Vasopressin regulation of sodium transport in the distal nephron and collecting duct

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Kortenoeven ML, Pedersen NB, Rosenbaek LL, Fenton RA. Vasopressin regulation of sodium transport in the distal nephron and collecting duct. Am J Physiol Renal Physiol 309: F280–F299, 2015. First published June 3, 2015; doi:10.1152/ajprenal.00093.2015.—Arginine vasopressin (AVP) is released from the posterior pituitary gland during states of hyperosmolality or hypovolemia. AVP is a peptide hormone, with antidiuretic and antinatriuretic properties. It allows the kidneys to increase body water retention predominantly by increasing the cell surface expression of aquaporin water channels in the collecting duct alongside increasing the osmotic driving forces for water reabsorption. The antinatriuretic effects of AVP are mediated by the regulation of sodium transport throughout the distal nephron, from the thick ascending limb through to the collecting duct, which in turn partially facilitates osmotic movement of water. In this review, we will discuss the regulatory role of AVP in sodium transport and summarize the effects of AVP on various molecular targets, including the sodium-potassium-chloride cotransporter NKCC2, the thiazide-sensitive sodium-chloride cotransporter NCC, and the epithelial sodium channel ENaC.

The peptide hormone arginine vasopressin (AVP) is synthesized in specialized neurons of the supraoptic and paraventricular nuclei of the hypothalamus and, before release, is stored in the posterior pituitary gland (293). The most important stimulus for AVP release into the circulation is the osmolality of the plasma, predominantly the sodium chloride concentration, with increased plasma osmolality rapidly resulting in AVP secretion. AVP is also released during hypovolemia or hypotension, but while AVP secretion responds to minute changes in plasma osmolality, larger changes in blood volume are required to elicit a secretory response (11). However, severe reductions in blood volume result in very high AVP levels, as volume-induced AVP secretion occurs in an exponential manner (reviewed in Ref. 4).

The most important role of AVP is to regulate the body’s retention of water by increasing 1) the osmotic driving force for water reabsorption; and 2) transcellular water transport in the kidneys, resulting in increased urine concentration. AVP also has other physiological roles, which include increasing blood pressure by inducing moderate vasoconstriction, enhancing blood coagulation, increasing the release of adrenocorticotropic hormone (ACTH), and influencing learning, memory, and social behavior (reviewed in Refs. 137 and 287).

Regulation of renal transcellular water transport mainly occurs via modulating cell surface expression of the water channel aquaporin-2 (AQP2) (reviewed in Ref. 204). In addition, the regulation of sodium and urea transport by the kidneys is crucial for the maintenance of systemic water balance. NaCl reabsorption (resulting in dilution of the ultrafiltrate) and concentration of NaCl in the renal medullary interstitium (through the process of countercurrent multiplication), coupled with accumulation of urea in the medullary interstitium (via countercurrent exchange), creates a luminal-to-interstitial osmotic gradient, allowing diffusion of water out of the connecting tubule (CNT) and collecting duct (CD) into the interstitium, from where it is subsequently returned to the general blood circulation (reviewed in Ref. 82).

In this article, we describe and evaluate the reported effects of AVP on the regulation of renal sodium transport. We describe in detail the molecular targets of AVP, the role of these transport proteins (Fig. 1) in antidiuretic and antinatriuretic states, and the potential contribution of AVP to various pathophysiological conditions. In addition, a brief overview of the role of AVP for modulating water and urea transport processes in the collecting duct is also provided, focusing on the associated roles of sodium, urea, and water transport for urine concentration.

Sodium Transport in the Distal Nephron

In the initial portion of the distal nephron, the thick ascending limb (TAL), active transport of Na⁺ out of the cell via the
basolateral Na-K-ATPase results in a concentration gradient for Na⁺, which drives sodium from the ultrafiltrate into the TAL cell via the apical sodium-potassium-chloride cotransporter 2 (NKCC2). Sodium removal in the TAL results in dilution of the tubule fluid. Since the epithelium of the TAL is relatively water impermeable, sodium reabsorption in this segment acts as the "single effect" for countercurrent multiplication, resulting in an increased medullary interstitial osmolality and providing the basis for osmotic removal of water from the adjacent collecting ducts and hence urine concentration (reviewed in Ref. 82).

At the transition of the TAL and the distal convoluted tubule (DCT), the luminal NaCl content of the tubule fluid is sensed by specialized macula densa cells, which act as sensors in the tubuloglomerular feedback (TGF) mechanism (213, 252). Here, the intraluminal levels of NaCl elicit adjustments in the resistance of the afferent arteriole entering the glomerulus of the same nephron, thereby altering the single-nephron glomerular filtration rate dependent on the concentration of NaCl at the macula densa. This allows the kidneys to maintain a relatively equal delivery of NaCl to the distal nephron (reviewed in Ref. 252).

In the DCT and latter segments of the distal nephron, fine tuning of water and electrolytes in the tubular fluid occurs. Filtrate arriving from the neighboring TAL to the DCT is always hypotonic (108, 171), which is independent of the body’s hydration status. The DCT plays an important role in regulating the content of sodium, as well as other electrolytes, reabsorbed from the tubular fluid (reviewed in Ref. 176). Micropuncture studies have demonstrated that the DCT reabsorbs ~5–10% of total filtered sodium (73, 138), and it is classically considered part of the diluting segment of the renal tubule as it, like the TAL, is relatively water impermeable. The majority of sodium reabsorption in the DCT is thiazide sensitive (53) and mediated via the thiazide-sensitive sodium-chloride cotransporter (NCC).

In the CNT and initial cortical collecting duct (iCCD), the majority of the sodium reabsorption is amiloride sensitive (52) and thus most likely mediated via the epithelial sodium channel (ENaC). Amiloride-sensitive sodium currents are most prominent in the CNT and are estimated to be 10-fold greater than in the CCD (94). In addition to ENaC, the CCD can transport sodium via an electroneutral mechanism, involving the parallel action of the Na⁺-driven Cl⁻/HCO₃⁻ exchanger (NDCBE) and the Na⁺-independent Cl⁻/HCO₃⁻ exchanger (pendrin), present in the intercalated cells (161). Regulated sodium reabsorption also occurs in latter segments of the CD, mainly dependent on the concentration of the steroid hormone aldosterone. Only 0.1–3% of the total filtered load of sodium is excreted in the urine (reviewed in Ref. 110). The CNT and CD epithelia are, under nonstimulated conditions, relatively impermeable to water. However, upon stimulation with AVP they become highly water permeable (202, 273), which allows the osmotically driven movement of water from luminal tubule fluid into the interstitium. During antidiuresis, the hyposmotic ultrafiltrate at the initial DCT can reach isotonicity in the latter portion of the distal convoluted tubule as it (probably the CNT or iCCD), while the final urine will be hyperosmotic, reaching an osmolality of ~1,200 mosmol/kgH₂O in humans during antidiuresis (108, 171).

