Body Water Homeostasis: Clinical Disorders of Urinary Dilution and Concentration

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The discovery of the aquaporin-1 (AQP1) water channel by Agre and colleagues (1,2), which led to the Nobel Prize in 2003, has revolutionized the understanding of body fluid water regulation by the kidney. Moreover, the identification of other water channels in the kidney, namely AQP2, 3, and 4, along with urea and ion transporters, has allowed a much improved understanding of urinary dilution and concentration in health and disease at the cellular and molecular levels (3–8).

The AQP have provided a pathway for water movement across cellular membranes that could not be explained by simple diffusion through the lipid bilayers of cell membranes. AQP1 has been found to be expressed constitutively on both the apical and basolateral membranes of the proximal tubule and descending limb of Henle’s loop. This water channel is not under control of vasopressin but is important in urinary concentration. Water efflux through these channels in the descending limb is an important factor in the countercurrent concentrating mechanism, and diminished maximal urinary osmolality has been shown in AQP1 knockout mice (9) and humans without the AQP1 gene (10).

AQP2, 3, and 4 are expressed in the cortical and medullary collecting duct (Figure 1) (11). AQP2 is found exclusively in the principal cells of the collecting tubule and collecting duct and is known to be regulated by arginine vasopressin (AVP). AQP3 and 4 are located on the basolateral membrane of the principal cells in the collecting duct. AQP3 knockout mice exhibit substantial polyuria secondary to vasopressin-resistant nephrogenic diabetes insipidus (NDI) (12). AQP3 is regulated by AVP. AQP4 predominates on the basolateral membrane of the inner medulla and is not regulated by AVP. AQP4 knockout mice also exhibit an NDI that is less severe than that observed in the AQP3 knockout mice (13). Whereas AQP3 and AQP4 constitute the exit channels for water movement across the basolateral membrane of the collecting duct, AQP2 is the water channel for water reabsorption across the apical membrane of the principal cells of the collecting duct. These transgenic mouse models of AQP deletion/mutation are of importance not only for kidney function but also for other epithelia (14).

AVP regulates AQP2 in both an acute (short term) (15) and chronic (long term) manner (16,17). The short-term regulation by AVP involves trafficking of vesicles that contain AQP2 to the apical membrane with resultant increased water permeability. Suppression of plasma AVP reverses this exocytosis of AQP2, and the vesicles are retrieved from the membrane (endocytosis) into the cytoplasm. Both the short- and long-term regulation of AQP2 by AVP is initiated by activation of the V2 receptor on the basolateral membrane of the collecting duct. The binding of AVP to the V2 receptor, which is coupled to a guanine-nucleotide–binding protein Gs, results in activation of adenylyl cyclase. This results in an increase in intracellular cAMP concentration that mediates the activation of protein kinase A (PKA). Activated PKA phosphorylates the serine 256 residue at the C-terminus of AQP2 protein (18,19). The phosphorylated AQP2 then is translocated to the collecting duct apical membrane, thus constituting short-term regulation of AQP2. The long-term regulation of AQP2 is mediated by the cAMP response element in the 5′ flanking region of the AQP2 gene in response to AVP stimulation (20,21). The AQP2 transcription and protein expression involves the phosphorylation of the cAMP response element–binding protein. In contrast to the involvement of PKA activation in the short-term regulation of AQP2, there is some evidence for PKA-independent long-term regulation of AQP2 expression by AVP (22). There also is recent in vitro (23) and in vivo (24) evidence for AQP2 regulation by hyperosmolality independent of AVP. The in vivo experiments were performed in Brattleboro rats, which have no detectable circulatory AVP.

Disorders of water balance can lead to either clinically relevant hyponatremia or hypernatremia. Hyponatremia is much more common and is the most frequent fluid and electrolyte disturbance in hospitalized patients. When defined as plasma sodium concentration <135 mEq/L, the prevalence of hyponatremia in hospitalized patients may be as high as 15 to 30%. Studies indicate that 40 to 75% of these cases are hospital acquired (25,26). It is clear that hyponatremia may occur in the presence of an increase in total body sodium, a decrease in total body sodium, or a near-normal total body sodium (Figure 2). In any of these three circumstances, hyponatremia has occurred because of a relatively greater amount of total body water as compared with total body solute. Recent advances of the mech-
anisms of impaired urinary diluting capacity that lead to these hyponatremic disorders are discussed.

