Aquaporin-2 abundance in the renal collecting duct: new insights from cultured cell models

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IN HUMANS, the vast majority of water of the glomerular filtrate (~180 l/day) is reabsorbed along the renal tubule and only ~1 liter of urine is excreted daily. About 70% of filtered water is reabsorbed by the proximal tubule through an isotonic mechanism. An additional 20% is passively reabsorbed along the thin descending limb of Henle’s loop. Because this part of the renal tubule is impermeable to solutes, the fluid of the tubule lumen to the interstitium initializes the generation of cortico-papillary osmotic gradient, generated by sodium reabsorption in the thick ascending limb. The antidiuretic hormone arginine vasopressin (AVP) increases collecting duct water permeability by enhancing aquaporin-2 (AQP2) water channel insertion in the apical membrane of principal cells, allowing water to passively flow along the osmotic gradient from the tubule lumen to the interstitium. In addition to short-term AQP2 redistribution between intracellular compartments and the cell surface, AQP2 whole cell abundance is tightly regulated. AVP is a major transcriptional activator of the AQP2 gene, and stimulation of insulin- and calcium-sensing receptors respectively potentiate and reduce its action. Extracellular tonicity is another key factor that determines the levels of AQP2 abundance. Its effect is dependent on activation of the tonicity-responsive enhancer binding protein that reinforces AVP-induced AQP2 transcriptional activation. Conversely, activation of the NF-κB transcriptional factor by proinflammatory factors reduces AQP2 gene transcription. Androgen additionally regulates AQP2 whole cell abundance by simultaneously reducing AQP2 gene transcription and stimulating AQP2 mRNA translation. These examples illustrate how cross talk between various stimuli regulates AQP2 abundance in collecting duct principal cells and consequently contributes to maintenance of body water homeostasis.

water transport; osmolality; vasopressin

The antidiuretic hormone arginine vasopressin (AVP) is a key hormone for osmoregulation and consequently plays a pivotal role in the maintenance of body homeostasis. The hormone is synthesized as a preprohormone precursor in the stroma of hypothalamic neurons of the supraoptic nucleus. Packaged in neurosecretory granules that traffic along the axon, it is stored in axon terminals of the posterior pituitary gland until being released into the blood in response to small variations of plasma osmolality. By binding to vasopressin receptor type 2 (V2R), AVP increases water reabsorption through multiple mechanisms. It enhances both active NaCl reabsorption by the thick ascending limb and passive urea reabsorption in medullary CD that together reinforce the cortico-papillary osmotic gradient required for water reabsorption in medullary CD. In addition, AVP directly increases the water...
permeabilization of the apical membrane of principal cells, thereby stimulating CD water reabsorption along the osmotic gradient (61). By enhancing NaCl reabsorption in the CD (2, 62), AVP secondarily increases interstitial osmolality at the basal side of cells, promoting vectorial water movement across CD principal cells.

Aquaporins (AQP) water channels are present in the membrane lipid bilayer of numerous cell types, endowing the membrane in which they are inserted with considerable water permeability. The group of Peter Agre (14) identified the first aquaporin in human erythrocytes, which earned him the 2003 Nobel Prize in chemistry. This gene was cloned (64) and later named aquaporin-1. To date, 13 AQP isoforms expressed in human tissue have been reported (44). Five AQP isoforms so far have been shown to be involved in renal water reabsorption. AQP1 insertion in the plasma membranes of epithelial cells of the proximal tubule and thin descending limb, and endothelial cells of the descending vasa recta, plays a major role in facilitating proximal water reabsorption (57, 87). While AQP1 expression is generally considered to be constitutively expressed in renal epithelial cells, it should be noted that its expression in cells of the thin descending limb was reported to augment in response to increased interstitial osmolality (78). Along with AQP1, AQP7 is expressed at the apical membrane of proximal straight tubules (S3 segment) (73). AQP7-knockout mice showed that while this AQP plays an important role in glycerol reabsorption, its role in proximal water reabsorption is small compared with that of AQP1 (73). At the level of the CD, principal cells express at least three different AQP isoforms. AQP2 is present in intracellular vesicles and at the cell surface, and both its intracellular redistribution and whole cell abundance are highly regulated (61). While AQP2 plasma membrane localization is mostly apical, it is also expressed at the basolateral cell surface (80). Indeed, the basolateral localization increases along the CD, with maximal levels observed in the distal inner medulla, suggesting that AQP2 basolateral expression is induced by extracellular tonicity. AQP3 and AQP4 are located at the basolateral side of CD principal cells. AQP4 expression is especially abundant in medullary parts of the CD (61). While the expressional regulation of these two aquaporins is not as important as that observed for AQP2, they are modulated by various factors such as AVP and extracellular tonicity (44, 72). Sodium, urea, and water transporters/channels that play key roles in renal water reabsorption are schematically depicted in Fig. 1. The remainder of this review focuses on factors that regulate AQP2 whole cell abundance.

