Nephrogenic Diabetes Insipidus: Essential Insights into the Molecular Background and Potential Therapies for Treatment

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The water channel aquaporin-2 (AQP2), expressed in the kidney collecting ducts, plays a pivotal role in maintaining body water balance. The channel is regulated by the peptide hormone arginine vasopressin (AVP), which exerts its effects through the type 2 vasopressin receptor (AVPR2). Disrupted function or regulation of AQP2 or the AVPR2 results in nephrogenic diabetes insipidus (NDI), a common clinical condition of renal origin characterized by polydipsia and polyuria. Over several years, major research efforts have advanced our understanding of NDI at the genetic, cellular, molecular, and biological levels. NDI is commonly characterized as hereditary (congenital) NDI, arising from genetic mutations in the AVPR2 or AQP2; or acquired NDI, due to for example medical treatment or electrolyte disturbances. In this article, we provide a comprehensive overview of the genetic, cell biological, and pathophysiological causes of NDI, with emphasis on the congenital forms and the acquired forms arising from lithium and other drug therapies, acute and chronic renal failure, and disturbed levels of calcium and potassium. Additionally, we provide an overview of the exciting new treatment strategies that have been recently proposed for alleviating the symptoms of some forms of the disease and for bypassing G protein-coupled receptor signaling. (Endocrine Reviews 34: 278–301, 2013)

I. Introduction

The maintenance of normal body water balance requires a system that ensures the daily intake of water matches the daily loss of water. Both water intake and water loss can vary considerably on a daily basis, e.g., due to limited access to water, water loss though breathing,

Abbreviations: adFNDI, Autosomal dominant FNDI; AQP2, aquaporin-2; ARF, acute renal failure; AVP, arginine vasopressin; AVPR2, type 2 vasopressin receptor; cGMP, cyclic GMP; CRF, chronic renal failure; dDAVP, desmopressin; DI, diabetes insipidus; ENaC, epithelial sodium channel; EP, subtype of prostanoid receptors; ER, endoplasmic reticulum; FNDI, familial neurohypophyseal DI; Hsp90, heat shock protein 90; KO, knockout; MRI, magnetic resonance imaging; NDI, nephrogenic DI; NKCC2, Na-K-Cl cotransporter 2; NPII, neurophysin II; ONO, ONO-AE-329; PDE, phosphodiesterase; PGE2, prostaglandin E2; PKA, protein kinase A; TAL, thick ascending limb; X-NDI, X-linked NDI; WT, wild type.
and sweating during exercise. Regulated water excretion by the kidney is one of the key factors in the body’s ability to adjust to these challenges and preserve body water balance. Increases in plasma osmolality or decreases in blood volume reflect a need for the body to conserve water. Even very minor changes, less than 1%, in plasma osmolality stimulate osmoreceptors in the hypothalamus, leading to secretion of the antidiuretic hormone arginine vasopressin (AVP) from the pituitary gland (1, 2). A similar response is elicited via baro-receptors due to a decrease in blood volume, although in this case, such blood volume changes must reach 5–10%.

The water-conserving effect of AVP is mediated predominantly by the binding of AVP to the type 2 vasopressin receptor (AVPR2), a class of G protein-coupled receptors localized to the basolateral side of the principal cell of the kidney collecting duct (Fig. 1). Binding of AVP to the AVPR2 results in receptor activation and interaction of the AVPR2 with the cytosolic G protein, GaS, which in turn activates adenylate cyclase. This results in increased cAMP levels and leads to a cascade of intracellular events, among which are protein kinase A (PKA) activation and movement of transport vesicles containing the water channel aquaporin-2 (AQP2) from intracellular storage compartments to the apical surface of the principal cells. At the apical plasma membrane, AQP2 functionally exists as homotetramers (3, 4) and is the rate-limiting entry site for water reabsorption along an osmotic gradient. The osmotic gradient is due to solute reabsorption in the medullary thick ascending limb (TAL), a process also regulated by AVP. Water entering the principal cell via AQP2 exits via AQP3 and AQP4 in the basolateral plasma membrane. Upon restoration of water balance, the levels of plasma AVP drop, and AQP2 levels in the apical plasma membrane decrease. In humans, the presence of AVP can increase urine osmolality to approximately 1200 mosmol/kg and reduce urine output to 0.5 ml/min. In contrast, in the absence of AVP, urine osmolality can be 50 mosmol/kg and the urine flow rate 20 ml/min (5). Under normal conditions, AVP-mediated activation of the AVPR2 leads to COOH-terminal phosphorylation of the AVPR2, β-arrestin recruitment, and AVPR2 internalization (6). This process negatively regulates the effects of the AVPR2 and prevents prolonged and excessive reabsorption of water.

Figure 1.

Figure 1. Illustration of AVP-mediated trafficking of AQP2 in the principal cell of the kidney collecting duct. Upon binding of AVP to the basolateral G protein-coupled vasopressin receptor AVP2R (V2R), Gs protein-mediated signaling leads to activation of adenylate cyclase (AC). This activation results in increased levels of intracellular cAMP, activation of PKA, and subsequent AQP2 phosphorylation and AQP2 accumulation in the apical plasma membrane of the cell. This event renders the cell permeable to water via the apically located AQP2 and the basolaterally located aquaporins, AQP3 and AQP4.
II. Pathophysiology of Diabetes Insipidus (DI)

DI is a clinical syndrome characterized by polyuria (due to a defect in the urinary concentrating mechanism) and compensatory polydipsia. In the general population, the prevalence of DI is approximately one per 25,000–30,000 (7–9). Upon restricted/inadequate water intake to compensate for the urinary loss of water, these patients are at risk of becoming severely dehydrated. There are four fundamental types of DI (Fig. 2) (5, 10). Two congenital forms of DI exist, namely familial neurohypophyseal DI (FNDI) and congenital nephrogenic DI (NDI). Together, these conditions account for less than 10% of all cases of DI in the clinic (11). FNDI occurs on the basis of decreased or defective secretion of AVP from the pituitary gland. NDI is primarily due to a decreased or defective action of AVP in the principal cell of the collecting duct, although a lack of AVP response in the TAL may also contribute to the condition. It must be emphasized that the urinary concentrating defect in both FNDI and NDI can vary considerably in severity. Both central DI and NDI can also be acquired. In contrast to the congenital forms, the acquired forms, especially the nephrogenic forms, are more common conditions in the clinic. Other forms of DI, which are not the focus of this review, include gestational diabetes caused by AVP deficiency due to increased metabolism of AVP in the placenta and primary polydipsia (dipsogenic and psychogenic), a form of DI resulting from AVP suppression due to excessive water intake (5, 10). Deficiency

Figure 2.

![Diagram of four fundamental causes and some of the underlying mechanisms of DI](image-url)
of AVP can be corrected by treatment with desmopressin, which is an AVP analog specific for the AVPR2 (trade names: desmopressin (dDAVP), DesmoMelt, Stimate, and Minirin). However, defects in the action of AVP at the kidney level can, at present, rarely be corrected. Treatment in these cases is aimed at using other therapeutic strategies to improve symptoms (Sections VIII and IX).

III. Diagnosis of DI

Effective treatment of DI requires accurate differentiation of the underlying cause. In DI, there are considerable variations in urine osmolality and urine output. DI is most commonly defined as a urine volume of more than 3–3.5 liters in 24 h in adults (or >50 ml/kg bodyweight/24 h) with a urine osmolality less than 300 mosmol/kg (5, 12, 13). Urine volume and osmolality are therefore essential measures in the diagnosis. A subject’s history is essential in differentiating DI from other causes of polyuria and determining the cause of the disease. In certain cases, the history (e.g. gestational onset or after brain surgery) and presentation of the symptoms (e.g. complete DI) make the differential diagnosis less complicated. However, in cases of doubt, there are some alternative diagnostic approaches that can be performed, such as a water deprivation test with or without hypertonic saline infusion, desmopressin challenge, and magnetic resonance imaging (MRI) of the brain.