Hypothyroidism
Advanced primary hypothyroidism can lead to hyponatremia, most frequently associated with myxedema. Milder forms of hypothyroidism can lead to a decrease in renal blood flow, glomerular filtration, and a decrease in maximal solute-free water excretion. However, these defects generally are not of sufficient magnitude to lead to hyponatremia in association with a normal fluid intake. With normal fluid intake, hyponatremia generally results from the inability to decrease urine osmolality below plasma rather than a defect in maximal solute-free water excretion. The failure to dilute the urine below plasma generally is due to a failure to suppress maximally the antidiuretic hormone AVP (27). The failure of some hypothyroid patients to suppress plasma AVP during an acute water load was reported initially by Skowsky et al. (28).

Recently, experimental studies in rats with advanced hypothyroidism (serum total T4 0.6 ± 0.02 ng/dl) further examined urinary dilution and maximal solute-free water excretion (29). There was a significant decrease in cardiac index and heart rate in these hypothyroid animals that was normalized with l-thyroxine replacement. During an acute water load, these hypothyroid animals exhibited a significantly lower urine flow and higher urinary osmolality than either control animals or hypothyroid animals that were treated with l-thyroxine. The hypothyroid animals also had significantly higher plasma AVP concentrations despite a lower plasma osmolality that normalized with l-thyroxine treatment. The impaired water excretion also was associated with an upregulation of AVP-mediated AQP2 expression and membrane trafficking in the inner medulla (Figure 3). Taken together, these results suggested a major role of nonosmotic AVP release in the hyponatremia associated with advanced hypothyroidism. Studies therefore were undertaken with a vasopressin V2 receptor antagonist (OPC-31260) in these animals with advanced hypothyroidism. Minimal urinary osmolality in hypothyroid rats that were treated with the V2

Figure 1. Nephron sites of aquaporin (AQP) water channels (blue), ion transporters (black), and urea transporters (green). ROMK, renal outer medullary potassium channel; UT, urea transporter.

Figure 2. Diagnostic and therapeutic approach to the hypovolemic, euvolemic, and hypervolemic hyponatremic patient.
receptor antagonist was normalized as compared with untreated hypothyroid rats (97 versus 430 mOsm/kg H2O; \( P < 0.0001 \)). Maximal solute-free water excretion in the hypothyroid rats significantly increased but did not reach euthyroid values. These experimental results therefore support the pivotal role of the baroreceptor-mediated nonosmotic AVP release in the hyponatremia associated with advanced hypothyroidism. The submaximal solute-free water excretion would not be of clinical significance unless other factors, such as diuretics or large fluid intakes, intervene.

Of interest, a defect in maximal urinary concentration was observed at a somewhat less prolonged stage of experimental hypothyroidism (30). Maximal urinary osmolality with fluid deprivation was associated with decreased Na-K-2Cl expression and diminished medullary osmolality. Even though AQP2 expression was decreased, the failure to exhibit a difference between the diminished urinary and medullary osmolality suggested that the primary mediator of the concentrating defect was a decrease in the Na-K-2Cl co-transporter expression and therefore impaired countercurrent concentration rather than the decreased AQP2 expression. This urine-concentrating defect in hypothyroidism would be of clinical relevance only in patients who were undergoing excessive extrarenal fluid losses (e.g., diarrhea).

**Addison’s Disease and Hypopituitarism**

Primary adrenal insufficiency (Addison’s disease) involves deficiency of glucocorticoid and mineralocorticoid hormones, both of which can be associated with hyponatremia. Hypopituitarism is associated with only glucocorticoid deficiency, because the renin-angiotensin-aldosterone system is intact. Although secondary hypothyroidism is associated with hypopituitarism, in contrast to primary hypothyroidism, the severity generally is not sufficient to be associated with hyponatremia. Therefore, the hyponatremia that is related to hypopituitarism generally is due to glucocorticoid deficiency rather than thyroid deficiency.