Critical Role of Vasopressin in Regulated AQP2 Abundance in Renal Collecting Duct Principal Cells

V2R is most expressed at the basolateral side of principal cells, where it couples with heteromultimeric G protein. After AVP binding, V2R and G proteins dissociate, liberating G protein αs-subunits. These activate plasma membrane-bound adenyl cyclases, leading to an increase of intracellular cAMP concentration and protein kinase A (PKA) activity. This signalization cascade leads to the translocation of AQP2, stored in intracellular vesicles, to the apical membrane of cells, thereby increasing apical membrane water permeability. This occurs within minutes of the initial stimulation. The mechanisms that govern this process are described in detail in several recent reviews (5–7, 79) and are beyond the scope of this review. This review highlights recent advances made on long-term control of AQP2 whole cell abundance by various stimuli that are schematically illustrated in Fig. 2.

Shortly after AQP2 was cloned by the group of Sei Sasaki (23), AVP was shown to control not only its cellular distribution but also its whole cell abundance. In Brattleboro rats, which have no circulating AVP and hypotonic urine, AQP2 expression is much lower than that of normal animals. Administration of AVP to these animals led to an increase of AQP2 synthesis and concentrated urine (15). The opposite effect was observed in normal animals administered a V2R antagonist (36). These results were among the first to show that AVP stimulates AQP2 expression in CD principal cells and that AQP2 is necessary for water reabsorption. Parallel variations of circulating AVP and AQP2 expression observed in both animal models and human pathologies further supported the regulatory role of AVP on AQP2 expression. Indeed, AQP2 expression in rats increases under conditions of water restriction and decreases under conditions of water overload (60). Similarly, AQP2 expression increases under conditions of nonosmotic stimulation of AVP secretion, as observed in states of chronic heart failure (83) and liver cirrhosis (21).

The identification of inactive AQP2 mutants in patients diagnosed with nephrogenic diabetes insipidus (NDI) (12) together with the creation of a mouse model of NDI produced by AQP2 gene deletion (84) confirmed the key role that AQP2 plays in water reabsorption along the CD. NDI patients excrete large amounts of hypotonic urine (up to 20 l/day) that must be compensated by high fluid intake. NDI can either be inherited or, more commonly, acquired (45, 61). Acquired NDI can be manifested in numerous pathophysiological conditions, such as hypokalemia, hypercalcemia, ureteral obstruction, and renal failure, or as a side effect of drug treatment such as lithium. Congenital NDI can be either dominant or recessive (4). Autosomally linked NDI is the less common form of inherited NDI and has been linked to various mutations in the AQP2 gene that render this type of NDI either dominant or recessive depending on the ability of mutant AQP2 to associate with wild-type AQP2 isoforms, leading to the retention of AQP2 heterotetramers in either the endoplasmic reticulum (ER) or the Golgi complex (45, 51). X-linked dominant NDI is a severe condition resulting from mutagenic loss of V2R function leading to decreased AQP2 expression (45). To date, more than 180 putative mutations have been identified in the V2R gene of families with X-linked NDI. Deficient V2R function can arise from insufficient expression of functional V2R protein at the plasma membrane reflecting defects in mRNA stability, aberrant splicing, or deletions of parts of the V2R protein arising from frameshift mutations or premature termination of mRNA translation. The majority of mutations found in X-linked NDI cause misfolding of the V2R protein, ER retention, and subsequent proteasomal degradation. A few mutations were additionally found to impair the coupling efficiency of the receptor to the stimulating G protein or to reduce AVP binding affinity. With the exception of a few rare cases of a milder form of NDI manifested around 10 yr of age, X-linked NDI is mostly manifested in the first weeks of life. Symptoms of congenital NDI during the first year of life, if not treated properly, include anorexia, vomiting, fever, growth retardation, and developmental delay. After infancy, the symptoms are dominated by...
polyuria and polydipsia, with mental retardation being the most severe complication of the disorder.