A. Water deprivation test and desmopressin challenge

Water deprivation test and desmopressin challenge can be helpful in differentiating between severe central DI and NDI (10). Due to the risk of pronounced dehydration and hypovolemia, this test must be performed under tight control. The principle of this test is to withhold all fluids from the patient and measure body weight, urine osmolality, and plasma osmolality frequently (hourly) to determine the subject’s dehydration status. When sufficiently dehydrated, i.e. body weight falls more than 5%, or when plasma osmolality rises from baseline, desmopressin is administered followed by determination of urine osmolality for at least 2 h. Commonly used doses are 2 μg iv, 20 μg intranasal, or 120 μg as a sublingual melt formulation. Normal individuals and those with primary polydipsia will have urine osmolality greater than plasma osmolality after water deprivation, and urine osmolality will further increase minimally after desmopressin administration. In central DI, urine osmolality will remain below plasma osmolality after water deprivation, but desmopressin administration results in increased urine osmolality (>50%). In NDI, urine osmolality remains below plasma osmolality, and dDAVP administration increases urine osmolality by less than 50% (13). One diagnostic pitfall in this approach is that all conditions with polyuria result in a reduced antidiuretic response to desmopressin due primarily to wash-out of the medullary concentration gradient. Furthermore, in response to fluid deprivation and desmopressin treatment, the moderate increases in urine osmolality make the diagnosis of primary polydipsia, partial neurohypophyseal DI, and partial NDI indistinguishable (14).

B. Measuring plasma AVP levels

During osmotic stimulation, measurement of plasma AVP levels can facilitate discrimination between the various forms of DI, especially the partial forms. The measurements are performed under basal conditions (ad libitum fluid intake), during fluid deprivation, and/or during hypertonic saline infusion. The levels of plasma AVP are compared with both plasma and urine osmolality. Elevated basal plasma AVP strongly suggests NDI. If plasma AVP levels during osmotic stimulation are normal or elevated relative to the corresponding plasma osmolality, neurohypophyseal DI can be excluded, and the level of urine osmolality relative to plasma AVP will distinguish NDI from primary polydipsia (5).

C. MRI scan

In cases of low plasma AVP levels, an MRI scan of the brain can help differentiate between primary polydipsia and DI. Under normal conditions (or primary polydipsia), an intense signal is detected in the neurohypophysis using T1-weighted imaging. In neurohypophyseal DI or NDI, this intense signal is absent (5, 15, 16).

D. Copeptin assays

AVP and copeptin share the same precursor peptide, which is 164 amino acids long and consists of a signal peptide, AVP, neurophysin II (NPII), and copeptin (Fig. 3). Thus, copeptin is released together with AVP during precursor processing. In contrast to AVP, copeptin is very stable in plasma at room temperature and is easy to measure (17). However, the value of plasma copeptin levels in the differential diagnosis of DI is not yet fully determined.

IV. Types of DI

A. Neurohypophyseal DI

In neurohypophyseal DI, the production and release of AVP from the posterior pituitary gland is impaired (11). AVP is produced by the magnocellular neurons located in the supraoptic and paraventricular nuclei of the hypothalamus and is transported to the posterior pituitary gland via axonal transport along long extensions. Commonly, central DI is an...
acquired condition due to for example neoplasm, infection, head trauma, or surgery that affects the pituitary gland. Alternatively, central DI can arise due to congenital defects.

B. Familial neurohypophyseal DI

In all cases studied to date, FNDI is due to variations (mutations) in the AVP gene (18). The AVP gene contains three exons encoding a signal peptide, AVP, NPII, and copeptin (Fig. 3) (19).

As of October 2012, 67 mutations in the AVP gene have been reported that result in FNDI [Table 1 and HGMD Professional 2012.3 for more information (20); HGMD Professional is a commercial database, a basic trial version of the database, which is not updated as frequently, is available from the same resource]. Of these, the majority of the mutations (>50 mutations) are in the NPII domain (18, 21), which is a carrier protein that promotes transport of AVP from the hypothalamus to the posterior pituitary gland. In all but three kindred’s, the disease has been transmitted in an autosomal dominant mode [autosomal dominant FNDI (adFNDI)] (18). In two kindred’s of FNDI, a recessive form was identified (22, 23), and one case of X-linked recessive transmission has been reported (24). Although affected family members with adFNDI have normal water balance at birth and during early infancy, symptoms of compulsive drinking progressively develop during childhood, and the polyuria and polydipsia continues throughout life. Two predominant hypotheses concerning the underlying mechanism for the dominant and progressive nature of the disease exist (21, 25, 26): 1) mutant proteins are aberrantly folded and accumulate in the endoplasmic reticulum (ER), leading to protein aggregation and progressive loss of magnocellular neurons (e.g. Ref. 27); and 2) there is a dominant negative effect of the mutant hormone on “wild-type” (WT) AVP secretion (e.g. Ref. 28). These two general hypotheses do not exclude one another but may occur in parallel.

Various animal models have been developed to examine the underlying mechanism of FNDI (for review see Ref. 21). A rat model containing an inducible Cys67stop variation known to cause adFNDI in humans suggested trapping of WT AVP products in the ER followed by lysosomal degradation (25). However, in this model, cell death was not observed, and the features of DI manifested only after repeated dehydration. An alternative knock-in mouse model with a truncated AVP precursor (C67X) showed symptoms of progressive DI, loss of AVP-producing neurons, and possible retention of both WT and mutant AVP precursors within the neuronal cell bodies (29). Another knock-in mouse model, expressing mutant NPII (Cys98stop) causing adFNDI in humans (30), illustrated that the prohormone aggregates accumulated in the ER and eventually caused cell death but that polyuria progressed before, and in the absence of, cell death, supporting a dominant negative effect of the mutant. These in vivo results indicate that both cell toxicity and a dominant negative effect of the mutant probably account for the cellular effects of the mutations. Along these lines, it has been proposed that adFNDI is closely related to other neurodegenerative diseases like Alzheimer’s disease and Parkinson’s disease (26). It is likely that different mutations cause adFNDI by different mechanisms. For example, knock-in

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**Table 1. Mutations of AVP, L1CAM, AVPR2, and AQP2 Classed by Type of Mutation**

<table>
<thead>
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<th>Chromosomal Location</th>
<th>Total no. of Mutations</th>
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<td></td>
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<td>Small deletions</td>
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<td>5</td>
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</tr>
<tr>
<td>Complex rearrangements</td>
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<tr>
<td>Total</td>
<td></td>
<td>51</td>
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Overview and classifications of mutations causing DI. HGMD Professional 2012.3; for more information see Ref. 18.
of a mutation in the signal peptide causing a mild phenotype in humans resulted in no apparent phenotype in mice, indicating that different mutations have different cell pathological explanations (29).

The Brattleboro rat (31), a model of neurohypophyseal DI, results from a single nucleotide deletion (guanosine) in the NPII region of the AVP gene resulting in a frameshift and loss of a normal stop codon (32). This defect results in abnormal processing of the precursor hormone and a failure in production, storage, and secretion of AVP (32, 33). The recessive trait suggests absolute deficiency, and indeed these rats have total lack of AVP secretion (31). The Brattleboro rat model has proven to be valuable in understanding the consequences of a lack of AVP for, among other things, renal urinary concentration. Studies of Brattleboro rats demonstrated that absence of AVP inhibits AQP2 trafficking and leads to decreased AQP2 expression levels, conditions that can be reversed by chronic AVP infusion (34). In addition to regulation of AQP2 expression, AVP also regulates other proteins involved in urine concentration, such as AQP3 (35), Na-K-Cl cotransporter-2 (NKCC2) and urea transporters (36). Studies of knockout (KO) mice deficient in these proteins emphasize their importance for urinary concentration (37).