Mineralocorticoid deficiency that is associated with primary adrenal insufficiency exhibits a different pathophysiology than glucocorticoid deficiency in causing hyponatremia. Because aldosterone increases potassium and hydrogen ion secretion, hyperkalemia and nonanion gap metabolic acidosis is charac-
teristic of primary but not secondary adrenal insufficiency as a result of hypopituitarism. The mineralocorticoid deficiency also is responsible for the sodium chloride wasting and extracellular fluid volume depletion in primary adrenal insufficiency. Selective mineralocorticoid deficiency has been studied in adrenalectomized animals that received replacement glucocorticoid hormone (31–33). With isolated mineralocorticoid hormone deficiency and hyponatremia, plasma AVP concentrations were not suppressed and collecting duct AQP2 and 3 expressions were upregulated. Outer medullary Na-K-2Cl co-transporter and Na-K-ATPase were decreased in mineralocorticoid animals (32). Administration of AVP V2 antagonist in these animals significantly improved urinary dilution, but a modest defect in maximal solute-free water excretion remained (33). This latter defect relates to effects of extracellular fluid volume depletion, including decreased GFR and increased proximal tubular sodium reabsorption with resultant diminished fluid delivery to the distal diluting segment of the nephron. Avoidance of negative sodium balance in mineralocorticoid-deficient rats normalized Na-K-2Cl co-transporter, Na-K-ATPase, and collecting duct AQP2 and 3 (32). It also is of interest that high sodium chloride intake may correct not only the hyponatremia but also the hyperkalemia and metabolic acidosis in Addison’s disease, thereby supporting an important role for mineralocorticoid deficiency.

If hyponatremia persists in primary adrenal insufficiency despite avoiding negative sodium balance, then the hyponatremia is due to glucocorticoid deficiency. The mechanisms of hyponatremia with glucocorticoid deficiency are different than with mineralocorticoid deficiency. Glucocorticoid deficiency does not cause a negative sodium balance, and in fact a positive sodium balance may occur. The absence of glucocorticoid hormone, however, has major effects on systemic hemodynamics, including a decrease in cardiac index with an inadequate response of systemic vascular resistance to maintain mean arterial pressure (34). These systemic effects result in several consequences. The resultant decrease in stretch on the arterial baroreceptors in the carotid sinus and aortic arch removes the tonic vagal and glossopharyngeal inhibition on the central release of AVP. Elevated plasma AVP concentrations have been demonstrated in glucocorticoid-deficient animals (33,35) and patients with hypopituitarism (36) despite a degree of hyponatremia that would maximally suppress AVP in normal individuals. The messenger RNA for AVP in the hypothalamus also has been shown to be increased during glucocorticoid deficiency (37). AVP-synthesizing neurons terminate in the median eminence of the hypothalamus; therefore, a role for ACTH also could be involved in the nonosmotic AVP stimulation that is associated with glucocorticoid deficiency. There also is evidence of a central effect of glucocorticoid hormone in the hypothalamus whereby hypo-osmolality does not maximally suppress AVP synthesis during glucocorticoid deficiency (38). In either case, the importance of this nonosmotic stimulation of AVP in glucocorticoid deficiency was documented using peptide and nonpeptide vasopressin V2 receptor antagonists, which profoundly reversed the water retention (33,35).

A recent molecular analysis of the impaired diluting capacity with glucocorticoid deficiency was undertaken in adrenalectomized rats that received replacement physiological concentrations of mineralocorticoid hormone (35). As compared with adrenalectomized rats that received replacement of both mineralocorticoid and glucocorticoid hormone, during an acute water load, the glucocorticoid-deficient animals exhibited higher plasma AVP concentrations, diminished solute-free water excretion, and increased protein expression in the inner medulla of AQP2, phosphorylated AQP2, and apical membrane trafficking of AQP2. The administration of a nonpeptide vasopressin V2 receptor antagonist reversed these events (Figure 4).