The creation by the group of Alain Vandewalle of a mouse cell line (mpkCCDcl4) that displays the essential functionalities characteristic of CD principal cells (3) has lent important insight to our understanding of mechanisms that govern AQP2-regulated expression. While these cells express very low levels of AQP2 expression under basal conditions, both AQP2 mRNA and protein expression are strongly induced in response to AVP. This induction is both dose- and time dependent (31, 33) and depends on basolateral V2R stimulation. Basolateral membrane voltage provides the energy necessary to couple sodium and bicarbonate transport out of the cell by sodium bicarbonate cotransporter (NBC). Transcellular water movement is mediated by aquaporin (AQP)1 inserted at both apical and basal membranes. The osmotic gradient increases along the cortico-medullary axis. The urine-concentrating mechanism is principally mediated by Na⁺-K⁺-2Cl⁻ cotransporter (NKCC2) expressed at the apical side of thick ascending limbs of Henle’s loop and secondarily by Na⁺-Cl⁻ cotransporter expressed at the apical side of cells of the distal tubule. Sodium transport across the basal membrane of these cells is mediated by NaK. In the inner medulla, interstitial accumulation of urea by urea transporter A isoform (UT-A1) and UT-A3 expressed in IMCD largely contributes to the osmotic gradient. Note that UT-A3 polarity appears to be species dependent. Driven by the osmotic gradient, water crosses cells via AQP1, expressed at the apical and basal sides of descending limbs of Henle’s loop, and AQP2 and AQP3s 3 and 4, expressed at the apical and basal sides of CD principal cells, respectively. Epithelial sodium channel (ENaC)- and NHE, -2, and -4-mediated sodium transport by CD principal cells may help water reabsorption via increased osmolality of the intercellular space. PCT, proximal convoluted tubule; CTAL, cortical thick ascending limb; MTAL, medullary thick ascending limb; DCT, distal convoluted tubule; CCD, cortical collecting duct; Na/Glucose, sodium-glucose cotransporter.

were made in rats (15, 36). The experimental data collected from animal and cell models show that AVP increases mRNA encoding the AQP2 protein (31, 60). Results from our lab (33) indicate that AVP does not affect the stability of AQP2 mRNA. Its increased expression is therefore most likely due to an increase of AQP2 gene transcription. This interpretation is consolidated by the presence of a binding site specific for cAMP-responsive element binding protein (CREB) in the AQP2 promoter located immediately upstream of the AQP2 transcription start site. Both CRE and AP-1 elements of the AQP2 promoter were shown to mediate AVP-induced AQP2 gene transcription (39, 56, 85). Our experimental data further suggest that AQP2 expression depends on an unknown factor that prevents AQP2 gene transcription and whose effect is relaxed by its proteasomal degradation. Indeed, the induction of AQP2 gene transcription by AVP was abolished in cells pretreated with pharmacological proteasomal inhibitors.
Previous observations based on in vitro data also suggested such negative control of AQP2 gene transcription mediated by factors acting on specific regions of the AQP2 promoter (22, 66).

During the preparation of this review, a report was published by the group of Mark Knepper (86) describing AQP2 transcription regulation with a systems biology-based approach. Among the numerous putative binding elements identified by computational analysis, including those that bind ETS, HOX, RXR, CREB, and GATA transcription factors, comprehensive transcriptomic profiling carried out in mpkCCDc14 cells established a positive correlation between AQP2 and Ets1 expression as well as a negative correlation between AQP2 and Elf1 (a member of the ETS family) and Nr1h2 (a member of the retinoid X receptor family) expression. Overexpression of three transcriptional regulators of the ETS family (Elf3, Elf5, and Elf6) increased AQP2-dependent luciferase activity in LLC-PK1 cells. Contrary to the effect of Elf3, those produced by Elf5 and Elf6 overexpression were dependent on high levels of cAMP. These results provide further evidence that AQP2 transcription is a multifactorial process (see below) that only partly depends on AVP.