C. Nephrogenic DI

The main characteristic of NDI is impaired AVP-induced water reabsorption. Most commonly, NDI is acquired and occurs as a complication to numerous common clinical conditions, such as electrolyte abnormalities (e.g. hypokalemia and hypercalcemia) or medical treatment (e.g. lithium and cisplatin therapy). NDI can also be due to primary genetic diseases caused by mutations in the AVPR2 or AQP2 gene, or secondary to other genetic renal diseases (38).

V. Congenital NDI

Congenital NDI is caused by mutations in the AVPR2 or the AQP2 gene. The distal nephron is in these cases insensitive to AVP resulting in blunted water reabsorption in the collecting ducts. The urine concentrating defect is present from birth, and symptoms arise during the first weeks of life. Infants often suffer from hypernatremic dehydration, with symptoms of irritability, poor feeding, and weight gain (39). Clinically, the signs of dehydration are dryness of the skin, loss of normal skin turgor, recessed eyeballs, increased periorbital folding, and depressed anterior fontanel. Intermittent high fever due to dehydration and constipation can sometimes be observed. In addition, seizures can occur (39). In so-called partial forms of NDI (see Section V.B.), patients retain some ability to concentrate urine, lowering their risk of developing severe dehydration. Left untreated, most patients fail to grow normally, but with initiation of treatment, most recover their initial weight loss (39, 40). Mental retardation, assumed to result from repeated episodes of brain dehydration and brain edema (brought about by attempts to rehydrate too quickly) can be a serious complication of NDI (41, 42). Such complications are mostly caused by de novo mutations, where delayed clinical diagnosis can occur. In contrast, children at risk of inheriting the disease gene, i.e. in known kindred’s with the disease, often are diagnosed and treated much earlier (42). Psychological development of these patients is adversely affected by the persistent need for drinking and frequent voiding. The persistent polyuria can cause development of megacystis, trabeculated bladder, hydroureter, and hydronephrosis (39).

A. Defects in the AVPR2

The AVPR2 gene was first described in 1992 (43). The AVPR2 contains seven membrane-spanning helices (Fig. 4). Upon binding of AVP, activation of the receptor is initiated, and allosteric structural rearrangements occur (44). AVP binds to the AVPR2 within the transmembrane helices II–IV (residues 88–96, 119–127, 284–291, and 311–317) (45, 46).

Mutations in the AVPR2 gene lead to X-linked NDI (X-NDI) (47). This is the cause of 90% of all diagnosed congenital NDI cases. In X-NDI, the symptoms (polyuria, thirst, and polydipsia) often present from birth. In the case of inadequate water supply, the polyuria can rapidly cause severe hypernatremia and dehydration. Newborns suffering from the condition frequently suffer from vomiting and poor weight gain due to the high water intake. Affected male patients do not concentrate urine even after administration of exogenous AVP (48), whereas due to skewed X-chromosome inactivation, some heterozygous females have variable degrees of polyuria and polydipsia (49–51). Significant variability has been described in the location of the mutations and in the severity of the disease (49). Thus, some patients are able to concentrate their urine in response to fluid deprivation or AVP/dDAVP administration, resulting in so-called partial or incomplete NDI (see Section V.B.) (5). As of October 2012, 222 mutations resulting in X-NDI have been identified (Fig. 4 and Table 1) (52, 53), and the number is constantly increasing (54). In addition to mutations in the AVPR2, gross gene deletions or complex rearrangement of the L1CAM gene, which lies adjacent to the AVPR2 (approximately 30 kb apart in humans), can result in NDI (54, 55). However, only L1CAM gene deletions that also encompass the AVPR2 are associated
with NDI, and isolated point mutations in the L1CAM gene are never associated with a polyuric phenotype.

Mutations in the AVPR2 leading to NDI can be classified as "loss of function" mutations. In addition, the AVPR2 can also be affected by "gain of function" mutations, e.g. arginine-137-cysteine and arginine-137-leucine (56–63). These mutations cause constitutive activation of the receptor (57, 64), resulting in the nephrogenic syndrome of inappropriate antidiuresis (57). The clinical presentations of nephrogenic syndrome of inappropriate antidiuresis vary from fully asymptomatic, with defective urine dilution only manifesting upon water loading, to severe neurological symptoms (59). Another gain of function mutation (G12E) in the AVPR2 has been associated to increased levels of von Willebrand factor and factor VIII plasma levels (65).

AVPR2 mutations are divided into five classes (4). Class I mutations lead to improperly processed/unstable mRNA, frameshifts, or nonsense mutations resulting in truncation of the receptor (4). Class II mutations (the most prevalent type of mutations) result in misfolding of the receptor and retention in the ER. Class III mutations also cause misfolding of the AVPR2, but although the AVPR2 reaches the plasma membrane and interacts with AVP, it does not interact fully with G proteins leading to impaired cAMP production. Class IV mutations also result in AVPR2 misfolding. In this case, although the AVPR2 is able to reach the plasma membrane, it does not interact properly with AVP. Class V mutations are missorted to an incorrect cellular compartment.

In vivo models for X-NDI are useful in terms of 1) elucidating potential compensatory or adaptive changes in the kidney and 2) examining novel treatment strategies for specific AVPR2 mutations. AVPR2-deficient male mice, with a mutation resulting in the premature insertion of a stop codon (E242X) known to cause defective AVPR2 function and X-NDI (66), are polyuric at birth. Three-day-old male pups (with E242X) have increased expression in a variety of genes, including AQ1P1, carbonic an-
hydrases, the Na-K-ATPase, and NaCl and HCO3 transporters, suggesting compensatory changes. The E242X mice also have up-regulation of cyclooxygenase 2 expression in both the kidney and hypothalamus (67). Despite these compensatory changes, E242X mice die within 1 wk, making them an unsuitable model for studying X-NDI in adult mice. Recently, a viable mouse model of X-NDI has been generated that has a conditional AVPR2 deletion upon tamoxifen treatment (68). After tamoxifen, adult mice present with polyuria, polydipsia, and a lack of response to AVP. This mouse model has been used to examine the potential beneficial effect of a selective EP4 prostanoid receptor agonist for treatment of X-NDI (68). It is likely that such models will prove useful for further studies to identify novel X-NDI treatment strategies (see Sections VIII and IX).

B. Partial NDI

Although almost all NDI mutations associated with a severe phenotype are characterized as complete DI, recent reports have revealed a number of mutations causing mild phenotypes with partial DI (69–77). Functional studies have underlined that some (e.g. p.Arg104Cys) decrease binding affinity of the AVPR2 with only minimal effects on receptor surface expression, whereas others (e.g. p.Ser329Arg) decrease cell surface expression of the AVPR2 due to accumulation of the receptor in the ER (73). Similarly, partial clinical NDI phenotypes have been described in patients carrying mutations in the AQP2 gene (78–84). The clinical and molecular characterization of partial NDI subtype have emphasized that altered ligand binding and signal transduction are dependent on the localization of the altered amino acid in the AVPR2. Striking divergences at the level of AVPR2 functionality may underlie similar clinical phenotypes in NDI. The majority of partial NDI cases have been uncovered after genetic testing of patients with a previous incorrect clinical diagnosis, e.g. primary polydipsia (due to the preserved urine concentration capacity during fluid deprivation). Thus, the studies of partial NDI have emphasized the value of molecular testing in determining the actual cause, and potential treatment strategy, of various forms of NDI.