The importance of the “molecular machinery” in the distal tubule and CD in driving NaCl and water transport can be appreciated through several genetic disorders (see also Role of Vasopressin in Pathophysiological Conditions). Loss-of-function autosomal recessive mutations in NKCC2 or the renal outer medullar potassium channel (ROMK), expressed in the proximal tubule and CD in driving NaCl and water transport can be appreciated through several genetic disorders (see also Role of Vasopressin in Pathophysiological Conditions). Loss-of-function autosomal recessive mutations in NKCC2 or the renal outer medullar potassium channel (ROMK), expressed in the proximal tubule and CD in driving NaCl and water transport can be appreciated through several genetic disorders (see also Role of Vasopressin in Pathophysiological Conditions). Loss-of-function autosomal recessive mutations in NKCC2 or the renal outer medullar potassium channel (ROMK), expressed in the proximal tubule and CD in driving NaCl and water transport can be appreciated through several genetic disorders (see also Role of Vasopressin in Pathophysiological Conditions). Loss-of-function autosomal recessive mutations in NKCC2 or the renal outer medullar potassium channel (ROMK), expressed in the proximal tubule and CD in driving NaCl and water transport can be appreciated through several genetic disorders (see also Role of Vasopressin in Pathophysiological Conditions). Loss-of-function autosomal recessive mutations in NKCC2 or the renal outer medullar potassium channel (ROMK), expressed in the proximal tubule and CD in driving NaCl and water transport can be appreciated through several genetic disorders (see also Role of Vasopressin in Pathophysiological Conditions). Loss-of-function autosomal recessive mutations in NKCC2 or the renal outer medullar potassium channel (ROMK), expressed in the proximal tubule and CD in driving NaCl and water transport can be appreciated through several genetic disorders (see also Role of Vasopressin in Pathophysiological Conditions).
reviewed in Ref. 148). Pseudohypoaldosteronism type II (PHAIII) is an autosomal dominant disorder in which patients present with hypertension, hyperkalemia, metabolic acidosis, and decreased renin activity. Patients suffer from increased renal NaCl reabsorption. Thiazide diuretics, that can block NCC, are known to normalize blood pressure and correct electrolyte abnormalities in these patients, implicating a prominent role for NCC in the disease (75, 298). PHAII can be caused by mutations in With-No-Lysine kinase WNK1 and WNK4, which are known to regulate NCC function. Recently, mutations in two other genes, Kelch-like 3 (KLHL3) or Cullin3, have been linked to PHAII. KLHL3 and Cullin3 can form a complex that acts as an E3 ligase, increasing ubiquitylation and degradation of WNK4, and possibly WNK1, a process that is decreased by mutations in KLHL3 or Cullin3 (reviewed in Ref. 289). Liddle’s syndrome, an autosomal dominant disorder, is caused by gain-of-function mutations in either the β- or γ-subunit of ENaC that are expressed in the CNT and CD. These mutations lead to an increased reabsorption of sodium and secretion of potassium, resulting in salt-sensitive hypertension associated with hypokalemia, metabolic alkalosis, and repressed renin activity and aldosterone secretion (reviewed in Refs. 33 and 239). Inactivating mutations in ENaC also cause autosomal recessive pseudohypoaldosteronism type 1 (PHAI). PHAI is characterized by salt loss, hypotension, hyperkalemia, and metabolic acidosis. Plasma renin and aldosterone concentrations are highly elevated. PHAII has a more severe clinical phenotype, due to the expression of ENaC in tissues other than the kidney, and is for example associated with recurrent pulmonary infections (reviewed in Ref. 232). Finally, mutations in either AQP2 or in the vasopressin receptor 2 (V2R), disabling AVP-mediated signaling, cause hereditary nephrogenic diabetes insipidus, in which patients are unable to concentrate their urine, resulting in polyuria and, consequently, polydipsia (reviewed in Ref. 234).

AVP Signaling and Receptor Expression in the Kidney

Three distinct AVP receptors, vasopressin receptor 1A (V1aR), vasopressin receptor 1B (V1bR, also named V3R), and the V2R are responsible for mediating the intracellular signaling effects of AVP in kidney epithelial cells (reviewed in Refs. 4 and 154). V1aR expression has been demonstrated in cells of the CNT, cortical and medullary portions of the CD, and (to a lesser degree) in cells of the TAL and thin ascending limb (38, 91, 105, 276). In the CD, the V1aR is exclusively expressed in intercalated cells in the medulla, but in both principal and intercalated cells in the CDD (38). The V1aR is predominantly coupled to Gq/11 proteins, and following AVP binding stimulates a phospholipase C-Ca2⁺-protein kinase C (PKC) pathway (reviewed in Ref. 154). Increased tubular V1aR activity results in increased urine flow and urinary sodium excretion (158). These effects are possibly a “feedback” mechanism to prevent too intense antidiuretic effects caused by V2R stimulation. Possible mechanisms for the V1aR’s attenuation of the positive effects of AVP on urine concentration (see below) include induction of PKC-mediated AQP2 ubiquitylation and internalization (132), or downregulation of V2R expression in principal cells via reduced gene transcription (130). In addition to the kidneys, the V1aR is expressed in several other organs and tissues, for example in vascular smooth muscle cells and has been shown to be responsible for the AVP-induced vessel constriction (155). V1bR is also involved in AVP-induced stimulation of the renin-angiotensin-aldosterone system (RAASS). The receptor is expressed in renal macula densa cells, where activation leads to increased renin production and activation of the RAASS system, and in the adrenal gland, where activation leads to aldosterone release (3). The V1aR is also abundantly expressed in the brain, where its activation influences a variety of brain functions such as social behavior and learning (reviewed in Ref. 154).

The V1bR has been detected in cells of the inner medullary collecting duct (IMCD), although the functional role of the receptor here remains to be clarified (242). The V1bR signals through similar pathways as the V1aR (reviewed in Ref. 154). Besides a possible role in the kidney, V1bR is abundantly expressed in the brain, where it is for example involved in social behavior and AVP-mediated stimulation of ACTH from the pituitary, and in the adrenal gland, where it is involved in promoting catecholamine release (118, 154).

The most abundant AVP receptor in the kidneys is the Gαq-coupled V2R, which is responsible for the majority of AVP-mediated signaling pathways occurring in the distal nephron and CD, as well as the antidiuretic action of AVP. AVP binding to the V2R activates adenylate cyclases (AC) to convert ATP to the intracellular second messenger cAMP, which elicits a variety of responses in principal cells due to activation of cAMP-dependent protein kinases, e.g., protein kinase A (PKA) (264). AC3, 4, and 6 have been shown to be expressed in CD principal cells, and knockdown of AC3 and AC6, but not AC4, has been shown to reduce AVP-stimulated cAMP generation in primary cultures mouse IMCD cells (117, 120, 272). CD-specific knockout of AC6 causes a urinary concentration defect in mice, while knockout of AC3 does not affect renal water excretion, suggesting that AC6 is responsible for the majority of AVP effects in the CD (143, 237). V2R mRNA and/or protein is detected in cells from the TAL through to the IMCD (78, 193, 247), which corresponds well with cAMP responsiveness to AVP in these cells (189). In the CD, the V2R is mainly expressed in the principal cells, but is also detected in a subset of type A intercalated cells in the CDD and all medullary type A intercalated cells (193). V2R activation also increases intracellular Ca2⁺ levels, and blocking intracellular Ca2⁺ by treatment with a Ca2⁺ chelator inhibits the AVP-mediated increased water permeability of isolated IMCDs (46, 63, 269, 306). The AVP-induced rise in intracellular Ca2⁺ levels in the CD might occur through the activation of the exchange protein directly activated by cAMP (EPAC), which can be activated independently of PKA, as an EPAC-selective agonist mimicked AVP in triggering Ca2⁺ mobilization and oscillations (306). The AVP analog dDAVP also increases intracellular Ca2⁺ levels in isolated DCT cells, as well as in the TAL (208, 214). The maximal increase in Ca2⁺ in response to AVP stimulation shows a delay of 20–40 s in the TAL, and in the CD this occurred after ~100 s (208). AVP stimulation has also been shown to lead to the release of norepinephrine in the TAL and CD, which oppose water and sodium transport activation via a local inhibitory feedback signal (26, 208, 258). In addition to the G protein-dependent signaling, the V2R, as well as the V1aR, activate a β-arrestin pathway, leading to the activation of ERKs (41, 279). AVP binding to the V2R generates cAMP and promotes PKA acti-
vation not only at the plasma membrane, but also after β-arrestin mediated receptor internalization into endosomes, and binding of arrestins to the V₂-R-AVP complex has been suggested to be necessary for this extended cAMP generation (76). Besides its role in the kidney, the V₂-R is expressed in other tissues such as the vascular endothelium, where activation leads to nitric oxide release and vasodilation, thereby counteracting the effect of V₂-R activation, and causing release of coagulation factors (131, 154).

