Newer insights into renal regulation of water homeostasis

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The regulation of high osmolality is an important driving force for water reabsorption and urinary concentration—the key functions of the kidney for maintaining optimum body fluid volume. New evidence shows that transcription factor tonicity responsive enhancer binding protein (TonEBP) and calcineurin-nuclear factor of activated T cells through cross-talk enhance Aquaporin 2 (AQP2) expression. AQP2 is the predominant vasopressin regulated water channel of the kidney collecting duct and is essential for urinary concentration. The serine/threonine phosphatase calcineurin is an important signaling molecule involved in kidney development and function. One potential target of calcineurin action is the water channel AQP3. The nuclear factor of activated T cells (NFAT) family has recently been expanded by the discovery of a new member, NFAT 5, or Ton EBP. Ton EBP is the only known mammalian transcription factor that regulates gene expression in response to hypertonicity. This review examines the importance of AQP2, calcineurin, NFATc and TonEBP in the renal regulation of water homeostasis.

Keywords: Aquaporin 2, Calcineurin, NFAT, Renal, TonEBP

Water excretion is dependent on the corticopapillary osmotic gradient, which arises from interstitial accumulation of both urea and NaCl. This osmotic gradient, decreases during water diuresis and increases under conditions of antiurexia. The ultimate checkpoint for renal water reabsorption occurs at the level of renal collecting duct. High water permeability in this nephron segment is largely due to the presence of AQP2 inserted in the apical membrane of principal collecting duct cells. Although the antidiuretic vasopressin plays a significant role in regulating AQP2 expression, the recent evidence indicates that this event is additionally influenced by hypertonicity. AQP2 is the major vasopressin-regulated water channel of the kidney collecting duct and is essential for urinary concentration. AQP2, a highly glycosylated protein, is processed through the endoplasmic reticulum (ER) and Golgi network, where it is folded correctly and then targeted to vesicles that are directed to the subapical region of the plasma membrane. After this, the water permeability of the inner medullary collecting duct (IMCD) cells can be rapidly regulated by the antidiuretic hormone the arginine vasopressin through binding to vasopressin V2 receptors (V2R). The vasopressin V2 receptor mediated signal transduction is dependent on the phosphorylation by calcineurin phosphatase enzyme, and this action is more prominent during the state of water deprivation. Water reabsorption increases along the osmotic gradient together with tonicity responsive enhancer binding protein (TonEBP) expression. TonEBP is consequently highly expressed in the kidney medulla. The importance of TonEBP in renal physiology is gaining importance. In contrast, the role of such calcineurin-NFATc pathway in kidney functions which has been extensively described in diverse areas as immune, nervous and cardiovascular system is relatively less known. These are the newer insights into the regulation of water homeostasis by the renal system. Despite severe hyperosmotic stress imposed by NaCl and urea on cells of the renal medulla, the elevated and highly variable osmolality in this part of the kidney is necessary for proper functioning of the urinary concentration mechanisms. Comprehensive knowledge about the signaling network activated by hyperosmolality, and its molecular targets is needed to fully understand the role of hyperosmolality in the development, physiology and pathophysiology of the mammalian kidney.

Role of TonEBP

The TonEBP regulates gene expression in response to hypertonicity. Water reabsorption increases along the osmotic gradient together with TonEBP...
expression. TonEBP is consequently highly expressed in the kidney medulla and the importance of TonEBP in renal physiology is well recognized. Changes in environmental tonicity increases TonEBP activity by increasing the TonEBP nuclear localization, transactivation and abundance. The activity of OREBP/TonEBP is regulated at multiple levels including nucleocytoplasmic trafficking. TonEBP protein can be detected in both cytoplasm and nucleus under isotonic conditions and nuclear import is regulated by a nuclear localization signal. TonEBP contributes to the corticopapillary osmotic gradient by stimulating urea recycling between the ascending limb of Henle’s loop and the inner medullary collecting duct mainly by protecting cells from the deleterious effects of high urea, by regulating heat shock protein 70 (hsp70) expression and also by enhancing AQP2 expression. TonEBP stimulates transcription of the hsp70 gene response to hypertonicity, and protects renal medulla against high osmolality and molecular chaperones. TonEBP also plays an important role in protecting cells against hypertonicity by stimulating transcription of genes whose products help accumulate compatible osmolytes that lower intracellular ionic strength. TonEBP is a transcriptional activator of the Rel family that includes nuclear factor κ B (NFκB) and nuclear factor of activated T cell (NFAT). TonEBP/NFAT5 resembles NfkB/rel proteins in forming a stable dimer in solution in the absence of DNA, the dimerization is obligatory for DNA binding and transcriptional activity. Hypertonic conditions regulate TonEBP/NFAT5 at multiple levels including translational regulation, postranslational modification and subcellular distribution.