C. Defects in the AQP2 gene

In humans, the gene for AQP2 is located on chromosome 12q13 and encodes a 271-amino acid protein (85). Normally, AQP2 consists of four identical protein subunits that form a stable tetramer in the plasma membrane. Each monomer consists of six transmembrane-spanning regions with the COOH terminus located within the cytosol (Fig. 5). Similar to the majority of other aquaporins, AQP2 contains two highly conserved NPA motifs (Asn-Pro-Ala motif), which are thought to “dip” into the membrane, overlap, and form the water pore of the channels (3, 9). The structure of AQP2 has been clarified at 4.9-Å resolution, but the structure of the intracellular domains is lacking (86). The trafficking of AQP2 is regulated by a variety of cellular processes, including complicated processing of AQP2 via posttranslational modifications (87). AVP mediates increased polyphosphorylation of AQP2 at the carboxyl-terminal tail at the serine residues S256, S264, and S269 and decreased phosphorylation at S261 (88). It is well established that AVP-induced S256 phosphorylation is critical for accumulation of AQP2 in the apical membrane (89–91). It was recently demonstrated that S269 phosphorylated AQP2 is only present in the apical plasma membrane. Along with S256 phosphorylation, S269 is likely involved in AQP2 membrane accumulation by decreasing the rate of internalization of AQP2 after AVP withdrawal (92–94), a result of decreased phosphorylation-dependent protein interactions (93).

Currently, 51 mutations in the AQP2 gene have been described to cause NDI (Fig. 5 and Table 1). These mutations result in two different molecular outcomes. First, a mutation in AQP2 can affect a sorting signal and inhibit the routing of functional AQP2 to the membrane. Secondly, a mutation can result in a defect in the formation of the pore-forming structure of AQP2 resulting in lack of function as a water channel. Since the role of AQP2 in NDI was first described (95), several of the AQP2 mutations resulting in NDI have been examined (4) and studied in various model systems. Studies of mammalian cell lines have provided information about the trafficking and targeting of mutant AQP2 protein, whereas the expression of AQP2 in the Xenopus laevis oocyte system has provided insight into function, i.e. the ability of mutations to alter AQP2-mediated water flux across membranes.

D. Autosomal recessive NDI

Autosomal NDI results from a recessive trait in more than 90% of cases. Patients are homozygous or compound heterozygous for mutations in AQP2. Predominantly, mutations are in the pore-forming region of AQP2, i.e. the core region (transmembrane domains and connecting loops) (4). These mutations result in AQP2 misfolding, retention in the ER, and rapid degradation of AQP2. Autosomal recessive NDI usually manifests at birth and affects males and females equally. Although the majority of cases are severe, a few cases of autosomal recessive partial NDI have been reported (5).

A variety of AQP2 KO/knock-in mice models of NDI have demonstrated the critical role of AQP2 in maintaining water balance (96, 97). It must also be mentioned that deletion or mutation of several other genes can result in
severe defects in the ability to concentrate urine and resistance of the kidney to AVP, suggesting an “NDI-like phenotype” (37, 98, 99). Several models for autosomal recessive NDI have been established (Table 2) (100–102), all with poor viability, suggesting that the mice are sensitive to the polyuria. Total AQP2 KO mice do not survive postnatally (96), and mimicking a human NDI causing mutation T126M in mice leads to early death within 6 d.

Figure 5. A schematic presentation of AQP2 in the membrane with indications of some of the mutations known to cause DI. Some mutations, such as splicing, are not indicated in the figure.

Table 2. Mouse Models of NDI

<table>
<thead>
<tr>
<th>Genetic Trait</th>
<th>Viability</th>
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<tr>
<td>AVP2R</td>
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<td></td>
</tr>
<tr>
<td>E242X (premature stop codon glu242stop) X-linked</td>
<td>Males, died postnatally (by d 7)</td>
<td>Yun et al. (66)</td>
</tr>
<tr>
<td>Tamoxifen inducible conditional AVPR2 knockout X-linked</td>
<td>Viable</td>
<td>Li et al. (68)</td>
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<tr>
<td>AQP2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genetic changes introduced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AQP2 knock-in (T126M) Aut. rec.</td>
<td>Died postnatally (by d 6)</td>
<td>Yang et al. (100)</td>
</tr>
<tr>
<td>Inducible AQP2 knock-in</td>
<td>Aut. rec.</td>
<td>Viable (to adulthood)</td>
</tr>
<tr>
<td>AQP2 knock-in (763–772 del) Aut. dom.</td>
<td>Viable (to adulthood)</td>
<td>Sohara et al. (111)</td>
</tr>
<tr>
<td>AQP2 total KO Aut. rec.</td>
<td>Died postnatally (5–12 d)</td>
<td>Rojek et al. (96)</td>
</tr>
<tr>
<td>Collecting duct-selective AQP2 conditional-KO Aut. rec.</td>
<td>Viable (to adulthood)</td>
<td>Rojek et al. (96)</td>
</tr>
<tr>
<td>COOH-terminal tail truncation Aut. rec.</td>
<td>Viable (to adulthood)</td>
<td>Shi et al. (199)</td>
</tr>
<tr>
<td>Spontaneous mutations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AQP2-F204V Aut. rec.</td>
<td>Viable (to adulthood)</td>
<td>Lloyd et al. (102)</td>
</tr>
<tr>
<td>AQP2-S256L Aut. rec.</td>
<td>90% postnatal mortality (2–4 wk)</td>
<td>Mc Dill et al. (200)</td>
</tr>
</tbody>
</table>

Examples of mouse models of NDI. Aut. rec., Autosomal recessive; aut. dom., autosomal dominant.
Mice with collecting duct-selective KO of AQP2 are viable but have a severe urinary concentration defect (96). These findings suggest that the collecting duct is necessary for urine concentration but that some compensation may occur, possibly via AQP2 in the connecting tube. More recently, an inducible knock-in mouse model of NDI was developed and used for identifying potential therapeutic compounds for NDI in adult mice (101). Furthermore, a mouse model with a F240V mutation resulting in recessive NDI supports the hypothesis that defective targeting of AQP2 is the basis for some forms of NDI (102).

Although animal models provide an ideal tool for studying the molecular basis of NDI and for investigating potential therapeutic strategies, the large number of different mutations necessitates use of other systems. Although several AQP2 gene mutations result in AQP2 protein being trapped in the ER (79, 82, 103–106), in oocytes, overexpression of particular AQP2 mutants results in some plasma membrane AQP2 expression, allowing investigations into the ability of the mutants to function as water channels (78, 79, 82, 106). In terms of treatment strategies, these results are critical, because they suggest that functional channels may be stimulated to reach the membrane by bypassing normal signaling. One of the problems with assessing AQP2 function and trafficking in different systems is that species differences can arise. For example, recently, two new autosomal recessive NDI causing mutations, K228E and V24A, were identified (107). Studies in oocytes of these mutations demonstrated that they were appropriately targeted to the membrane and that they were “functional variants” of AQP2, which could therefore not readily explain the NDI phenotype. However, studies of the mutants in mammalian cells demonstrated defective trafficking, emphasizing the importance of supplemental studies of AQP2 mutants in cell lines that contain the relevant machinery for trafficking (93).

### E. Autosomal dominant NDI

Ten percent of autosomal NDI cases are inherited in a dominant trait. The urinary concentrating defect in these cases is due to mutations in the carboxyl-terminal tail of AQP2 (Table 3). Although these mutations do not affect the water-transporting properties of the protein, the carboxyl tail is essential for correct intracellular routing of the channel. In this class of NDI, heterotetramers of AQP2 monomers (the functional unit at the membrane) are formed between the WT and the mutated form, causing misrouting of AQP2 (108), retention in the Golgi apparatus, or sorting of AQP2 to late endosomes, lysosomes, or the basolateral plasma membrane (109). Thus, the mutations act in a dominant negative mechanism and prevent WT-AQP2 from reaching the apical plasma membrane. Although classified as “dominant,” the condition is usually only partial, suggesting that at least some WT-AQP2 forms functional homotetramers and reaches the apical plasma membrane (80, 81, 83, 84). Fluid restriction or treatment with desmopressin usually increases urine osmolality and only in some cases is the resistance to AVP severe (5).