Antinatriuretic Effects of Vasopressin

Although AVP has a multitude of effects on sodium transport mechanisms, the antinatriuretic effects of AVP are not widely acknowledged. In rats (216) and humans (7), acute administration of dDAVP not only increases urine osmolality and reduces urine volume but also reduces Na⁺ excretion. As patients with nephrogenic diabetes insipidus due to mutations in AQP2 have reduced Na⁺ excretion following dDAVP administration, but not patients suffering from NDI due to V₂-R mutations (7), the antinatriuretic effects of AVP are likely mediated via a mechanism involving the V₂-R. Evidence for an intrarenal mechanism is provided by ex vivo experiments using perfused rat kidneys, where addition of dDAVP to the perfusate increased urinary osmolality and decreased urine flow and fractional sodium excretion (163).

The potent effects of AVP for maintaining water homeostasis are well established, and are due to a “double-effect”: 1) AVP increases the water permeability of the CNT and CD epithelium; and 2) AVP increases sodium transport mechanisms throughout the distal nephron and CD and urea transport in the IMCD, providing a driving force for osmotic removal of water from the CNT and CD principal cells. Several of the increased sodium transport mechanisms ultimately result in the antinatriuretic effects of AVP. Physiologically, it is likely that this parallel regulation of water and sodium transport by AVP is an evolutionary homeostatic mechanism to limit the simultaneous loss of water and salt during certain conditions, e.g., sweating. However, experimental data suggest that the levels of AVP required to stimulate sodium or water transport differ. In isolated, perfused rat CDs, stimulation of sodium transport required higher AVP concentrations relative to the AVP concentrations required to increase water fluxes (4, 115). Similarly, although acute V₂-R antagonist administration to Wistar rats increased urine flow rate and Na⁺ excretion in a dose-dependent manner, a higher antagonist concentration was required to elicit effects of a lesser magnitude on urinary Na⁺ levels (216). Administration of the V₂-R agonist dDAVP decreased both urine flow and sodium excretion; however, an effect on sodium excretion was only seen at higher concentrations of dDAVP (216). Furthermore, healthy subjects on water diuresis receiving a very low infusion rate of AVP had a modest reduction in urine flow, whereas with higher AVP infusion rates, reductions in both urine flow and sodium excretion were observed (1, 7). Combined, these data suggest that increased Na⁺ reabsorption, relative to water reabsorption, requires a higher level of AVP. However, the reason for this difference in response is not known. In the following sections, focus is on the molecular targets of AVP for modulating sodium transport in the distal nephron, and the roles of individual proteins in antiuresis.

**Na-K-ATPase**

The Na-K-ATPase, located at the basolateral membrane of kidney epithelial cells, provides the driving force for secondary active Na⁺ and K⁺ transport, as well as the secondary active transport of other solutes. AVP increases the mRNA expression and subsequent protein abundance of the Na-K-ATPase α-subunit in rat CD and in rat outer medulla (19, 23, 60). Short-term AVP administration stimulates the activity of the Na-K-ATPase in the medullary TAL, an effect that is mediated via the V₂-R (42, 220). In addition, cAMP analogs and forskolin stimulate Na-K-ATPase activity in medullary TAL suspensions (142). Similarly, AVP increases Na-K-ATPase activity in isolated CDs (54), and synergistic stimulatory effects on the Na-K-ATPase are observed with AVP and aldosterone (54). The effects of AVP on the Na-K-ATPase in the CCD can be reproduced by addition of the V₂-R agonist dDAVP, cAMP analogs, or forskolin, indicating that AVP acts via a V₂-R pathway (54, 104). AVP or cAMP stimulation of CD principal cells is associated with an increase in the cell surface expression of the Na-K-ATPase (104, 188), which is not related to changes in Na-K-ATPase internalization or the total pool of Na-K-ATPase. This suggests that AVP increases the translocation of Na-K-ATPase units from intracellular stores to the plasma membrane (104). AVP induced increases in activity and cell surface expression can be inhibited by the PKA blocker H89 (188). PKA phosphorylates the α-subunit of the Na-K-ATPase on Ser943 in vitro and in intact cells (13). However, mutation of the PKA phosphorylation site does not alter the time course of increased cell surface expression of the Na-K-ATPase in response to AVP or cAMP, indicating that effects of AVP are not dependent on phosphorylation of the α-subunit on Ser943 (188). It is likely that the effects of PKA are via phosphorylation of an intermediate target. In conclusion, AVP stimulates both Na-K-ATPase activity and expression levels in the TAL and CD.

**NKCC2**

The medullary TAL plays a major role in generation of a hypertonic medullary interstitium, which creates the luminal-to-interstitial concentration gradient in the CD required for the osmotic diffusion of water. This hypertonic interstitium is created via secondary active transport of sodium out of the tubule fluid along the length of the TAL, resulting in reabsorption of ~25% of the filtered load of sodium. The majority is transported by the cation-chloride cotransporter NKCC2, which is encoded by the **SLC12A1** gene (69, 98, 133). (Figure 2 is a schematic representation of a TAL cell and highlights some of the regulatory mechanisms discussed in the following text.) NKCC2 is expressed throughout cells along the medullary and cortical TAL, as well as in macula densa cells, where it is associated with the apical plasma membrane and subapical vesicles (133, 205). AVP has an overall positive effect on transepithelial sodium transport in the TAL. During chronic exposure to high levels of AVP, marked hypertrophy of medullary TAL cells occurs alongside a doubling in NaCl transport (20, 29). These effects are likely due to an effect of AVP on several transport proteins in TAL cells; however, the effects of AVP on NKCC2 are likely to be the major determinant of Na⁺ transport rates across the apical membrane of TAL cells. The expression of NKCC2 increases significantly both with re-
NKCC2 can be phosphorylated by the kinases Ste20/SPS1-related proline/alanine-rich kinase (SPAK) and oxidative stress-response 1 (OSR1), which directly interact with NKCC2 via a (R/K)fx(V/I) binding motif in NKCC2 (219). SPAK and OSR1 directly phosphorylate at least three of the identified phosphorylation sites in NKCC2: Thr91, Thr96, and Thr101. Acute dDAVP treatment increased phosphorylation of OSR1/SPAK in the medullary TAL in mice (246). SPAK and OSR1 are activated by WNKs, with a WNK3/SPAK interaction resulting in phosphorylation and activation of SPAK (222). WNK3 protein, which is expressed in TAL cells, can also increase phosphorylation of Thr96 and Thr101 and activity of NKCC2 (233). These data indicate a possible role for WNK3 and/or SPAK/OSR1 as downstream effectors of AVP for regulation of NKCC2. However, studies in SPAK knockout mice concluded that SPAK is not essential for the AVP-mediated increase in NKCC2 surface expression, Thr96/Thr101 phosphorylation, or AVP-stimulated sodium reabsorption in the TAL, suggesting that the effects of AVP depend on activation of OSR1 or another kinase (45, 246).

AVP stimulates the exocytosis of NKCC2 from subapical vesicles to the plasma membrane, and this effect is dependent on AVP-mediated activation of PKA. However, it is unclear whether PKA indeed phosphorylates NKCC2 directly in vivo or whether a different kinase is responsible for phosphorylation of Thr96 following AVP exposure.
Annexin A2, a Ca$^{2+}$-dependent phospholipid-binding protein that localizes to lipid rafts, interacts with nonphosphorylated NKCC2 and promotes NKCC2 apical trafficking in response to AVP (56).