Hypokalemia causes a significant decrease in the tonicity of the renal medullary interstitium in association with reduced expression of sodium transporters in the distal tubule. TonEBP expression decreased significantly in the outer and inner medullas of hypokalemic rats. In the renal medulla, TonEBP plays a key role in protection of cells from the deleterious effects of hypertonicity and high urea. In addition to these protective effects, TonEBP stimulates expression of a renal medullary specific gene encoding the vasopressin-regulated urea transporters UT-A1, UT-A3, UT-A4. In cultured kidney cells, activity of TonEBP is stimulated in response to hypertonicity via several pathways. Hypertonicity increases mRNA and protein abundance of the transcription factor TonEBP activity by increasing the TonEBP nuclear localization, transactivation and abundance. The activity of OREBP/TonEBP is regulated at multiple levels including nucleocytoplasmic trafficking. TonEBP protein can be detected in both cytoplasm and nucleus under isotonic conditions and nuclear import is regulated by a nuclear localization signal. TonEBP contributes to the corticopapillary osmotic gradient by stimulating urea recycling between the ascending limb of Henle’s loop and the inner medullary collecting duct mainly by protecting cells from the deleterious effects of high urea, by regulating heat shock protein 70 (hsp70) expression and also by enhancing AQP2 expression. TonEBP stimulates transcription of the hsp70 gene response to hypertonicity, and protects renal medulla against high osmolality and molecular chaperones. TonEBP also plays an important role in protecting cells against hypertonicity by stimulating transcription of genes whose products help accumulate compatible osmolytes that lower intracellular ionic strength. TonEBP is a transcriptional activator of the Rel family that includes nuclear factor κ B (NFκB) and nuclear factor of activated T cell (NFAT). TonEBP/NFAT5 resembles NfkB/rel proteins in forming a stable dimer in solution in the absence of DNA, the dimerization is obligatory for DNA binding and transcriptional activity. Hypertonic conditions regulate TonEBP/NFAT5 at multiple levels including translational regulation, postranslational modification and subcellular distribution.

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**Importance of calcineurin-NFATc pathway**

Although TonEBP/NFAT5 shares amino acid sequence similarity with NFATc transcription factors, this similarity is limited to the DNA binding domain. NFATc proteins are substrates for calcineurin, a calcium – regulated serine-threonine phosphatase comprising of a catalytic subunit, calcineurin A (CNa) and calcineurin B (CNb). Calcium signaling activates the phosphatase calcineurin and induces movement of NFATc proteins into the nucleus, where they cooperate with other proteins to form complexes on DNA. Nuclear import is opposed by kinases such as GSK3, thereby rendering transcription continuously responsive to receptor occupancy.

Interplay between TonEBP and calcineurin-NFATc pathways may confer greater regulatory control over gene expression in response to diverse environmental signals. For instance, exposure of cells to conditions of high osmolarity initiates a series of events, including compatible osmolyte accumulation and cytokine elicitation, as part of an adaptation process that ultimately leads to cell survival. It can be speculated that converging TonEBP and calcineurin-NFATc pathways may provide the cell with the means to quickly adapt to a hyperosmotic environment. We are only beginning to understand the complex interplay between these pathways. Li et al suggested that water reabsorption is modulated by both TonEBP and calcineurin-NFATc pathways via controlled AQP2 transcriptional activity. NFATc activity, on the other hand, is mediated by a large number of environmental signals that induce a receptor mediated rise of intracellular Ca2+. The ensuing activation of the protein phosphatase calcineurin dephosphorylates NFATc leading to
exposure of NFATc nuclear localization sequences and nuclear import of NFATc protein. Several observations suggest to the proposal that NFATc is implicated in salt and water homeostasis by decreasing the COX-2 dependent mechanism, sodium reabsorption as a consequence of decreased Na⁺K⁺2Cl⁻ co-transporter activity in the medullary thick ascending limb and decreased activity in other nephron segments\textsuperscript{24}. Calcineurin inhibition interferes with T cell signaling by preventing activation of the transcription factor NFATc (Fig.1).