The underlying cause for AQP2 missorting in autosomal dominant NDI reflects the many potential signaling and regulatory sites in the carboxyl terminus. In AQP2-R254L mutations, it has been suggested that the loss of the consensus site for AVP-induced S256 phosphorylation results in defective trafficking to the apical plasma membrane (80, 81). In contrast, although the E258K mutation is similarly close to the S256 phosphorylation site in AQP2, the mutation is suggested to cause NDI due to interference with an RRXxxxKL motif in the carboxyl terminus rather than due to interfering with phosphorylation (110). Another mutation, AQP2-insA, causes a frameshift mutation in the carboxyl terminus resulting in basolateral targeting of AQP2 in cells (108). Similar to the human condition, a mouse model that mimics autosomal

### Table 3. Mutations Causing Dominant NDI

<table>
<thead>
<tr>
<th>Mutations Causing Dominant NDI</th>
<th>Mutation Type</th>
<th>Molecular Diagnosis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E258K</td>
<td>Substitution</td>
<td>Retained in Golgi, does not affect AQP2-S256 phosphorylation in oocytes</td>
<td>Mulders et al. (84)</td>
</tr>
<tr>
<td>721delG</td>
<td>Frameshift</td>
<td>Defective trafficking in oocytes</td>
<td>Kuwahara et al. (83)</td>
</tr>
<tr>
<td>763–772del</td>
<td>Frameshift</td>
<td>Defective trafficking in oocytes</td>
<td>Kuwahara et al. (83)</td>
</tr>
<tr>
<td>812–818del</td>
<td>Frameshift</td>
<td>Defective trafficking in oocytes</td>
<td>Kuwahara et al. (83)</td>
</tr>
<tr>
<td>R254L</td>
<td>Substitution</td>
<td>Defective trafficking in oocytes and MDCK cells, defective AQP2-S256 phosphorylation</td>
<td>de Mattia et al. (80)</td>
</tr>
<tr>
<td>R254Q</td>
<td>Substitution</td>
<td>Defective trafficking in oocytes and MDCK cells, defective AQP2-S256 phosphorylation</td>
<td>Savelkoul et al. (81)</td>
</tr>
<tr>
<td>AQP2-insA (frameshift c779–780insA)</td>
<td>Frameshift</td>
<td>Misrouted to basolateral membrane in polarized renal cells</td>
<td>Kamsteeg et al. (108)</td>
</tr>
<tr>
<td>7278G</td>
<td>Frameshift</td>
<td>Defective trafficking in oocytes, renal cells in endosomes and lysosomes</td>
<td>Marr et al. (82)</td>
</tr>
</tbody>
</table>

Current mutations causing dominant NDI.
dominant NDI, with an AQP2 763–772 deletion, displayed impaired urinary concentrating ability in heterozygous mice. However, the mice responded to fluid deprivation by increasing urine osmolality (111). Additionally, this model confirmed the theory of misorting, in this case basolateral AQP2 missorting, as the cause for autosomal dominant NDI.

VI. Genetic Testing in DI

Genetic testing for DI can be very useful to verify the initial diagnosis and should be performed in all patients with a family history of the disease. Because the clinical DI phenotype of FNDI develops during childhood, mutations in either the AVPR2 or AQP2 genes must be suspected in newborns or infants presenting with DI. As mentioned, de novo AQP2 and AVPR2 mutations often result in delayed diagnosis with a significant risk of cerebral damage due to severe, prolonged dehydration, and in such cases, a genetic characterization enables early (even prenatal) diagnosis during subsequent pregnancies as well as genetic counseling of other family members. In children with neurohypophyseal DI occurring during childhood without an identifiable cause (e.g. thickening of the pituitary stalk), who do not have a family history, should also be tested genetically.

VII. Acquired Forms of NDI

Acquired NDI is a frequently occurring condition that is much more common than hereditary NDI. Acquired NDI results from a variety of conditions (see for example Refs. 112, 113 for reviews), but for the purposes of this article, only NDI associated with drug treatment (lithium therapy, antibiotic treatment, and other drug-induced forms), acute and chronic renal failure (CRF), and electrolyte abnormalities are briefly discussed. Although the underlying molecular basis for different forms of acquired NDI may be different, all forms of acquired NDI have been found to be associated with decreased expression of AQP2 or dysregulated AQP2 trafficking to the apical plasma membrane.

A. Lithium-induced NDI

Lithium is a common treatment in bipolar affective disorders, with approximately 0.5% of the Western population currently under lithium therapy (113). Unfortunately, up to 40% of individuals treated with lithium develop NDI as a side effect (114). The mechanisms behind lithium-induced NDI have been long sought, but it is likely to be multifactorial (112). In humans, lithium can result in a reduced capacity to concentrate urine as early as 8 wk after onset of treatment. In some individuals, prolonged use (10–20 yr) can result in chronic kidney disease. Treatment strategies for patients being treated with lithium that develop NDI include thiazide and amiloride (115, 116) or modulation of the renin-angiotensin-aldosterone system via captopril (angiotensin-converting enzyme inhibitor), spironolactone (mineralocorticoid receptor blocker), or candesartan (angiotensin II receptor antagonist) (112).

Lithium is filtered and reabsorbed by the kidney similarly to sodium and can enter the collecting duct principal cells via the apical amiloride-sensitive epithelial sodium channel (ENaC). In fact, ENaC’s permeability to lithium is up to 2-fold higher for lithium than sodium (114, 117), and mice lacking αENaC in the collecting ducts do not develop polyuria after lithium treatment (118). Accumulation of cytotoxic concentrations of lithium within principal cells ultimately results in decreased AQP2 and AQP3 expression, resulting in NDI (114, 119–121).

Despite numerous studies, the molecular mechanism for the onset of lithium-induced NDI is not clear. One hypothesis derived from early cell studies suggests that lithium causes decreased AQP2 transcription (117, 122). Lithium-based interruption of normal AVP signaling and impaired cAMP production (123–126) would result in drastically reduced AQP2 expression levels and/or membrane targeting. However, recent studies in a kidney cortical collecting duct cell line (mpkCCD cells) contradicted this hypothesis and demonstrated that lithium decreases AQP2 transcription without changes in cAMP levels (122). Other factors that may influence lithium’s effects on water balance could be: altered prostaglandin E2 (PGE2) production or secretion, cyclooxygenase 2-mediated signaling, AVP-independent mechanisms, β-catenin-mediated gene transcription, or glycogen synthase kinase type 3β-mediated cell signaling (for reviews see Refs. 112, 114). Thus, it is likely that lithium-induced NDI is not just a direct effect on AQP2 but a complex cascade of events that results from alterations in various signaling pathways, cell death, cell proliferation, altered principal cell morphology, and cellular reorganization of the tubular system (124, 127, 128).

B. Other drugs

Apart from lithium treatment, a large number of drugs have been proposed to, at least temporarily, induce NDI (113). Most of this evidence is based on single case reports, with vague diagnostic criteria of DI, and without rigorous proof of causality and reversibility. In 2005, a metaanalysis demonstrated that out of 155 published reports, only 58 provided a definite NDI diagnosis (113). Apart from lithium, 29 agents were causative of NDI, of which antibiotics, antifungals, and antineoplastic agents were the
most frequent categories. There is some evidence that most of these drugs cause reversible NDI, with the period until recovery dependent on the duration of drug exposure. Little is known regarding the molecular effects of these drugs on antidiuretic function.