There also was insight into AVP-independent effects on water excretion during glucocorticoid deficiency. Pair feeding during glucocorticoid deficiency was associated with an upregulation of the Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) co-transporter, Na\(^{+}\)/H\(^{+}\) exchanger isoform 3, and cortical \(\beta\) and \(\gamma\) subunits of the epithelial sodium channel and sodium retention (35). These events, along with the effect of a decrease in renal perfusion pressure, would lead to decreased fluid delivery to the distal nephron diluting segments and attenuate maximal solute-free water excretion. This AVP-independent effect on maximal water excretion probably would not be of clinical significance except in the circumstance of large increases in water intake.

Of interest, maximal urinary concentration also was diminished in glucocorticoid-deficient rats after 36 h of fluid deprivation (34). The concentrating defect involved primarily an impairment of the countercurrent concentrating mechanism with comparable diminutions in medullary and urinary osmolalities. With the fluid deprivation, lower expression of the urea transporter and ion transporters (e.g., Na-K-2Cl) in the outer medulla seemed to account for the diminished medullary osmotic gradient.

**Primary Polydipsia or Compulsive Water Drinking**

The renal capacity to excrete solute-free water is substantial (27), yet the average daily fluid intake for hyponatremic patients is only 2.4 L (39). The delivery of tubular fluid to the distal diluting segment of the nephron is estimated to be approximately 20% of glomerular filtrate. The diluting segment begins with the water-impermeable ascending limb of Henle’s loop and in the absence of vasopressin includes virtually the remainder of the nephron, i.e., connecting tubule, collecting tubule, and cortical and medullary collecting ducts. With a GFR of 100 ml/min, 140 L of filtrate occurs daily. If 20% of this filtrate reaches the distal diluting segment and plasma AVP is maximally suppressed, then 28 L of solute-free water theoretically could be excreted. Therefore, the normal capacity to excrete solute-free water may approximate 1 L/h if administered consistently over 24 h. Patients with primary polydipsia, however, ingest their water intake mostly during their awake hours of the day. Even so, individuals who have primary polydipsia and normal renal, endocrine, cardiac, and hepatic function and are not volume depleted or taking diuretics do not become hyponatremic from drinking up to 10 to 12 L of water per day. Their plasma osmolalities, however, may be in the lower nor-
mal range (275 to 285 mOsm/kg H2O). If any of the above circumstances intervene, then patients with primary polydipsia are in danger of developing “water intoxication” with severe central nervous system symptoms, including headache, nausea, vomiting, decreased mentation, confusion, obtundation, seizures, and even cerebral hernia, cardiopulmonary arrest, and death. Pure water intoxication rarely can occur without an intervening event with massive water intake, e.g., drinking directly from a faucet or with an open hose in the stomach.

Psychiatric patients in hospital frequently have polydipsia, because of dry mouth from medications with anticholinergic properties and/or delusions (e.g., “water cleanses the soul”). In fact, another term that has been used for primary polydipsia has been psychogenic water drinking. In 1973, acute psychoses with hyponatremia mimicking the syndrome of antidiuretic syndrome first was described (40). Subsequently, hyponatremia and water intoxication with failure to suppress plasma AVP have been described with psychotic disorders, such as schizophrenia (41). Psychiatric patients also have a high incidence of smoking, and nicotine is a very potent acute stimulus for AVP release (42,43).

Recently, molecular and cellular events were analyzed in a unique rat model of primary polydipsia in which the same daily food intake was ingested in control animals with ad libitum water intake and polyuric rats that drank 100 ml/d (44). This model therefore allowed assessment of the renal effects of polyuria at comparable electrolyte, caloric, and protein intakes. As compared with controls, serum osmolality was lower in the polydipsic rats (293 versus 277 mOsm/kg H2O; P < 0.04) as was urine osmolality (1365 versus 139 mOsm/kg H2O; P < 0.001). As occurs in humans after 10 d of increased water intake (45), a form of AVP-resistant NDI emerged after 10 d in the polydipsic rats. With 36 h of fluid deprivation, the polydipsic animals exhibited significantly higher urine output associated with a lower urine osmolality despite significant higher plasma AVP concentrations (Figure 5). The ion and urea transporters with potential to alter the integrity of the countercurrent concentrating mechanism were unaltered, including outer medullary Na-K-2Cl, Na-K-ATPase, and inner medullary urea transporter, in polydipsic as compared with control rats. The AQP1 water channel expression, which if mutated can cause a concentrating defect (9,10), also was no different in the polydipsic animals. What was remarkably different was a highly significant suppression of AQP2 protein expression in the outer and