In conclusion, 1) acute AVP increases NKCC2 phosphorylation and exocytosis to the apical plasma membrane, and 2) chronically elevated AVP levels increase NKCC2 expression. Together, these processes increase sodium chloride reabsorption, medullary osmolality, the luminal-to-interstitial concentration gradient, and the osmotic diffusion of water from the collecting duct, resulting in an overall increase in urine concentrating ability.

**ROMK**

In the TAL, NKCC2 reabsorbs K$^+$ parallel to NaCl into the TAL cells. Apical potassium channels are responsible for the secretion of this K$^+$ out of the same cells into the tubule lumen, which is essential for the maintenance of an adequate supply of luminal K$^+$ and efficient function of NKCC2 (Fig. 2). In addition, K$^+$ secretion from TAL cells leads to a luminal-positive voltage, driving paracellular uptake of Na$^+$ and other cations (109, 111, 116). The ATP-sensitive, inward rectifier ROMK potassium channel (also known as Kir1.1), expressed in the apical membrane of cells from the TAL, DCT, and CNT, in addition to principal cells of the cortical and outer medullary CD (159, 177, 299), plays an essential role in K$^+$ secretion in the TAL. ROMK’s contribution to sodium reabsorption in the TAL is emphasized by patients suffering from Bartter syndrome type II due to mutations in ROMK (260). Bartter syndrome type II patients suffer from similar symptoms as patients with Bartter syndrome type I resulting from mutations in NKCC2, including salt wasting, hypokalemic alkalosis, polyuria, and polydipsia (259).

AVP increases the apical potassium conductance and thus transepithelial NaCl transport in the TAL, presumably via ROMK (295). This K$^+$ conductance can also be increased in vitro by cAMP, forskolin, or by addition of the catalytic subunit of PKA, suggesting that AVP increases ROMK activity via the cAMP-PKA pathway (224, 295). PKA can directly phosphorylate three serine residues of ROMK, one residue at the NH$_2$ terminus (Ser25) and two at the COOH terminal (Ser200 and Ser294; numbers relative to the rat ROMK2 isoform and correspond to Ser44, Ser219, and Ser313 in the ROMK1 isoform). All three serine residues have to be phosphorylated to obtain full activation of ROMK by cAMP, with phosphorylation of at least two sites required for detectable activity of the channel (301). In addition, long-term AVP stimulation via water restriction or dDAVP treatment increases ROMK protein abundance in the TAL (64). Although expressed in the DCT (159, 177, 299), the role of ROMK in this segment is unclear. Similar to the TAL, AVP or forskolin increases K$^+$ conductance via ROMK in the CCD (39).

In conclusion, AVP regulates the luminal secretion of K$^+$ in the TAL and ensures essential Na$^+$ reabsorption. This process results from cAMP-mediated activation of PKA, increased phosphorylation and activation of ROMK, alongside increased ROMK abundance.

**Sodium Hydrogen Exchanger 3**

Sodium hydrogen exchanger 3 (NHE3) is expressed in the apical membrane of cells in the proximal tubule, the outer medullary thin descending limb of Henle’s loop, and the TAL (21, 27). NHE3 reabsorbs a large proportion of sodium from the proximal tubule, but is also responsible for a small fraction of the sodium reabsorbed from the TAL (175). Mice with kidney tubule-specific deletion of NHE3 display relatively normal fluid homeostasis under basal conditions (85). In the medullary TAL, NHE3 activity, determined as a function of bicarbonate reabsorption, is decreased by AVP, an effect mediated by cAMP (106, 107). In medullary TAL suspensions, dDAVP increases NHE3 phosphorylation at Ser552 (112). Ser552 phosphorylation is involved in the inhibitory effect of cAMP or PKA on NHE3 (310). Although PKA-mediated Ser552 phosphorylation does not affect NHE3 transporter activity, Ser552 phosphorylated NHE3 specifically localizes to the coated pit region of the brush-border membrane of the proximal tubule, where NHE3 is inactive, while total NHE3 is found throughout the brush-border membrane, suggesting that phosphorylation of NHE3 plays a role in its subcellular trafficking (150, 151). In conclusion, NHE3-mediated Na$^+$ reabsorption appears to be inhibited by AVP.

**CFTR**

Although not a sodium transporter, CFTR is AVP sensitive and is an important regulator of several salt transporters in the distal nephron and CD due to its role in chloride secretion. Furthermore, as SPAK/OSR1 activation can occur via a reduction in intracellular Cl$^-$ levels, CFTR is a possible regulator of this effect (see the DISCUSSION). CFTR is localized to the apical plasma membrane of renal epithelial cells throughout the renal tubule (55, 58, 186). In the CD, CFTR expression is found in both principal and intercalated cells (283). AVP increases CFTR mRNA levels in the rat kidney and Madin-Darby canine kidney cells, suggesting an effect of AVP on CFTR transcription (187). CFTR is activated by a cAMP-dependent PKA pathway, leading to phosphorylation of the channel at seven serine residues (Ser660, Ser700, Ser712, Ser737, Ser753, Ser795, and Ser813), increased open probability, and greater secretion of Cl$^-$ into the tubule fluid (2, 198, 218, 254). Mouse cell lines derived from the DCT, CCD, and IMCD respond to dDAVP treatment with increased basolateral-to-apical Cl$^-$ conductance, which can be inhibited by Cl$^-$ channel blockers (61, 62). Furthermore, microdissected CCDs from CFTR knockout mice do not display the dDAVP-induced basal-to-apical Cl$^-$ flux seen in wild-type mice (15). These data indicate that luminal Cl$^-$ secretion through CFTR is stimulated via the V$_2$R in the distal tubule and CD. Activation of CFTR occurs alongside an amiloride-sensitive apical-to-basolateral Cl$^-$ flux of greater magnitude than the reverse current, corresponding to the activation of ENaC.

CFTR plays a modulatory role in several salt transporters in the distal tubule. ROMK activity is regulated by CFTR, as demonstrated by reduced ROMK activity in CFTR knockout mice and in transgenic mice with cystic fibrosis-causing mutations in CFTR (167). The Na$^+$/H$^+$ exchange regulatory factors NHERF-1 and -2 modulate formation of a ROMK-CFTR complex via PDZ-domain interactions (307). This complex increases ROMK abundance at the apical plasma mem-
brane of COS cells. CFTR also modulates ENaC (discussed in ENaC) and may be important for modulation of NCC activity (see discussion in NCC).

**NCC**

V2R mRNA and protein are detectable in the DCT, although at lower levels than in the TAL (193). Micropuncture studies have demonstrated dDAVP-mediated sodium reabsorption in the distal convolution of Brattleboro rats (71, 72). The major sodium transporter in this segment is the thiazide-sensitive NaCl cotransporter [TSC or NCC, solute carrier family 12 member A3 (SLC12A3)], which is localized to the apical plasma membrane and subapical compartments of DCT cells (207, 221). (Figure 3 is a schematic representation of a DCT cell and highlights some of the regulatory mechanisms discussed in the following text.) NCC is the target for the commonly used thiazide diuretics, which produce mild natriuresis. Mutations in NCC can cause Gitelman’s syndrome (160, 173, 185, 261). Phosphorylation sites in the N terminus of NCC are important for transporter activity (212, 227). Phosphorylation of Thr58 (rat/mouse nomenclature) appears to be an essential site, and a missense mutation to methionine of the corresponding residue in humans (Thr60) is frequently observed in patients with Gitelman’s syndrome (164, 170, 256).