Fig. 2. Controlled AQP2 abundance in the renal collecting duct principal cell. Numerous extracellular stimuli contribute to controlling AQP2 whole cell abundance by acting on AQP2 gene transcription, AQP2 mRNA translation, or AQP2 protein degradation. Shown are pathways mediated by arginine vasopressin (AVP, red), hypertonicity and/or inflammation (blue), insulin (orange), aldosterone (green), and extracellular calcium (brown). Increased AQP2 abundance by AVP stimulation results from adenyl cyclase activation, increased intracellular cAMP concentration, and protein kinase A (PKA) activation. PKA in turn increases both AQP2 gene transcription, via increased cAMP-responsive element binding protein (CREB) and AP-1 activity, and AQP2 lysosomal and proteasomal protein degradation. NF-κB activation by inflammatory stimuli, such as lipopolysaccharide (LPS) and tumor necrosis factor-α (TNF-α), or hypertonicity reduces AQP2 gene transcription. After longer periods of hypertonic challenge, tonicity-responsive enhancer binding protein (TonEBP) increases AQP2 gene transcription. Insulin increases AQP2 gene transcription via a mechanism that depends on ERK and p38 MAP kinase activity as well as phosphatidylinositol 3-kinase (PI3-kinase) activity. Aldosterone decreases AQP2 gene transcription, possibly via NF-κB, and increases AQP2 mRNA translation via mechanisms that depend on mineralocorticoid receptor (MR) activity. Activation of extracellular calcium-sensing receptor (CaSR) by extracellular calcium decreases AQP2 transcription via a calmodulin-dependent mechanism by reducing coupling efficiency between vasopressin type 2 receptor (V2R) and adenyl cyclase. PM, plasma membrane; ER, endoplasmic reticulum; TLR-4, toll-like receptor 4; TRFR, TNF-α receptor; IR, insulin receptor; IRS, insulin receptor substrate protein; MAPK, mitogen-activated protein kinase; VP, vasopressin; IP3, inositol 1,4,5-trisphosphate.
Calpain-mediated AQP2 proteolysis has additionally been demonstrated in purified inner medullary CD (IMCD) endosomes (65). Transcriptional induction is not the only way by which AVP modulates AQP2 whole cell abundance. Indeed, uncoupled expression between AQP2 mRNA and protein has been described under certain conditions. For instance, hypercalcemic rats display polyuria accompanied by decreased expression of AQP2 protein, but not mRNA, in IMCD (69). Similarly, decreased expression of AQP2 protein, but not mRNA, was observed in cortical CD of fasted animals (1, 82). An analysis of time-dependent AQP2 mRNA and protein kinetics following AVP challenge performed by our group on mpkCCD14 cells (33) revealed that while AQP2 mRNA steadily increased over time, AQP2 protein first decreased immediately after AVP challenge and then increased after longer periods of time. Our analysis further revealed that AVP increases PKA-dependent lysosomal and proteasomal AQP2 protein degradation (33). PKA therefore not only promotes AQP2 gene transcription, but retroactively controls AQP2 protein abundance by promoting its degradation. Mechanisms induced by AVP and other stimuli described below that control AQP2 whole cell abundance are schematically depicted in Fig. 2.