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**Figure 4.** Effect of V2 receptor antagonist (OPC) in glucocorticoid-deficient rats to reverse the impaired water excretion (A) and urinary dilution (B) as well as the increased AQP2 (C) and phosphorylated AQP2 expression (D). Reprinted from reference (33), with permission.
inner medulla. The AQP2 expression in the membrane fraction, as an index of trafficking to the collecting duct membrane, was decreased. The AQP3 protein abundance in the outer medulla also was significantly diminished in the polydipsic rats. After the 36-h fluid deprivation, the medullary osmolalities in the control and polydipsic rats were no different, even though the polydipsic rats demonstrated a significant decrease in maximal urinary osmolality. This failure of osmotic equilibration between the medullary interstitium and urine in the polydipsic rats supported a critical role of the downregulation of AQP2 and, possibly, AQP3 in the impaired urine-concentrating capacity associated with polydipsia.

Pregnancy
A decrease in plasma osmolality of 8 to 10 mOsm/kg H2O is an early occurrence in normal pregnancy. Sodium and water retention occurs during pregnancy with an expansion of extracellular fluid ranging from 30 to 50% (46). Water retention exceeds the sodium retention in normal pregnancy and thus the fall in plasma osmolality and sodium concentration. Recent studies have shown that systemic arterial vasodilation occurs early in the first trimester of pregnancy (47). The mediator(s) of this decrease in vascular resistance is(are) not well defined, but some experimental results indicate a role of estrogen-mediated nitric oxide (48) and relaxin (49). In any case, the standard responses to arterial underfilling secondary to arterial vasodilation also occur in pregnancy. There is a compensatory rise in cardiac output and stimulation of the renin-angiotensin-aldosterone system that attenuates the vasodilation-mediated fall in BP during the first trimester (47). In other circumstances of arterial vasodilation, such as cirrhosis or a large arteriovenous fistula, the activation of arterial baroreceptors is accompanied by the nonosmotic release of AVP (50,51). In pregnancy, plasma AVP concentrations are still detectable in the presence of a degree of hypo-osmolality that normally would suppress AVP release, i.e., 1 to 2% decrease in plasma osmolality. The hypo-osmolality of pregnancy has been termed a “resetting” of the hypothalamic osmoreceptor threshold to a lower level (52) but may be due merely to the nonosmotic release of AVP that occurs with arterial vasodilation (50,51). It also is of interest that early in pregnancy, there is an increase in thirst and fluid intake despite a fall in plasma osmolality, which normally suppresses thirst. This also is compatible with a nonosmotic stimulation of thirst as a result of arterial underfilling.