In AVP-deficient Brattleboro rats, short-term dDAVP treatment increased the abundance of phosphorylated NCC at Thr53 and Thr58 (214), suggesting that NCC is responsible for the dDAVP-mediated sodium reabsorption in the DCT (71, 72). Similar effects of dDAVP were observed in normal Wistar rats during blockade of the angiotensin II receptor, type 1 using candesartan alongside feeding of a high-NaCl diet to eliminate the effects of angiotensin and aldosterone that independently affect NCC (141, 157, 200, 201, 214). A parallel study in Brattleboro rats found similar effects of short-term dDAVP treatment on the phosphorylation of Thr53 and Ser71 in NCC (195), thus validating the short-term regulatory role for AVP in NCC. An additional phosphorylation site in NCC at Ser124, which plays a role in NCC activity, is also increased in abundance by acute dDAVP treatment of Brattleboro or normal rats (238). AVP treatment has no effect on phosphorylation of Thr58 NCC in AC6 knockout mice, indicating that AVP activates NCC via a V2R-cAMP pathway (231). NCC phosphorylated at Thr53 and Thr58 is only detected in the apical plasma membrane of DCT cells, underlining the importance of NCC phosphorylation in relation to function (214). Although the fraction of NCC protein in the luminal plasma membrane was significantly increased upon dDAVP treatment in one study (195), this could not be confirmed in other models or studies (214, 238). Long-term dDAVP treatment of Wistar rats on a high-NaCl diet and candesartan infusion also resulted in increased pNCC (pThr53 and pThr58) levels of a similar magnitude to the short-term effects (214). Total abundance of NCC was unaffected in this study, suggesting that the previous observation of increased NCC abundance with chronic dDAVP treatment of Brattleboro rats was dependent on the RAAS (66).

The precise molecular mechanisms behind AVP-mediated effects on NCC are not known (see discussion below), but the WNK/SPAK/OSR1 signal transduction pathways regulating NKCC2 in the TAL are likely to play a similar role for modulation of NCC via AVP in the DCT. The phosphorylated Thr53 and Thr58 residues in NCC that are increased in abundance by AVP correspond to the Thr96 and Thr101 residues in NKCC2 that are phosphorylated by SPAK/OSR1 (195, 214). These sites are conserved among the sodium chloride transporters NKCC1, NKCC2, and NCC (229). SPAK/OSR1 phosphorylate NCC at Thr53, Thr58, and Ser71 (190, 227), and phosphorylation/activation of SPAK/OSR1 in the DCT is increased by acute dDAVP treatment (214, 246). Finally, dDAVP-induced phosphorylation of Ser71 or Thr58 was greatly reduced or abolished, respectively, in SPAK knockout mice (246). Although these data indicate that SPAK plays a major role in the AVP-mediated signaling pathway regulating NCC in the DCT, whether the effects of AVP occur via the WNKs, which are potent activators of NCC via phosphorylation of SPAK/OSR1, is unknown (227, 233, 244, 292).

As indicated, although AVP has clear effects on phosphorylation of NCC, several steps of the AVP-mediated signaling pathways in DCT cells remain unknown. The presence of the V2R and cAMP responsiveness in the DCT suggests that PKA...
is activated in DCT cells upon AVP stimulation, although direct evidence of a role of PKA in NCC regulation is lacking. In addition, similarly to the collecting duct, intracellular Ca\(^{2+}\) levels in the DCT are increased upon dDAVP treatment (214), suggesting that Ca\(^{2+}\)-mediated signaling networks may also play an important role.

One area for speculation is a role for WNKs and CFTR in mediating some of the effects of AVP for increasing NCC phosphorylation. WNKs regulate the function of several distal tubule transporters, such as NCC, ROMK, and NKCC2 (reviewed in Ref. 125, and CFTR. In Xenopus laevis oocytes, WNK1 and –4 reduce CFTR-mediated Cl\(^{-}\) fluxes by decreasing CFTR abundance in the plasma membrane (303). In isolated pancreatic duct epithelia using both \(^{32}\)P-labeling and antibody-mediated \(\text{pSer/pThr}\) detection in immunoprecipitates, CFTR can be phosphorylated and, thereby inhibited, via a SPAK-WNK scaffold. This effect could be opposed by the CFTR activator IRBIT, leading to recruitment of the protein phosphatase PPI to CFTR, resulting in dephosphorylation and reactivation of the channel (304). These data, combined with the activation of CFTR by AVP through PKA-mediated increases in cAMP, provide a plausible explanation to combine the observed effects of short-term AVP stimulation of NCC phosphorylation in vivo (195, 214) and the observations that low-CI\(^{-}\) treatment of cells increases NCC phosphorylation by SPAK/OSR1 in vitro (212, 227). AVP-stimulated increases in cAMP and activation of PKA would result in phosphorylation of CFTR, increased open probability, and greater Cl\(^{-}\) secretion, resulting in transient low intracellular Cl\(^{-}\) levels. As WNKs are activated at low ambient Cl\(^{-}\) (12, 217, 222), the low intracellular Cl\(^{-}\) levels would activate the WNK-SPAK cascade, increasing the phosphorylation/activity of NCC, and simultaneously turning off the initial response by SPAK-mediated phosphorylation of CFTR. Recently Terker et al. (278) showed that incubation of HEK cells in medium with low potassium increased NCC phosphorylation, and this effect was dependent on modulation of membrane voltage and, in turn, a decline in intracellular chloride, leading to WNK activation. HEK cells transfected with mutated CIC-K2, a Cl\(^{-}\) channel expressed at the basolateral membrane of the DCT, had attenuated low-potassium-induced NCC phosphorylation compared with cells expressing the wild-type channel (149, 278), confirming the importance of intracellular chloride for NCC regulation and suggesting a possible role for CIC-K2. However, this protein is not known to be regulated by AVP. In agreement with a regulation of NCC by low chloride is a recent study showing that lack of functional Kcnj10, a potassium channel expressed at the basolateral membrane of the DCT, reduced Cl\(^{-}\) exit across the basolateral membrane of mice DCT cells (309). This is expected to increase intracellular Cl\(^{-}\) concentrations and inhibit WNK. In agreement with this, Kcnj10 knockout showed lower expression of SPAK and NCC. A critical role of intracellular Cl\(^{-}\) is supported by a study from Mutig and colleagues (192), which demonstrated a regulatory mechanism for NKCC2 via Tamm-Horsfall protein (THP). THP-mediated extrusion of Cl\(^{-}\) mediated a reduction in intracellular Cl\(^{-}\) in TAL cells, resulting in increased NKCC2 phosphorylation (and activity). Furthermore, the effects of short-term dDAVP treatment, represented by N-terminal phosphorylation of NKCC2, were blunted in THP-KO mice compared with wild-type or in cells lacking THP.

In conclusion, 1) acute AVP increases the phosphorylation and thereby activity of NCC, 2) long-term AVP indirectly increases NCC abundance via the RAAS. An AVP-mediated increase in sodium reabsorption in the relatively water-impermeable DCT likely aids the reabsorption of water from the downstream CNT (being the first highly water-permeable segment of the distal nephron) by reducing the tonicity of the tubular fluid and increasing the tonicity (albeit only marginally) in the cortical interstitium, which together increase the driving force for water reabsorption.

**ENaC**

The ENaC is expressed in cells of the late DCT and in the principal cells of the CNT. ENaC is also expressed in the principal cells of the CD, predominantly in the CCD and the outer medullary collecting duct. ENaC is the primary sodium transport pathway in CNT and CCD cells and represents the final major transport protein that regulates sodium excretion (165). (Figure 4 is a schematic representation of a CD principal cell and highlights some of the regulatory mechanisms discussed in the following text.) There is an axial decline in the distribution of ENaC and the Na-K-ATPase from the CNT to the CD (88, 94, 166). Apical recruitment of ENaC in response to sodium restriction is initiated in the CNT and progresses to the downstream CCD only with lower levels of dietary sodium intake (166), suggesting that ENaC-mediated Na\(^{+}\) reabsorption normally occurs mainly in the CNT. Both the V\(_{7}Ra\) and AQP2 are expressed alongside ENaC in these cells.