C. Antibiotics/antifungals

Demeclocycline is an antibiotic in the tetracycline class used most frequently in the treatment of resistant infections or acne. The nephrotoxic effects of demeclocycline seem to be an isolated tubular dysfunction that lasts only a few weeks after discontinuation and in a dose-dependent manner (129–132). Although the exact mechanism behind the increased free water excretion is unclear, demeclocycline has been used in the treatment of the syndrome of inappropriate antidiuretic hormone (e.g., Ref. 133). Foscarnet is an antiviral agent used to treat cytomegalovirus infection in immunosuppressed patients but has also been attributed to drug-induced reversible NDI (134). Amphoterin B is a commonly used antifungal agent used in the treatment of localized or systemic mycotic infections, most commonly seen in immunocompromised patients. Several reports have provided solid evidence that NDI and renal tubular acidosis are reversible side effects of Amphoterin B treatment (135). Replacement with liposomal amphotericin B was suggested to decrease the risk of NDI, although several reports have challenged this hypothesis.

D. Neoplastic agents

Nephrotoxicity is a frequent complication of treatment with ifosfamide, especially in children (136). Although this drug occasionally induces NDI, it more often results in glomerular impairment and proximal tubular dysfunction with a Fanconi’s syndrome. In one study of a cohort of 12 children, considerable nephrotoxicity was still present 10 yr after completion of ifosfamide treatment. Total drug dose, patient age, and concomitant treatment with another neoplastic drug, cisplatin, were identified as risk factors (136).

E. Electrolyte disorders

Hypokalemia and hypercalcemia can cause mild NDI, and animal models have supported that decreased AQP2 expression and targeting is one of the underlying causes (137–140). Similarly to lithium-induced NDI, the precise underlying molecular mechanisms of hypokalemia- and hypercalcemia-induced NDI remain to be resolved, and the condition probably reflects a complicated “mixture” of events. In a mouse model of Gitelman’s syndrome with hypokalemia, the mice display drastically reduced AQP2 levels and severe polyuria (140). Furthermore, a recent report of a patient with Fanconi’s syndrome and NDI suggests that hypokalemia may be the underlying cause of the NDI (141).

In the case of hypercalcemia, in addition to AQP2, several different proteins involved in the urinary concentrating mechanism are affected. Expression of AQP1 and AQP3 are reduced in hypercalcemia (138), and the sodium-potassium-chloride cotransporter NKCC2 in the TAL, which plays a critical role in generation of a medullary osmotic gradient, was down-regulated in rats with PTH-induced hypercalcemia (142). NKCC2 down-regulation is also observed in hypokalemia (143). Hypercalcemia can be accompanied by hypercalciuria, leading to decreased AQP2 expression and targeting. This effect is likely mediated via the luminal calcium-sensing receptor in collecting duct principal cells (144–146), which has previously been shown to regulate AQP2 expression and targeting (144, 147). A recent study demonstrated that in humans, hypercalciuria resulted in a reduced excretion of urinary AQP2 after desmopressin treatment and a lower urinary concentrating ability, effects mediated by the calcium-sensing receptor (148). Thus, in hypercalciuric patients, the reduction in AQP2 and the subsequent NDI may be an internal defense mechanism to reduce the risk of calcium renal stones. Calcium-dependent activation of calpain, a proteolytic enzyme expressed ubiquitously, has also been proposed to reduce AQP2 levels by proteolysis (149).

F. Acute and CRF

Polyuria and impaired urinary concentration are seen in patients with acute and CRF. In both conditions, multiple renal abnormalities contribute to the renal dysfunction. Experimentally, in rats, a widely used model for acute renal failure (ARF) is ischemia and reperfusion (150, 151). These are a major cause of ARF in humans (151). Ischemia can occur after, for example, aortic surgery or renal transplantsations, leading to renal dysfunction (152, 153), which is further complicated by the necessary reperfusion that can cause additional cellular injury (154). ARF is complicated by defects of both water and solute reabsorption in both the kidney proximal tubule and the collecting duct (155–157). In one model of experimentally induced ARF, reduced AQP1 expression in the proximal tubule and reduced AQP2 and AQP3 expression in the collecting duct were observed 24 h after onset of polyuria (158). In an alternative model, although AQP2 and AQP3 abundances were reduced in collecting ducts 18 h after onset of polyuria, AQP1 expression was not reduced until 36 h after urine volume increased (159). Hemorrhagic shock-induced ARF is also associated with decreased expression of AQP2 and AQP3 (160). In patients with CRF, the urine remains dilute despite administration of high doses of AVP, suggesting a defect in the AVPR2 response or sen-
The hypovolemic state induces activation of the ENaC in the connecting tubule Na-Cl-cotransporter in the distal tubule and amiloride that thiazide blocks the sodium chloride cotransporter proposed mechanism behind the effect of the treatment is drochlorothiazide and amiloride) seems a paradox. The due to gastrointestinal symptoms) (39).

well, or patients who do not tolerate indomethacin (often treatment of young children who do not tolerate amiloride (171). Alternative approaches must be considered for (39, 169, 170) can be as effective with fewer side effects due to excessive drinking and urine voiding. Other treatment strategies aim to reduce the symptoms of polydipsia and polyuria (18, 164). Additionally, treatment with the diuretic thiazide, sometimes in combination with a cyclooxygenase inhibitor (indomethacin), can also efficiently decrease the degree of polyuria due to skewed X-inactivation with dDAVP is usually not effective; except in heterozygous females with polyuria due to misfolding of the receptor and ER/Golgi retention. How-er, the receptor may still be functional. In these cases, the receptor can aid folding and promote trafficking to the membrane (Fig. 6), thereby allowing functional AVPR2 receptors. The main strategy for treating NDI is to replace the urinary water loss with sufficient water intake, yet this can seriously impact on quality of life due to gastrointestinal symptoms. Low-sodium diets reduce the solute load to the kidney, thereby minimizing the obligatory water excretion. Additionally, treatment with the diuretic thiazide, sometimes in combination with a cyclooxygenase inhibitor (indomethacin), can also efficiently decrease the degree of polyuria (5). Other reports suggest that the use of hydrochlorothiazide in combination with amiloride (39, 169, 170) can be as effective with fewer side effects (171). Alternative approaches must be considered for treatment of young children who do not tolerate amiloride, or patients who do not tolerate indomethacin (often due to gastrointestinal symptoms) (39).

Treatment of a polyuric condition with diuretics (hydrochlorothiazide and amiloride) seems a paradox. The proposed mechanism behind the effect of the treatment is that thiazide blocks the sodium chloride cotransporter Na-Cl-cotransporter in the distal tubule and amiloride blocks the sodium channel ENaC in the connecting tubule and collecting ducts. Blocking these channels together leads to decreased sodium reabsorption in the proximal tubule, which is followed by water reabsorption via AQP1. The net effect is a decreased load of prourine reaching the distal tubule and collecting duct, making the role of these segments in water reabsorption less important (39). Additionally, thiazide may increase AQP2 levels in some cases of NDI (172).

Although these treatment regimens can cause some relief of NDI symptoms, they most often do not eliminate them. In the few reports available, urine osmolality increased and urine volume decreased by 30–70% in patients on diuretic therapy (173–177). Due to the insufficient control of the polyuria by conventional treatments, recent focus has been on new and alternative methods to induce antidiuresis in NDI patients. Some of these strategies are currently under clinical evaluation.