Arterial underfilling as a result of arterial vasodilation, as occurs in cirrhosis and pregnancy, is associated with compensatory increases in total blood volume and cardiac output. Moreover, arterial vasodilation stimulates the nonosmotic AVP release and activates the renin-angiotensin-aldosterone axis, as occurs in cirrhosis and pregnancy. These hormonal responses also compensate for arterial underfilling, at the expense of hyponatremia and edema (50,51,53).
With the discovery of the renal water channels, i.e., AQP, body water regulation in pregnancy could be investigated in more depth. Pregnancy in the rat exhibits most of the characteristics of human pregnancy and therefore has been the standard experimental model. Studies therefore were undertaken in pregnant rats to examine the effect on renal AQP (54). If the nonosmotic release of AVP secondary to arterial vasodilation is involved in the water retention in pregnancy (55), then vasopressin-mediated upregulation and trafficking of AQP2 should be demonstrable. Alternatively, if pregnancy "resets" the osmotic threshold for AVP release, then the regulation of the AQP2 around this reset osmostat should mimic the nonpregnant state. As compared with nonpregnant littersmates, pregnant rats were found to have in the first trimester a profound upregulation of inner medullary AQP2 mRNA and protein expression that persisted throughout the pregnancy (Figure 6). In the pregnant rats, there also was an increase in AQP2 in the membrane fraction, indicating increased AQP2 trafficking to the apical membrane of the collecting duct. It is known that the effect of AVP on AQP2 protein expression and trafficking is mediated by vasopressin V2 receptor. Studies therefore were undertaken with a nonpeptide V2 receptor antagonist in pregnant and nonpregnant animals. The AQP2 protein expression and apical membrane location by immunofluorescence were returned to the nonpregnant state during the V2 receptor antagonist administration to pregnant rats (53). These experimental results therefore strongly support a role of the nonosmotic AVP release in regulation AQP2 expression and trafficking in pregnancy. This was supported further by the observation that increased urinary AQP2 occurs during pregnancy (56). It must be remembered, however, that the antidiuretic effect of oxytocin also is mediated via the V2 receptor on the basolateral membrane of the collecting duct and therefore could be a contributing factor in the water retention of pregnancy (57). It also has been shown that circulating vasopressinase from the placenta, which increases AVP degradation, can uncover or cause diabetes insipidus in pregnancy (58).

**Cardiac Failure**

Advanced heart failure frequently is associated with hyponatremia. In fact, hyponatremia has been found to be a risk factor for poor survival for patients with congestive heart failure (59). The pathogenesis of the hyponatremia of cardiac failure initially seemed not to involve AVP, primarily because the bioassay for antidiuretic hormone was relatively insensitive. With the development of the RIA to measure plasma AVP, studies were undertaken to examine the cause of hyponatremia in patients with heart failure. In the first study, the hypo-osmolality in patients with heart failure was of a degree that would maximally suppress plasma AVP in normal individuals, yet 30 (81%) of 37 patients had detectable plasma AVP by RIA (60). Therefore, the term nonosmotic AVP release emerged to describe hyponatremic patients with heart failure and other edematous disorders. There also is evidence for increased AVP synthesis in the hypothalamus in experimental heart failure (61). Hyponatremia may occur both in low-output cardiac failure (e.g., ischemic or nonischemic cardiomyopathy) and in high-output cardiac failure (e.g., thyrotoxicosis, beriberi, large arteriovenous fistula) (62). This apparent dilemma is understandable because arterial underfilling with baroreceptor-mediated AVP stimulation can occur with either a decrease in cardiac output or systemic arterial vasodilation as occurs with high-output cardiac failure (50,51,53).

With the discovery of water channels, investigations were undertaken to examine whether increased plasma AVP in cardiac failure would be associated with increased renal AQP2 protein expression and trafficking to the apical membrane of the collecting duct (63,64). As in humans, plasma AVP increased in advanced cardiac failure in rats despite hypo-osmolality (63). In these animals with heart failure secondary to coronary artery ligation, AQP2 expression and membrane trafficking were increased in the inner medulla of the kidney (63,64). Administration of a nonpeptide V2 receptor antagonist to these animals with heart failure reversed the increased expression and trafficking of AQP2 (63). Support for this relationship between nonosmotic AVP and AQP2 expression has been demonstrated by studies in patients with heart failure by measurement of urinary AQP2 (65). Approximately 3 to 6% of AQP2 can be measured by RIA or Western immunoblotting in the urine. In patients with New York Heart Association class II or III heart failure, a V2 receptor antagonist caused a solute-free water diuresis and increased plasma sodium concentration. Urinary AQP2 decreased in these patients during the V2 receptor antagonist administration (Figure 7) (65), thus indicating that less of this water channel reached the apical membrane of the collecting duct. An orally active, nonpeptide vasopressin V2 receptor antagonist was shown recently in patients with cardiac