**Insulin and Extracellular Calcium Modulate Effects of Vasopressin on AQP2 Abundance**

In addition to AVP, AQP2 whole cell abundance depends on the net effect of numerous stimuli acting in tandem with each other. These either affect the AVP-cAMP-PKA pathway or act independently of it. This review will now focus on these stimulatory factors. In the 1970s it was shown that diuresis is reduced by insulin under hyperinsulinemic eglycemic conditions (13). An increase of water permeability in isolated medullary CD perfused with insulin was demonstrated later on (53). Using mpkCCD14 cells, we were able to show (8) that insulin, acting alone, induces a slight increase of AQP2 protein whole cell abundance and significantly enhances AVP-induced AQP2 mRNA content without altering AQP2 mRNA stability, its transcription, or AQP2 protein degradation. In the light of these findings, insulin most likely contributes to the transcriptional activation of the AQP2 gene induced by the cAMP-dependent signalization pathway. The enhancing effect of insulin on AVP-induced AQP2 expression depends on the simultaneous activation of several signalization pathways, notably those involving phosphatidylinositol 3-kinase, ERK MAP kinase, and p38 MAP kinase activation (8). Insulin may enhance water reabsorption not only by acting on AQP2 expression but also by potentiating sodium reabsorption by stimulating both epithelial sodium channel (17) and Na⁺-K⁺-ATPase (19). Together, these events may enhance the water-sparing effect of AVP during the postprandial period.

In contrast to insulin, extracellular calcium attenuates the effect of AVP on AQP2 whole cell abundance. NDI associated with hypercalcemia is well known to clinicians and is at least partly due to activation of the extracellular calcium-sensing receptor (CaSR) present in basolateral membranes of cells of the thick ascending limb (68). After ligand binding, this receptor, coupled to heteromultimeric G protein, activates the phospholipase C-PKC pathway (38). This leads to a reduction of active ion transport along the thick ascending limb (10), which in turn reduces the magnitude of the cortico-papillary osmotic gradient and subsequently the ability to concentrate urine. CD cells, which express the CaSR at their apical pole, also play a role in hypercalcemia-linked polyuria. This was deduced from observations made on microperfused, isolated medullary rat CD exposed to high levels of luminal calcium that displayed decreased levels of water reabsorption (70) and observations made in hypercalcemic rats that displayed decreased renal CD AQP2 expression (16, 69). Our group has recently confirmed this hypothesis (9) by showing that an increase of calcium concentration at the apical medium of mpkCCD14 cells grown on filters attenuated the enhancing effect of AVP on AQP2 expression at both mRNA and protein levels. This effect relies on V₁R and adenylylate cyclase decoupling and is mediated by CaSR activation. Indeed, an increase of extracellular calcium attenuated, in a calmodulin-dependent manner, AQP2 transcription mediated by AVP but not that mediated by forskolin, a direct activator of adenylylate cyclases (9). Altogether, these observations indicate that an increase of luminal calcium concentration decreases CD water permeability and urine-concentrating ability by decreasing AQP2 whole cell abundance. This regulatory mechanism could alleviate calcium salt precipitation and nephrolithiasis.

**Extracellular Tonicity Is a Key Factor for Regulated AQP2 Abundance**

A role for extracellular tonicity in the regulation of AQP2 expression was first suspected from animal studies. Water restriction was found to increase AQP2 whole cell abundance in normal rats administered with V₁R antagonists (54), while water loading decreased AQP2 content in rats administered AVP, i.e., under conditions of AVP escape (18). In aging rats, AQP2 whole cell abundance was found to decrease with medullary osmolality despite unchanged levels in cAMP concentration (63). Using mpkCCD14 cells exposed to medium made hypertonic by the addition of NaCl or sucrose, we were able to show in vitro that extracellular hypertonicity exerts a time-dependent biphasic effect on AQP2 expression (35). AQP2 mRNA expression and protein abundance first decrease during the first 3 h of hypertonic challenge and then increase after 24 h of exposure. To distinguish between hyperosmotic and hypertonic effects, we determined AQP2 expression levels in cells challenged with medium made hyperosmotic by the addition of urea. Urea decreased AQP2 mRNA and protein expression after 3 h of hyperosmotic challenge but did not stimulate their expression after longer periods of time (35).