ENaC is regulated by AVP and aldosterone in a synergistic manner. However, AVP acts within minutes, whereas the aldosterone-induced effects take hours (249, 250, 284). ENaC-mediated transport in the CNT and CD is also upregulated by long-term administration of AVP or water restriction, and AVP-deficient Brattleboro rats treated long term or acutely with dDAVP greatly elevate their mRNA and protein abundance of the ENaC \(\beta\)- and \(\gamma\)-subunits (65, 66, 199). Stimulation of perfused CCDs with AVP also increases sodium reabsorption, likely via ENaC (225, 226, 251, 284). Furthermore, dDAVP has amiloride-sensitive antinatriuretic effects in humans, indicating that they are due to activation of ENaC (22). AVP, acting through the V\(_{7}R\), increases cAMP levels and PKA activation, increasing the density of ENaC subunits at the apical plasma membrane and thus sodium transport (14, 66, 74, 99, 265). AC6 knockout mice do not show similar changes in AVP-mediated increases in ENaC open probability or apical plasma membrane channel number, suggesting a major role of AC6 for AVP-stimulated ENaC activity (236). ENaC is translocated to the membrane via targeting and fusion of a subcellular pool of ENaC-containing vesicles (35). AVP-mediated increases in the expression of the ubiquitin-specific protease (Usp10) stabilize sorting nexin 3 (SNX3) and facilitate this vesicle fusion (31).

Removal of the AVP stimulus internalizes ENaC from the apical membrane, following which the channel is reorganized into recycling vesicles or is degraded (35). cAMP inhibits this internalization through PKA-dependent phosphorylation of Nedd4-2 (an E3 ubiquitin-protein ligase), rendering Nedd4-2 inactive and unable to target ENaC for ubiquitylation and degradation (266). Similar phosphorylation sites in Nedd4-2 are also the target of the serum and glucocorticoid-induced kinase-1 (SGK-1), which is...
Fig. 4. Regulation of ENaC by AVP. AVP increases the density of ENaC at the apical plasma membrane by increasing the expression of Uspl0, which stabilizes SNX3, thereby facilitating vesicle fusion. AVP-mediated PKA-activation leads also to phosphorylation of Nedd4-2, rendering Nedd4-2 inactive and unable to target ENaC for ubiquitylation and degradation, also leading to increased ENaC levels at the plasma membrane. AVP also increases prostasin activity, leading to cleavage of inhibitory peptides of the ENaC subunits, increasing activity of the channel. In addition, AVP increases the mRNA and protein abundance of the ENaC β- and γ-subunits. Activation of PKA increases phosphorylation and activity of ROMK, resulting in increased potassium excretion. Activation of PKA results in increased activity of the Na-K-ATPase.

regulated by aldosterone (44, 57, 265). Hence, Nedd4-2 is part of the synergistic regulation of ENaC by AVP and aldosterone. PKA and SGK-1 have mechanistic differences by virtue of different affinities for three characterized phosphorylation sites in Nedd4-2. Ser327 is phosphorylated by both kinases, whereas Ser221 is only phosphorylated by PKA and Thr246 only by SGK-1 (266). Due to the synergistic effects on ENaC of AVP and aldosterone demonstrated in vitro, only small AVP effects on ENaC activity might be observed in low-aldosterone conditions (34, 43, 225).

In addition to increasing the density of ENaC on the plasma membrane, AVP also increases the open probability of the channel, an effect dependent on AC and PKA. This increased open channel probability is fast but sustained, and followed by a slower but greater elevation in the density of active ENaC at the apical plasma membrane (34). One mechanism whereby AVP could influence the open probability of ENaC in the long term is by affecting prostasin. The gene expression of prostasin (a glycosylphosphatidylinositol-anchored serine protease) is increased by AVP, and prostasin is known to activate ENaC by cleavage of inhibitory peptides from the γ-subunits, subsequently increasing the open probability of the channel (95, 147, 248).

A role of CFTR in modulation of ENaC function has been identified. In X. laevis oocytes, both CFTR open probability and surface expression are increased when CFTR is coexpressed with ENaC, and, conversely, the activity of ENaC is inhibited by CFTR (152, 302). In addition, ENaC increases K+ currents via ROMK in a CFTR-dependent manner, an effect blunted when a Liddle’s syndrome-causing mutation in ENaC is introduced (152). Although electrophysiological studies demonstrate conflicting results regarding a direct interaction between CFTR and ENaC (reviewed in Ref. 17), fluorescence resonance energy transfer (FRET), photobleaching, and communoprecipitation studies in HEK293 cells suggested a direct interaction between the two channels (16).

To summarize the effects of AVP on antinatriuresis and antidiuresis, AVP increases ENaC abundance at the apical plasma membrane by increasing exocytosis and inhibiting endocytosis and degradation via inhibition of degradative pathways such as the Nedd4-2-mediated ubiquitylation, as well as increasing ENaC open probability and gene expression (Fig. 4). By increasing sodium reabsorption in the cortex via ENaC (and NCC), AVP will facilitate an osmotic movement of water into the isotonic cortical interstitium of the CNT and CCD along with sodium (reviewed in Ref. 5). This theory is supported by work on CNT- and CD-specific α-ENaC knockout mice, which have increased urine output and decreased urine osmolality, due to impaired ENaC-mediated sodium reabsorption in the CNT and CD (47). In contrast, CD-specific α-ENaC knockout does not have impaired sodium reabsorption or differences in urine osmolality (240), arguing that α-ENaC function in the CNT is essential for full urine concentrating ability (47). In mice on a high-sodium diet treated with the ENaC blocker benamil, urine osmolality was decreased alongside increased urine flow (178). Although benamil increased urinary sodium excretion, plasma sodium concentrations were increased. Benamil treatment of water-deprived mice and dDAVP-treated mice also resulted in reduced urinary concentration (178), demonstrating that stimulation of ENaC by AVP plays an important role in water homeostasis. Similarly to the mechanisms described for NCC (see above), stimulation of ENaC is predicted to decrease the tonicity of the tubular fluid and (marginally) increase the tonicity in the surrounding interstitium. Together, these effects will favor increased water movement through CD principal cells into the interstitium via aquaporins, thereby enhancing total water conservation.

In addition to antinatriotic and antidiuretic effects, by increasing ENaC and ROMK activity in the late DCT and/or CNT and CD, AVP likely has a physiological function for regulating potassium homeostasis during different states of hydration. Although potassium excretion is typically a flow-dependent process, AVP stimulates potassium secretion independently of flow rate (90), suggesting that AVP increases Na+/K+ exchange to prevent potassium retention during antidiuresis. Similarly, elevated AVP levels are linked to better survival following severe hemorrhage, by allowing increased potassium excretion and plasma potassium levels to remain within normal limits (290).
Role of Vasopressin in Pathophysiological Conditions

AVP has been implicated in a variety of disorders, including suppression of the innate immune system, impairment of the kidney’s ability to respond to infections, promotion of renal tumor growth, decrease in longevity of rodents, impairment of cognitive performance, alteration of mood, and perception of task difficulties (reviewed in Ref. 5). In the following, a brief overview of some clinical aspects of AVP and the role of AVP and AVP-mediated sodium retention in influencing the severity of various pathophysiological disorders is provided. Readers are further directed to various excellent and extensive review articles on this area.