![Figure 6. Inner medulla AQP2 protein expression (densitometry above and immunoblots below) in nonpregnant (NP) rats and first-trimester (P7), second-trimester (P14), and third-trimester (P20) pregnant rats. Glycosylated (36 to 45 kD) and nonglycosylated (29 kD) expression is shown. Reprinted from reference (54), with permission.](image_url)
failure to cause a substantial loss of body weight over 30 d of treatment (66). Acute reversal of water retention with another nonpeptide V2 antagonist also was shown recently (67). Cardiac afterload reduction with either hydralazine or an angiotensin-converting enzyme inhibitor in patients with cardiac failure increased cardiac output, thereby attenuating arterial underfilling. This was associated with an increase in solute-free water excretion in association with a decrease in plasma and platelet AVP concentration (68).

Cirrhosis

Hyponatremia frequently occurs in decompensated patients with cirrhosis and ascites (69), whereas patients with compensated cirrhosis and no ascites rarely are hyponatremic (70). Hyponatremia also is a risk factor for poor survival in patients with cirrhosis (71). As with heart failure, not until measurement of plasma AVP by RIA was the nonosmotic AVP incriminated in the hyponatremia of cirrhosis (72). Early in cirrhosis, portal hypertension is associated with an increased splanchnic blood flow (73). The associated decrease in systemic vascular resistance causes arterial underfilling and baroreceptor-mediated increase in AVP release (74). An increase in AVP synthesis in the hypothalamus also has been found in experimental cirrhosis (75). The increase in plasma AVP and water excretion in cirrhosis were shown to correlate directly with plasma norepinephrine and renin activity (76). This observation provides evidence that cirrhosis activates the sympathetic and renin-angiotensin systems, as well as the nonosmotic stimulation of AVP, during arterial underfilling as a result of arterial vasodilation. Increased AQ2P2 expression and trafficking also have been shown to be present in experimental cirrhosis (77), a finding that is compatible with the observed increased urinary excretion of AQ2P2 in patients with cirrhosis (78). Vasopressin V2 receptor antagonists also have been shown to increase solute-free water excretion in patients with cirrhosis (79) and experimental cirrhosis (80). These effects on the nonosmotic stimulation of AVP and AQ2P2 seem to be manifest with more severe cirrhosis, because milder experimental forms of cirrhosis (e.g., bile duct ligation, inhalation of carbon tetrachloride) did not show these changes (81,82).

There are several candidates as mediators of the splanchnic vasodilation and arterial underfilling in cirrhosis. Recent evidence, however, indicates a prominent role of endothelial and inducible nitric oxide synthesis (83). In this regard, 7 d of treatment with a nonspecific nitric oxide synthase inhibitor at a dose to reverse the arterial vasodilation and thus the hyperdynamic circulation of experimental cirrhosis was found to suppress plasma AVP, increase solute-free water diuresis, and correct the hyponatremia (84).

NDI: Genetic and Acquired

NDI is when the kidney does not respond normally to the antidiuretic effect of AVP. NDI can be due to genetic or acquired causes. The cellular and molecular defects that cause the absolute or relative renal unresponsiveness to vasopressin may involve impairment of the water permeability across the collecting duct, the generation of the medullary osmotic gradient for water transport, or both.

Congenital NDI

The genetic or congenital NDI can be due primarily to mutations in two areas. The most common defect relates to mutations of the vasopressin V2 receptor on the basolateral membrane of the collecting duct. More than 180 mutations of the V2 receptor have been found in chromosome region Xq28 (Figure 8), and together these abnormalities account for 90% of patients...
with congenital NDI (85). Studies suggest that most mutations involve protein misfolding, which traps the V2 receptor intracellularly, thereby not allowing the receptor to translocate to the basolateral membrane of the collecting duct (86). Therefore, the adenylate cyclase-cAMP signaling pathway, which mediates AQP2 expression and trafficking to the apical membrane, is not activated. Recent in vivo studies indicate that nonpeptide V2, V1, and V1/V2 receptor antagonists may allow some of the mutant V2 receptors to be transported to the basolateral membrane and to function normally (87). These antagonists act as chaperones for the misfolded V2 receptors to reach the membrane. A recent study in patients with NDI demonstrated an approximate decrease in daily urine flow from 12 to 8 L and an increase in urinary osmolality from 98 to 170 mOsm/kg using such a receptor antagonist (88). This variety of congenital recessive NDI has an X-linked recessive mode of inheritance, and affected male individuals cannot concentrate their urine in response to vasopressin. Heterozygous female individuals generally are asymptomatic but may have a modest degree of polyuria and polydipsia. In contrast, male individuals may have up to 20 L of urine every day. The need to drink sufficient water to maintain water balance, therefore, is challenging and certainly affects the quality of life of the individual. Before genetic screening of newborns in affected families, dehydration and mental retardation frequently developed. Now affected individuals who receive adequate water intake progress to normal adulthood without any mental or physical retardation.

