L to the plasma membrane. Using this technique,
the authors have elegantly demonstrated how an important clinical finding can be addressed experimentally by directly showing the physiologic impact of a mutation in the AQP2 gene at the cellular and molecular level. In particular, the results of this study strongly indicate that dominant NDI in this family is due to a R254L mutation, resulting in loss of vasopressin-mediated phosphorylation of AQP2 at S256, and for the first time illustrates the in vivo importance of phosphorylation of AQP2 at S256.

References