**IX. Therapeutic Strategies for the Treatment of NDI**

A. Promoting AVPR2 signaling, AVPR2 antagonists and agonists

The most prevalent AVPR2 mutations (class II) result in misfolding of the receptor and ER/Golgi retention. However, the receptor may still be functional. In these cases, the principles of the treatment strategies include:

1) Rescue of the AVPR2 insertion into the plasma membrane. This approach is based on aiding AVPR2 folding in the ER and AVPR2 escape from the ER quality control system. This allows the AVPR2 to reach the basolateral plasma membrane where endogenous AVP can activate the receptor. This strategy has been attempted with limited success using chemical chaperones, chemical compounds that in an unspecific way aid protein folding, e.g. glycerol and dimethylsulfoxide (178). Another strategy is to rescue membrane expression of the AVPR2 via treatment with cell-permeable AVPR2 antagonists that function as pharmacochaperones and thereby aid folding and membrane expression of the AVPR2 (Fig. 6). However, once the AVPR2 is inserted in the membrane, the antagonist needs to be washed away (competed out) to allow the AVP-AVPR2 interaction and signaling to occur. In some cases, cell-permeable agonists that do not need to be released from the receptor can aid folding and promote trafficking to the membrane (Fig. 6), thereby allowing normal signaling (179).

2) Cell-permeable agonists that can bind to intracellular AVPR2 and activate signaling without leading to alterations in membrane expression (180).
Figure 6. Schematic presentation of various potential strategies for treating NDI. A, Rescue of plasma membrane expression of the AVP2R (V2R) in NDI by cell-permeable antagonists. Antagonists (red circles) can enter the cell and bind to a class II mutant V2R that is misfolded in the rough ER (RER). This aids stabilization of the protein conformation and allows the V2R to escape the RER and Golgi and reach the cell plasma membrane. In the plasma membrane, the antagonist is displaced by AVP (green circles) and normal signaling occurs, leading to increased cAMP and AQP2 trafficking. B, Activation of mutated and misfolded V2R by cell-permeable agonists. The agonists (blue circles) enter the cell and reach the misfolded V2R in the RER. This allows normal signaling to occur, leading to increased cAMP and AQP2 trafficking. C, Rescue of mutant V2R plasma membrane expression and signaling via cell-permeable agonists. This class of agonists (yellow circles) enter the cell and aid proper folding of the V2R in the RER, which results in rescue of the V2R to the plasma membrane. The compounds secondarily act as agonists and induce normal V2R signaling from the plasma membrane. D, Mechanisms to bypass V2R signaling and allow translocation of AQP2 to the plasma membrane. 1, EP2 and EP4 prostanoid receptor agonists have been shown to induce membrane expression and abundance of AQP2; 2, increased abundance of cGMP via PDE5 inhibitors or cGMP addition has been shown to induce AVP independent AQP2 trafficking to the plasma membrane; 3, increasing cAMP levels via prevention of cAMP degradation leads to activation of PKA and subsequently AQP2 membrane insertion; 4, AQP2 membrane accumulation can be increased by preventing AQP2 internalization (one class of compounds suggested to work via this effect are statins); and 5, inhibition of the molecular chaperone Hsp90 partially allows escape of misfolded AQP2 from the RER to the plasma membrane, where it retains some of its water transport properties.
B. Nonpeptide antagonists (pharmacological chaperones)

The cell-permeable AVPR2 antagonists S121463 and VPA-985 (Table 4) can stabilize ER-retained AVPR2 mutants, allowing the receptor to escape from the ER and reach the plasma membrane (181, 182). Other antagonists have also been proposed, e.g., SR49059 (a V1a receptor antagonist with moderate affinity for AVPR2) (182–184), YM087 (conivaptan, a combined V1a and AVPR2 antagonist) (184), and OPC31260 and OPC41061 (high-affinity AVPR2 antagonists) (178). SR49059 has been tested in patients for “proof of principle” and shown some beneficial effects and therefore remains a potential for future treatment of X-NDI (185). However, because there are some side effects from its use, further studies using modified antagonists or treatment regimes are required (185).

One limitation of pharmacological chaperones is that their effects are often significantly dependent on the nature of the AVPR2 mutation. Thus, different mutations may require different compounds to achieve AVPR2 rescue (179, 182). Secondly, if the compound is not a completely selective AVPR2 agonist, side effects via other receptors may arise (e.g., undesired antipressor effects via V1A receptor). Third, the trade-off in affinity required for these components, i.e., the need for sufficient receptor binding combined with easy release from the receptor, may constitute a weak point in this treatment strategy. Fourth, stimulation of the AVPR2 by AVP promotes termination of the response by inducing receptor internalization and its delivery to and degradation in lysosomes. In the presence of high levels of AVP, this could counteract the effect of the rescued receptor.

C. Nonpeptide agonists

The principle of a cell-permeable nonpeptide agonist is that it can enter the cell, reach the mutant AVPR2 receptor, and initiate a cAMP response, potentially leading to AQP2 translocation (Fig. 6) (179, 180). Although nonpeptide agonists do not necessarily need to rescue misfolded and mislocalized AVPR2 mutants, some agonists can also rescue membrane expression of the AVPR2 leading to additional beneficial effects, e.g., antagonistic effects on β-arrestin recruitment that can down-regulate AVPR2 signaling (186). The compounds MCF14, MCF18, and MCF57 are high-affinity agonists for the AVPR2 and capable of inducing receptor maturation, translocation to the plasma membrane (some mutations), and initiating a cAMP response. Additionally, receptor internalization with these compounds via arrestins was not induced (186). Other compounds include VA999088, VA999089, and OPC51803 that can functionally serve as AVPR2 agonists but do not rescue membrane expression (180). Again, the beneficial effects of such compounds may be mutation and compound dependent (179).

In the cases of NDI where the above strategies cannot be used, e.g., mutations where the AVPR2 is not functional or is insensitive to rescue, an alternative strategy for treatment is to bypass the AVPR2-mediated signaling pathway and promote AQP2 trafficking to the membrane via other intracellular pathways (187).

D. Bypassing vasopressin receptor signaling

Navigating around a nonfunctional or mislocalized AVPR2 to induce AQP2 accumulation in the apical membrane is a potential way to treat X-NDI or some forms of NDI arising from AQP2 mutations that still have the ability to transport water. Additionally, bypassing the AVPR2 could be an effective treatment strategy for treatment of some of the acquired forms of NDI (for extensive review see Ref. 187).

E. Phosphodiesterase (PDE) inhibitors

1. Cyclic GMP (cGMP) pathway activation

Intracellular cGMP levels can be increased by sodium nitroprusside (which breaks down to release NO), L-arginine, and atrial natriuretic peptide. All these substances can increase AQP2 abundance in the apical membrane (187–189). The selective cGMP PDE (PDE5) inhibitor sildenafil citrate (Viagra) prevents degradation of cGMP, resulting in increased membrane expression of AQP2 in vitro and in vivo (190). Recently, it was shown that sildenafil citrate reduces polyuria in rats with lithium-induced NDI (191). However, no decreases in urine volume or increases in urine osmolality were observed in a small number of NDI patients subjected to clinical trials with sildenafil citrate (D. Bichet, Université de Montréal, Montreal, Canada; personal communication).