Another cause of congenital NDI involves mutations of the AQP2 gene in chromosome region 12q13 (89). This accounts for 10% of families with congenital NDI. Currently, more than 30 such mutations have been identified (Figure 9). In the autosomal recessive variety, the mutant AQP2 proteins are trapped in the endoplasmic reticulum, whereas the autosomal dominant varieties are mutations of the carboxy terminus.

**Acquired NDI**

Hypokalemia and hypercalcemia are electrolyte disorders that may be associated with polyuria and polydipsia as a result of a defect in the renal response of vasopressin to concentrate the urine maximally. Experimental studies in rats have been undertaken to study the mechanisms involved. A potassium-deficient diet for 11 d caused hypokalemic-related NDI (90), and 7 d of vitamin D (dihydrotachysterol) caused hypercalcemic-related NDI (91). In both experimental models, there was a downregulation of AQP2 expression in the inner medulla. In the hypercalcemic model, there also was a decrease in the Na-K-2Cl co-transporter in the outer medulla. As the inhibitor of the countercurrent concentrating mechanism for generation of the corticomedullary osmotic gradient, this decrease in the Na-K-2Cl co-transporter no doubt also contributed to the acquired NDI.

Polyuria secondary to NDI also may accompany bilateral urinary tract obstruction, in part due to the resultant volume expansion and urea retention. Experimental studies, however, have shown that unilateral ureteral obstruction, which avoids these sequelae of bilateral ureteral obstruction, also causes NDI (92). As with the above electrolyte disorders, AQP2 downregulation has been demonstrated to be associated with acquired NDI related to urinary tract obstruction. Lithium also has been demonstrated in experimental animals to be associated with downregulation of AQP2 expression and trafficking (93). Lithium therapy has been a worldwide, effective treatment for bipolar affective disorder for many years. Therefore, understanding the mechanism for the NDI that is associated with this medication is important, particularly because it occurs in approximately 20% of treated patients. In this regard, recent in vitro and in vivo experiments suggest that the effect of lithium to downregulate AQP2 may occur independent of adenyllyl cyclase activity (94,95).

A vasopressin-resistant urine-concentrating defect is one of the earliest abnormalities associated with acute renal failure. This variety of acquired NDI also seems to involve an inability to establish a high medullary solute content, i.e., the osmotic driving force for water reabsorption. Moreover, AQP1, 2, and 3 expression has been shown to be reduced in rats with both oliguric and nonoliguric ischemic acute renal failure (96). Similar results have been found in the 5/6 nephrectomy–induced model of chronic renal failure (97). In patients with advanced chronic renal failure, vasopressin-resistant hypotonic urine has been described (98). This finding suggests a defect in water transport across the medullary collecting duct, because absolute impairment of the countercurrent concentrating mechanism still would be associated with an isotonic, not hypotonic, medullary interstitium. In contrast to congenital NDI, the polyuria of acquired NDI generally is of a moderate degree (e.g., 3 to 4 L/24 h). The thirst mechanism generally protects against hypernatremia in NDI states unless age (newborn, elderly) or illness restricts availability of fluid intake.

**Conclusion**

The ability to analyze the renal water channels and ion and urea transporters has allowed for the better understanding of
several clinical disorders with impaired urinary dilution and/or concentration.

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Access to UpToDate on-line is available for additional clinical information at http://www.jasn.org/