Edematous disorders. During cardiac failure, liver cirrhosis, and pregnancy, sodium and water retention often results in hyponatremia. However, plasma AVP concentrations are not consistently decreased in these conditions, as would be expected from the hyponatremia, suggesting involvement of “non-osmotic AVP” (release of AVP by factors other than increased plasma osmolality). For example, patients with hyponatremia and hyposmolality due to cardiac failure have increased plasma AVP levels relative to their plasma osmolality (reviewed in Ref. 253). In these patients, AVP actions on the V2R have been proposed, with administration of the V2R antagonist OPC-31260 to heminephrectomized rats with DOCA-salt-induced AVP actions (235, 267, 268), alongside altered NHE3, ROMK1, AQP2, and Na-K-ATPase protein expression (268). Despite such evidence for effects of AVP on blood pressure, the presence of other aggravating factors, such as increased plasma renin activity or aldosterone secretion, inhibition of the V1aR, or decreased plasma atrial natriuretic peptide levels, also appear to play a substantial role (reviewed in Ref. 5).

Chronic kidney disease. In 1990, studies by Bouby et al. (28) indicated a role for AVP in chronic kidney disease (CKD). In % nephrectomized rats with a high water intake relative to normal water intake, reduced AVP concentrations and thus urine concentration occurred alongside major reductions in proteinuria, kidney hypertrophy, glomerular lesions, interstitial injury, and mortality. Furthermore, in % nephrectomized Brattleboro rats infused with dDAVP, CKD progression was aggravated (30), suggesting that AVP is one factor that promotes CKD progression. This theory is supported by epidemiological studies that confirmed the linkage between AVP and renal damage in humans, where high water intake and urine volume were associated with a lower risk of CKD and a slower annual decline in estimated glomerular filtration rate (eGFR) (50, 274). Similarly, in patients with autosomal dominant polycystic kidney disease (ADPKD) a faster decline in GFR was correlated with higher baseline urine osmolality (286), and tolvaptan (selective V2R antagonist) slowed kidney growth and functional decline in ADPKD patients (119, 285).

Diabetes mellitus. A higher plasma AVP concentration is consistently found in patients with either type I or type II diabetes mellitus (DM) or in animal models of DM (reviewed in Ref. 6). Following induction of DM in either normal Long-Evans or AVP-deficient Brattleboro rats by streptozotocin, Long-Evans rats exhibit more severe diabetic nephropathy and associated parameters relative to Brattleboro rats (10). Furthermore, in Wistar rats with streptococci-induced DM, a rise in urinary albumin excretion (UAE) was prevented by treatment with the V2R antagonist SR121463 (9). In healthy humans, administration of dDAVP results in decreased urine flow and increased urine osmolality, but this is also accompanied by increased UAE without any change in creatinine or β2-microglobulin excretion. A similar increase in UAE occurs in patients with central diabetes insipidus or hereditary nephrogenic diabetes insipidus caused by AQP2 mutations, but not in patients with hereditary nephrogenic diabetes insipidus caused by V2R mutations (8). Combined, these studies suggest that the genesis of renal damage in DM is a result of AVP effects via the V2R.

Regulation of Water and Urea Transport by Vasopressin

Increases in AVP-mediated Na⁺ transport occur parallel to increases in AVP-regulated urea and water transport. As the molecular mechanisms behind these processes are similar, and because antidiuresis is partially dependent on AVP-mediated increases in Na⁺ and urea transport, an overview of the regulation of water and urea transport by AVP is informative.

Apical membrane expression and thus water flux through AQP2 in CD principal cells is the major factor determining water permeability of the CD epithelium. AQP2 is abundantly expressed throughout the principal cells along the whole CNT and CD (97, 203). Although mainly associated with the apical plasma membrane, AQP2 is, especially in the CNT and IMCD, also found in the basolateral plasma membrane of cells (48).
(see Fig. 5 for a schematic representation of AQP2 in a CD principle cell and some of the regulatory mechanisms discussed in the following text). Loss-of-function mutations in the AQP2 gene lead to a severe urinary concentrating defect and nephrogenic diabetes insipidus, confirming the importance of AQP2 in renal water handling (184, 234).

AVP binding to the V2R in renal CD principal cells and CNT cells induces a signaling cascade, involving Gs protein-mediated activation of adenylate cyclase (most likely AC6) (230), a rise in intracellular cAMP, activation of PKA, and subsequent phosphorylation of AQP2 (180). This results in the redistribution of AQP2 from intracellular vesicles to the apical membrane. Driven by the transcellular osmotic gradient, water will then enter principal cells through AQP2 and leave the cell via AQP3 and AQP4, which are expressed in the basolateral membrane, resulting in concentrated urine. In the long term, vasopressin also increases AQP2 expression via phosphorylation of cAMP-responsive element binding protein (CREB), which stimulates transcription from the AQP2 promoter. Once the water balance is restored, AVP levels drop and AQP2 is internalized via ubiquitylation. Internalized AQP2 can either be targeted to recycling pathways or to degradation via lysosomes. Modified from Ref. 152a with permission.

![Fig. 5. Regulation of aquaporin-2 (AQP2)-mediated water reabsorption by AVP. AVP binds to the V2R at the basolateral membrane of the CD and CD principal cells. This induces a signaling cascade, involving Gs protein-mediated activation of adenylate cyclase (AC), a rise in intracellular cAMP, activation of PKA and possibly the exchange protein directly activated by cAMP (EPAC), and subsequent phosphorylation of AQP2. This results in the redistribution of AQP2 from intracellular vesicles to the apical membrane. Driven by the transcellular osmotic gradient, water will then enter principal cells through AQP2 and leave the cell via AQP3 and AQP4, which are expressed in the basolateral membrane, resulting in concentrated urine. In the long term, vasopressin also increases AQP2 expression via phosphorylation of cAMP-responsive element binding protein (CREB), which stimulates transcription from the AQP2 promoter. Once the water balance is restored, AVP levels drop and AQP2 is internalized via ubiquitylation. Internalized AQP2 can either be targeted to recycling pathways or to degradation via lysosomes. Modified from Ref. 152a with permission.](http://ajprenal.physiology.org/)

Fig. 5. Regulation of aquaporin-2 (AQP2)-mediated water reabsorption by AVP. AVP binds to the V2R at the basolateral membrane of the CD and CD principal cells. This induces a signaling cascade, involving Gs protein-mediated activation of adenylate cyclase (AC), a rise in intracellular cAMP, activation of PKA and possibly the exchange protein directly activated by cAMP (EPAC), and subsequent phosphorylation of AQP2. This results in the redistribution of AQP2 from intracellular vesicles to the apical membrane. Driven by the transcellular osmotic gradient, water will then enter principal cells through AQP2 and leave the cell via AQP3 and AQP4, which are expressed in the basolateral membrane, resulting in concentrated urine. In the long term, vasopressin also increases AQP2 expression via phosphorylation of cAMP-responsive element binding protein (CREB), which stimulates transcription from the AQP2 promoter. Once the water balance is restored, AVP levels drop and AQP2 is internalized via ubiquitylation. Internalized AQP2 can either be targeted to recycling pathways or to degradation via lysosomes. Modified from Ref. 152a with permission.