2. Cyclic AMP pathway activation

Rolipram, a PDE4 inhibitor, increased urine osmolality in a mouse model of autosomal dominant NDI. In contrast, PDE3 and PDE5 inhibitors had no significant effects (111). The effects of rolipram are likely due to increased cAMP levels leading to increased AQP2 phosphorylation and translocation (Fig. 6). Naturally, because PDEs are abundant in almost all cell types, the potential for PDE inhibitors in treatment of NDI needs to be clarified further, e.g., determining the long-term effects on urine output, effects of PDE inhibition in other cell types, and the potential side effects of sustained treatment. Clinically, rolipram treatment of two male patients suffering from NDI due to AVPR2 mutations did not cause any relief of symptoms (192). In this case, it is plausible that there are differences in AMP metabolism between mice and humans.
Table 4. Various Peptide and Nonpeptide Agonists With Potential for Treatment of NDI

<table>
<thead>
<tr>
<th>Compound Name in Text</th>
<th>Synonyms</th>
<th>IUPAC Name</th>
<th>CAS Registry Numbers</th>
<th>Chemical Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>VPA-985</td>
<td>VPA985</td>
<td>N-[3-chloro-4,6,11-dihydropyrrol[2,1-c][1,4]benzodiazepine-5-carbonyl]phenyl]-5-fluoro-2-methylbenzamide</td>
<td>168079-32-1</td>
<td>C_{27}H_{21}ClFN_{3}O_{2}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lixivaptan</td>
<td>5-fluoro-2-methyl-N-(4-[5H-pyrrolo[2,1-c][1,4]benzodiazepin-10(11H)-ylcarbonyl]-3-chlorophenyl)benzamide</td>
<td>185913-78-4</td>
</tr>
<tr>
<td>SR121463</td>
<td>Satavaptan</td>
<td>N-tert-butyl-4-(5H-pyrrolo[2,1-c][1,4]benzodiazepin-10(11H)-ylcarbonyl)-3-chlorophenyl)benzamide</td>
<td>185913-78-4</td>
<td>C_{33}H_{45}N_{3}O_{8}S</td>
</tr>
<tr>
<td></td>
<td>Aquilda</td>
<td>1-(4-Boc-2-methoxybenzenesulfonyl)-5-ethoxy-3-spiro-(4-[5H-pyrrolo[2,1-c][1,4]benzodiazepin-10(11H)-ylcarbonyl]-3-chlorophenyl)benzamide</td>
<td>150375-75-0</td>
<td>C_{28}H_{27}Cl_{2}N_{3}O_{7}S</td>
</tr>
<tr>
<td>OPC31260</td>
<td>Relcovaptan</td>
<td>(2S)-1-[(2R,3S)-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulphonyl)-3-hydroxy-2,3-dihydro-1H-indole-2-carbonyl]pyrrolidine-2-carboxamide</td>
<td>137975-06-5</td>
<td>C_{23}H_{29}N_{2}O_{3}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mozavaptan</td>
<td>5-dimethylamino-1-(4-[2-methylbenzoylamino] benzoyl]-2,3,4,5-tetrahydro-1H-benzazepine</td>
<td>150683-30-0</td>
</tr>
<tr>
<td>OPC41061</td>
<td>OPC-41061</td>
<td>N-[4-(7-chloro-5-hydroxy-2,3,4,5-tetrahydro-1-benzazepine-1-carbonyl]phenyl]-2-methylbenzamide</td>
<td>150683-30-0</td>
<td>C_{28}H_{25}ClN_{2}O_{3}</td>
</tr>
<tr>
<td>Tolvaptan</td>
<td></td>
<td>N-[4-[6R]-9-chloro-6-hydroxy-2-azabicyclo[5.4.0]undeca-8,10,12-triene-2-carbonyl]-3-methyl-phenyl]-2-methylbenzamide</td>
<td>210101-16-9</td>
<td>C_{32}H_{26}N_{4}O_{2}</td>
</tr>
<tr>
<td>Samsca</td>
<td>Conivaptan</td>
<td>N-[4-(4,5-dihydro-2-methylimidazao[4,5-d][1]benzazepin-6(1H)-yl]carbonyl]phenyl]- (1,1'-biphenyl)-2-carboxamide</td>
<td>210101-16-9</td>
<td>C_{32}H_{26}N_{4}O_{2}</td>
</tr>
<tr>
<td>Vaprisol</td>
<td>YM087</td>
<td>N-[4-(4,5-dihydro-2-methylimidazao[4,5-d][1]benzazepin-6(1H)-yl]carbonyl]phenyl]- (1,1'-biphenyl)-2-carboxamide</td>
<td>210101-16-9</td>
<td>C_{32}H_{26}N_{4}O_{2}</td>
</tr>
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</table>

IUPAC, International Union of Pure and Applied Chemistry; CAS, Chemical Abstracts Service; N/A, not available.
and that alternative PDE4 inhibitors may be more suitable. An alternative strategy is the use of calcitonin, a 32-amino acid linear polypeptide hormone that is produced in humans primarily by the parafollicular cells of the thyroid. Calcitonin acts via a seven-transmembrane domain receptor, which is coupled to GoS and can increase intracellular cAMP levels. Calcitonin has been demonstrated to induce AQP2 membrane accumulation in vitro and in vivo via a cAMP-mediated mechanism (193, 194).

F. Statins

Various statins, used in the treatment of hypercholesterolemia, have been proposed for treatment of NDI. Statins exert their effect by inhibiting the activity of 3-hydroxy-3-methyl-glutaryl-CoA reductase, which results in decreased biosynthesis of cholesterol. Acute exposure to simvastatin can increase apical membrane AQP2 in cultured cells and kidney slices from Brattleboro rats (195). In other cell systems, it has been reported that both fluvastatin and lovastatin can induce apical plasma membrane expression of AQP2 (196). In mice, fluvastatin was able to increase AQP2 expression and water reabsorption in the kidney in an AVP-independent manner (196). The molecular mechanisms behind these effects are not fully understood, but it has been suggested to be due to various indirect effects, e.g., changes in prenylation of Rho-family proteins that are involved in AQP2 trafficking or regulation of the cytoskeleton (187, 196). Whether the effect of statins is specific for AQP2 or all other classes of membrane channels/transporters are influenced by statin treatment remains to be resolved.

G. Prostaglandins

E-prostanoid-specific receptor agonists provide evidence for PGE2 being able to decrease diuresis and AQP2 internalization (68, 197). An EP4 prostanoid receptor agonist [ONO-AE-329 (ONO)] has been shown to be a potential drug candidate in X-NDI. In a mouse model for X-NDI, the ONO compound transiently increased urine osmolality, reduced polyuria, and reduced the dilation of the renal pelvis (68). Long term, the ONO compound was able to increase AQP2 protein abundance in X-NDI. Other agonists specific for EP2 (butaprost) and EP4 (CAY10580) were shown to increase AQP2 trafficking in MDCK cells (197), although the mechanisms of action are likely to be different, because only EP2 stimulation resulted in increased cAMP (197). In the same study, butaprost was able to reduce urine volume and increase urine osmolality by up to 65% in a rat model of X-NDI. A promising treatment strategy of NDI could be to target EP2 and/or EP4 to increase collecting duct water permeability, alongside inhibition of potential PGE2 negative effects, e.g., use of EP3 antagonists. EP2 agonists have been approved by the Food and Drug Administration and have been tested on humans for the treatment of primary dysmenorrhea with good tolerability observed in the subjects (198).

H. Heat shock protein 90 (Hsp90)

Hsp90 is, among other functions, considered a “molecular chaperone,” an ER-resident/cyttoplasmic protein that aids proper folding of proteins (178). An Hsp90 inhibitor (17-allylamino-17-demethoxygeldanamycin) was shown to partially correct NDI in a mouse model of autosomal recessive NDI where the mutant, AQP2-T126M, is retained in the ER. However, the precise molecular basis for this effect remains to be established (101).

X. Conclusions

In the past two decades, our understanding of DI from a clinical, genetic, molecular, and cell biological viewpoint has increased enormously. Although the identification of the main types and causes of DI has become simpler, the great variety in the severity of the disease and the genetic basis means that no current treatment regime exists that fully alleviates the symptoms in all sufferers. Thus, although several potential new forms of therapy have been proposed, several...
more years of basic, translational, and clinical science endeavors are required to fully understand and treat this disease of ever expanding complexity.

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