**Importance of calcineurin and aquaporin 2 in kidney**

Elucidation of the specificity of calcineurin action in kidney is therefore an area of great interest. One potential level of calcineurin signaling specificity is in differential expression and/or action of calcineurin isoforms\textsuperscript{25}. Calcineurin is a serine/threonine phosphatase comprised of two subunits: the catalytic subunit A, which contains the phosphatase domain, the regulatory subunit B, which binds calcium and the A subunit. There are three closely related isoforms of the A subunit: α, β, and γ\textsuperscript{25}. In kidney, the expression of both α and β isoforms are detected in proximal tubules, collecting ducts (CD) and medulla\textsuperscript{26,27}. Areas of highest calcineurin activity are the proximal and distal tubules and correspond to predominant expression of α isoform. Recent data suggest that the serine/threonine phosphates calcineurin also regulates the trafficking of AQP\textsubscript{2}\textsuperscript{28}. First, calcineurin binds to at least two A kinase anchoring proteins (AKAP) including AKAP79, which is expressed in the kidney, in close proximity to PKA. Jo et al\textsuperscript{29} identified calcineurin in a complex with AQP\textsubscript{2} and an AKAP scaffold protein, and showed that calcineurin dephosphorylates AQP\textsubscript{2} in an *in vitro* assay. Calcineurin causes a net decrease in phosphorylation of AQP\textsubscript{2} and is associated with alterations in subcellular localization. It is possible that the increase in AQP\textsubscript{2} protein, observed with the inhibition of calcineurin, may be due to lower turnover of AQP\textsubscript{2} protein, either by shedding the apical vesicles into urinary space or by degradation after endocytosis, since the protein fails to complete its normal trafficking cycle. Lack of functional AQP\textsubscript{2} is seen in primary forms of DI and reduced expression and targeting are seen in several diseases associated with urinary concentrating defects such as acquired nephrogenic diabetes insipidus (NDI), post obstructive polyuria and in acute and chronic renal failure\textsuperscript{30}. Recent studies suggest that functional consequence of calcineurin inhibition with cyclosporin A was a decrease in phosphorylation and a redistribution of AQP\textsubscript{2} away from the apical membrane. Long term inhibition of calcineurin with cyclosporine A results in polyuria and decreased urine osmolality\textsuperscript{31}, and down regulation of several aquaporins including AQP\textsubscript{2}. Calcineurin knock out mice have been created by Zhang et al\textsuperscript{32}. These are a new model of NDI characterized by an impaired response to vasopressin as a result of altered trafficking and phosphorylation of AQP\textsubscript{2}. Previous studies suggest that α isoforms of calcineurin A subunit (CnAα) and AQP\textsubscript{2} colocalize in collecting duct principal cells of normal and diabetic rats\textsuperscript{33}. Gooch et al\textsuperscript{34} have reported that CnAα absent mice shows decreased phosphorylation of AQP\textsubscript{2} in response to vasopressin and significantly less AQP\textsubscript{2} protein is found in inner medulla collecting duct
vesicles, and consequently in the apical membrane. As a result of this, Cnα- absent mice becomes a model of NDI.

Apart from these recent observations that renal actions of cardiac vasoactive peptides may be possibly regulating water homeostasis by regulating some of the above mentioned mechanisms. In conclusion it can be stated that TonEBP and calcineurin-NFATc pathways may confer greater regulatory control and may provide the renal cell with a means to quickly adapt to a hyperosmotic environment.

Future studies should be on understanding the significance of high TonEBP expression in medullary endothelial cells and should provide us with more complete knowledge of renal functions of this transcription factor and also further defining the signaling mechanisms that underlie the TonEBP/NFAT osmotic stress response pathway.

References