Our observations showing an increase of AQP2 abundance in response to long-term hypertonic challenge are in good agreement with data collected from cultured outer and inner medullary CD cells (22, 76) and correlate well with observations made in water-restricted animals (54, 60). The increase of AQP2 expression in response to extracellular hypertonicity is independent of the cAMP-PKA pathway (35). A possible role played by tonicity-responsive enhancer binding protein (TonEBP), a transcription factor whose activity is regulated by extracellular tonicity, was first suggested by observations in mice lacking TonEBP activity (47, 52). By increasing the intracellular concentration of “compatible” organic osmolytes and heat shock protein 70, TonEBP plays a key role in protecting cells from the deleterious effects of hypertonicity.
and urea, respectively (40). Without these protective measures, cell function is perturbed and apoptotic cell death is initiated. This explains why the majority of animals lacking TonEBP activity die. Those that do survive display a defective urinary concentration ability associated with severe renal tubular atrophy (47, 52). Because the phenotype of renal CD cells of these animals is severely altered, we reassessed a regulatory role of TonEBP on AQP2 gene transcription, using mpkCCD_{14} cells transiently transfected with either TonEBP small interfering RNA (siRNA) or a dominant-negative TonEBP (DN-TonEBP) that lacks COOH-terminal transactivation domains. The phenotype of transfected cells is largely retained, as reflected by AVP- and aldosterone-sensitive electrogenic Na^+ transport (58, 74). Reduced TonEBP activity in mpkCCD_{14} cells was accompanied by an important loss of AQP2 mRNA and protein expression under both isotonic and hypertonic conditions (29). While AQP2 mRNA was further reduced after 3 h of hypertonic challenge in cells transfected with either TonEBP siRNA or DN-TonEBP, downregulated TonEBP activity abolished the hypertonicity-induced increase of AQP2 expression that occurred after longer periods of hypertonic stimulation. A TonEBP binding site is located within the first 600 bp of the AQP2 promoter, and mutation of this site abolished the hypertonicity-induced increase of luciferase activity in cells expressing AQP2 promoter-luciferase constructs (29). These observations indicate that TonEBP not only modulates AQP2 gene transcription in conditions of prevailing extracellular tonicity but also adjusts baseline levels of AQP2 gene transcription.

Recently, NFATc, which belongs to the same family of transcription factors as TonEBP, was found to increase AQP2 expression via an intracellular Ca^{2+} - and calcineurin-dependent pathway in response to hypertonicity (50). This process was found to depend on binding of this transcription factor to the AQP2 promoter. In addition to its effect on AQP2 abundance, acute hypertonic challenge induces the recruitment of intracellular vesicles containing AQP2 to the apical surface of principal CD cells within minutes through a mechanism that occurs independently of an increase of cAMP but depends on MAP kinase activation and AQP2 phosphorylation at S256 (34). Chronic hypertonic challenge, on the other hand, leads to its recruitment at the basolateral surface (80). Interestingly, loss of calcineurin activity decreases AVP-mediated AQP2 accumulation at the apical surface of IMCD cells despite increased cAMP levels in calcineurin α-null mice (26).

**Proinflammatory NF-κB Pathway Strongly Represses AQP2 Gene Transcription**

Polyuria and a decreased capacity to concentrate urine are frequently associated with both acute and chronic renal tubulointerstitial inflammation. Polyuria might result not only from anatomic but also functional abnormalities of the renal medulla that would lead to decreased expression of sodium transporters and aquaporins, as suggested by experimental animal models (27, 49). These observations led us to investigate a possible regulation of AQP2 expression by the proinflammatory NF-κB pathway. Generally, NF-κB subunits are sequestered in the cytoplasm as an inactive form when associated with the NF-κB inhibitor IκB, predominantly IκBα. Classical NF-κB activators, such as bacterial lipopolysaccharides (LPS) or tumor necrosis factor-alpha, induce activation of IKK kinases, leading to the phosphorylation, polyubiquitination, and subsequent proteasomal degradation of IκB, allowing translocation of released NF-κB to the nucleus. These bind to promoters of target genes, which activate or inhibit their transcription (25, 59). Polyuria associated with a strong decrease of AQP2 expression is observed both in animal models of obstructive uropathy induced by ureteral ligation and in sepsis models induced by intraperitoneal LPS injection (20, 27, 49). Our in vitro results from mpkCCD_{14} cells and the more recent mCCD_{11} (24) cell line as well as our ex vivo results obtained from rat kidney slices show that NF-κB activation by LPS induces a decrease of both AQP2 mRNA and protein (30). Decreased AQP2 expression induced by LPS occurred under basal conditions, in cells stimulated with either AVP or hypertonic medium, and in cells pretreated with either a V_{2}R antagonist or bromo-cAMP. Decreased AQP2 expression was found to result from binding of NF-κB complexes consisting of p50 and/or p52 to specific κB elements of the AQP2 promoter.