AVP mediates the majority of its effects on AQP2 via modulating a variety of phosphorylation sites in AQP2 that facilitate trafficking of AQP2 to/from the apical plasma membrane (reviewed in Refs. 84 and 182). AQP2 can be phosphorylated at five residues: Thr244, Ser256, Ser261, Ser264, and Ser269 (123, 124). Phosphorylation of Thr244, Ser256, Ser264, and Ser269 is increased upon stimulation with dDAVP, while phosphorylation of Ser261 is decreased (83, 121, 122). The role of Thr244 phosphorylation is unknown. Ser261 phosphorylation can stabilize AQP2 ubiquitylation (275), with phosphorylated Ser261-AQP2 mainly observed in intracellular vesicles (122). Phosphorylated Ser256-AQP2 and Ser264-AQP2 reside in intracellular vesicles and the plasma membrane. Upon stimulation with dDAVP, there is a change in distribution of these phospho-forms (or an increase in abundance) to the plasma membrane (49, 83). Although the function of Ser264-AQP2 phosphorylation is currently unknown, Ser256 phosphorylation is increased by PKA upon AVP stimulation and appears essential for rapid AQP2 translocation to the apical plasma membrane following AVP (96, 136, 291). Finally, phosphorylated Ser269-AQP2 is solely found in the apical plasma membrane (181), and phosphorylation at this site is involved in apical plasma membrane retention of AQP2 (121, 179) in a process partly dependent on reduced interaction of AQP2 with proteins of the endocytic machinery (183). Although various kinases have been identified that may play a role in AQP2 phosphorylation, e.g., Ca2+/calmodulin-dependent protein kinase II (CAMK2) and SGK (Ref. 32 and reviewed in Ref. 182), PKA is the most widely recognized protein kinase for modulating AQP2 phosphorylation. The importance of PKA is highlighted in a recent study, where expression of a dominant negative form of inactive PKA in mice resulted in greatly reduced levels of AQP2 and phosphorylated AQP2 and a diabetes insipidus phenotype (100). It would be informative to analyze these mice with respect to the function of the various other AVP-modulated transporters/channels discussed in this article to confirm the role of PKA in their regulation.
AVP-mediated increases in cAMP also increase total AQP2 expression within several hours (114, 281). cAMP increases AQP2 transcription via PKA-mediated phosphorylation of the cAMP-responsive element binding protein (CREB), which stimulates transcription from the AQP2 promoter (126, 174, 305). Although the PKA-CREB pathway is involved in the initial increase in AQP2 abundance after AVP stimulation, it is not involved in the long-term effect of AVP (153), which instead may be mediated by activation of EPAC (153). EPAC may also play a role in the regulation of AQP2 trafficking, as perfusion of mouse IMCDs with an EPAC activator resulted in translocation of AQP2 to the apical plasma membrane (306).

The water channels AQP3 and AQP4 are constitutively expressed in the basolateral membrane of the CNT and CD principal cells (51, 68, 280), where they function as exit pathways for water reabsorbed via AQP2. As such, AQP3 and AQP4 are also important for the urine concentrating mechanism, and both AQP3 and AQP4 knockout mice have decreased urine concentrating ability (168, 169). Water deprivation or AVP infusion, resulting in sustained increases in AQP3 levels, upregulate AQP3 and AQP4 protein and mRNA levels (129, 191, 223, 281). However, there is no evidence for short-term regulation of AQP3 or AQP4 by AVP-induced trafficking.

AVP also regulates renal urea transport. Urea is generated in large quantities by the liver as a result of protein metabolism. Urea is freely filtered by the glomerulus, and excretion of this urea constitutes a large osmotic load to the kidney. Part of this filtered urea is reabsorbed in the proximal tubule via a constitutive process, and part is reabsorbed in the IMCD (reviewed in Ref. 77). The permeability of the majority of the distal nephron and CD to urea is very low. Although the terminal part of the IMCD has the highest urea permeability, exposure of the IMCD to AVP rapidly increases this urea permeability by several orders of magnitude. In the presence of AVP, urea concentrations within the tubule fluid at the level of the IMCD and the interstitium nearly equilibrate, thereby preventing osmotic diuresis. Recycling of some of the urea accumulated within the interstitium and “trapping” of this urea to maintain high interstitial concentrations occurs when urea is secreted into the long loop of Henle and is returned to the medullary CD with the tubule fluid, allowing reentry of this urea into the medullary interstitium (reviewed in Ref. 77).

Regulated urea transport occurs via urea transporters, which in mammals are encoded by two genes; UT-A (Slc14a1) and UT-B (Slc14a2). Although classically called “transporters,” the crystal structure of two evolutionarily distant urea transporters have been solved, indicating that urea flux occurs via a channel-like mechanism (reviewed in Ref. 162). The UT-A gene encodes several mRNA transcripts, of which UT-A1 and UT-A3 are expressed in IMCD cells. In the IMCD, both UT-A1 and UT-A3 localize to intracellular vesicles, while UT-A1 in addition is expressed at the apical plasma membrane and UT-A3 at the basolateral plasma membrane (24, 86, 206, 270) (see Fig. 6 for a schematic representation of urea transporters in an IMCD cell and some of the regulatory mechanisms discussed in the following text). UT-A1/3 knockout mice show a complete absence of AVP-regulated urea transport in IMCD segments (reviewed in Ref. 87), demonstrating that UT-A1 and UT-A3 are responsible for the AVP-regulated high urea permeability of the IMCD (79, 81). UT-A2 mRNA is expressed in the thin descending limbs of Henle’s loop, where interstitial urea is secreted into the nephron during urea recycling (86, 206, 294), while UT-B is expressed in the descending vasa recta, where it is important for exchange of urea between ascending and descending vasa recta (288, 300).

AVP increases urea permeability of the IMCD by stimulation of the classic V2R-adenylate cyclase-cAMP pathway, which leads to modulation of UT-A1 and UT-A3 function (86, 134, 245, 257, 269). Increased cAMP results in two mechanisms that act synergistically to modulate urea transporter function. One mechanism requires increased PKA-dependent stimulation of UT-A1 and UT-A3 phosphorylation, which occurs within minutes and is consistent with the time course for increased urea transport in AVP-treated IMCDs (144, 308). Second, increased cAMP activates EPAC, which also mediates
UT-A1 function (296). Maximal UT-A1 activation following AVP action likely occurs through both pathways (296). In UT-A1, the major AVP-regulated phosphorylation sites are Ser486 and Ser499, activation of which increases urea transport activity and membrane accumulation (25, 127, 144). In addition, AVP induces phosphorylation at Ser84 in both UT-A1 and UT-A3 (127), but the role of this phosphorylation site is not known.

AVP stimulation increases the accumulation of UT-A1 and UT-A3 in the IMCD plasma membrane (24, 146). After AVP stimulation, a proportion of UT-A3, which has a predominantly basolateral localization under normal conditions (270), is also found at the apical plasma membrane, where it may further increase urea influx of IMCD cells (24). However, it has yet to be shown whether phosphorylation of UT-A1 or UT-A3 results in rapid trafficking of the transporters in a similar mechanism as for AQP2.

Prolonged elevation of AVP levels also modulates urea transporter expression in rodents. The effects of AVP appear to be time dependent. For example, in AVP-deficient Brattleboro rats administered AVP for 5 days, UT-A1 protein abundance decreased (139, 282), but after administration for 12 days UT-A1 protein abundance increased. This delay in increased UT-A1 expression corresponds with the time course for increased interstitial urea and osmolality in AVP-treated Brattleboro rats (113, 139), suggesting that the long-term effects of AVP on UT-A1 are due to hyperosmolality-enhanced alterations in UT-A1 gene promoter activity via a tonicity enhancer (TonE) element (196, 197). UT-A2 and UT-A3 expression are also regulated by AVP. For example, chronic infusion of dDAVP in rats increased the abundance of UT-A2 in the thin descending limb of Henle’s loop (294), and in mice UT-A2 and UT-A3 mRNA expression are increased following 20 h of water restriction (80). Similarly to UT-A1, these effects may be an indirect response to dDAVP via enhanced interstitial osmolality, as the V2R has not been detected in the thin descending limb (78, 277).

Conclusion

It is clear from multiple studies in vitro, ex vivo, and in vivo, both in rodent species and humans, that AVP has a multitude of effects on sodium transport mechanisms in the distal nephron. Relative to, for example, the well-described effects of aldosterone on sodium transport which occur predominantly several hours after stimulation, the effects of AVP on sodium transport can occur within minutes. These rapid responses to AVP play a crucial role in water homeostasis, by allowing a similar time frame of AVP response as the effect of AVP on AQP2 regulation.

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AUTHOR CONTRIBUTIONS


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