Because inflammatory diseases of the kidney are associated with increased NF-κB activity in response to cytokines secreted by leukocytes, our results strongly support the hypothesis that polyuria arises from a NF-κB-induced decrease of AQP2 gene transcription. This may be part of a generalized mechanism that counteracts extracellular volume expansion. Since TonEBP activity increases along the CD as a consequence of increased interstitial toxicity (28), decreased AQP2 expression in tubulointerstitial inflammatory diseases should be predominant in isotonic cortical regions, where most water reabsorption occurs. Our results further indicate that in more medullary parts of the CD NF-κB activity is additionally increased on the onset of hypertonic challenge (30). We speculate that decreased AQP2 expression mediated by NF-κB may help to reduce water loss via AQP2 inserted at the basolateral membrane of cells exposed to conditions of high interstitial toxicity (80), adding another protective role of NF-κB in renal CD principal cells. After longer periods of hypertonic stimulation, i.e., at a time when cells are fully adapted to their hypertonic environment, the stimulatory effect of TonEBP on AQP2 expression would supersede the repressive effect of NF-κB, resulting in an increase of AQP2 gene transcription (29, 30) and allowing the organism to cope with conditions of water restriction.

**An Example of Complex Regulation: Aldosterone**

Classically, sodium transport in CD principal cells is controlled by aldosterone, while AVP controls water transport. However, several pieces of evidence collectively show that AVP additionally stimulates NaCl reabsorption (2, 62, 67, 77) and that aldosterone influences water reabsorption (71, 81). Interpretation of the effect of aldosterone on AQP2 expression based on data collected from animals is complicated since aldosterone has been shown to either decrease (11, 46) or increase (41) AQP2 whole cell abundance and/or affect its subcellular localization (11). These contrasting observations most likely result from the effects of different mechanisms that regulate AQP2 expression, which themselves vary depending on the experimental conditions used. Indeed, using mpkCCD_{14} cells, we were able to show that aldosterone controls AQP2 expression via two distinct mechanisms whose final effects oppose each other (32). Aldosterone first decreases both AQP2
mRNA and protein expression after short intervals of time. After 48 h of incubation, however, aldosterone increases AQP2 protein expression by increasing AQP2 mRNA translation. These effects are mediated by the mineralocorticoid receptor since they occur in response to physiological concentrations of aldosterone (1 nM) and are inhibited by carbenoxolone, an aldosterone-competitive antagonist. In agreement with observations that suggest cross talk occurring between aldosterone and NF-κB signaling pathways (88), we recently demonstrated mineralocorticoid receptor-dependent NF-κB activation in CD principal cells (48). This may suggest that the NF-κB pathway mediates decreased AQP2 gene transcription by aldosterone.

As described above, we found that AQP2 protein content first decreases on the onset of either AVP or hypertonic stimulation as a result of increased AQP2 protein degradation and decreased AQP2 gene transcription, respectively. Aldosterone may contribute to this effect by decreasing AQP2 gene transcription. Similarly, increased AQP2 abundance after sustained periods of AVP or hypertonic stimulation may be enhanced by chronic aldosterone stimulation through an increase of AQP2 mRNA translation. Modulation of AQP2 abundance by aldosterone may be necessary for the prevention of hypo- or hyperosmolar states.

Conclusion

While the majority of studies have focused on regulatory mechanisms that control AQP2 distribution between subcellular compartments and the plasma membrane, new cell lines derived from mouse CD principal cells that display high mineralocorticoid receptor-dependent NF-κB signaling pathways (88), we recently demonstrated that this pathway mediates decreased AQP2 gene transcription. Similarly, increased AQP2 abundance after sustained periods of AVP or hypertonic stimulation may be enhanced by chronic aldosterone stimulation through an increase of AQP2 mRNA translation. Modulation of AQP2 abundance by aldosterone may be necessary for the prevention of hypo- or hyperosmolar states.

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Review

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