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Abstract

Under normal circumstances, about 90% of the 180 L/day glomerular filtrate is constitutively reabsorbed in the proximal tubule and descending limb of Henle's loop. According to the needs, the remaining 10% of the fluid is reabsorbed in the collecting duct by a tightly regulated process under control of arginine vasopressin (AVP). After binding of AVP to arginine vasopressin type 2 receptors (AVPR2) in the basolateral membrane of collecting duct cells, aquaporin-2 (AQP2) water channels are inserted into the luminal membrane of these cells, allowing water reabsorption and urine concentration. Disorders of water handling are characterized by disturbances of this AVP-regulated system. In congenital nephrogenic diabetes insipidus (NDI), the kidney cannot concentrate urine in response to AVP, as a result of loss-of-function mutations in genes encoding AVPR2 and AQP2, resulting in polyuria and polydipsia. In recent years, extensive research has led to increased understanding of the cellular defects in NDI, with important implications for future development of targeted treatment of the disorder, with hope for better outcomes in comparison to the conventional symptomatic therapy. The very rare nephrogenic syndrome of inappropriate antidiuresis (NSIAD), caused by gain-of-function mutations in the gene encoding AVPR2, is the mirror image of NDI. In this disorder, urinary dilution is impaired, independent of the presence or absence of AVP. In this chapter, the focus will be on the physiology of water handling in the collecting duct and on its disturbances in congenital NDI. The clinical details, differential diagnosis, genetics, and conventional and possible future therapies of NDI will be discussed in detail.

Keywords

Congenital nephrogenic diabetes insipidus · Arginine vasopressin · Anticipatory thirst · Polyuria · Arginine vasopressin type 2 receptor · AQP2 water channel · X-linked · Autosomal · Nephrogenic syndrome of inappropriate antidiuresis · Acquired nephrogenic diabetes insipidus

Introduction

Disorders of water handling in the kidney lead either to dehydration or to overhydration. The vast majority of these disorders are acquired. The inherited disorders of water handling comprise congenital nephrogenic diabetes insipidus, which is associated with dehydration, and its mirror image, the nephrogenic syndrome of

inappropriate antidiuresis, associated with overhydration and the risk of hyponatremia. In this chapter, after a detailed discussion on the physiology of urine concentration, we will mainly focus on the clinical details, diagnostics, differential diagnosis, genetics, and treatment of congenital nephrogenic diabetes insipidus.

Physiology of Urine Concentration, Including Vasopressin-Regulated Water Permeability

The physiologic action of vasopressin on the renal collecting duct is one of the most intensively studied processes in the kidney. Arginine vasopressin (AVP, or antidiuretic hormone (ADH)) is synthesized on the ribosomes of the magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus as a large biologically inactive bound form. Within storage granules, the hormone is cleaved into the biologically active form and transported down the neuronal axons to the posterior pituitary and stored there. Following appropriate stimuli, AVP is secreted from the posterior pituitary to the circulation as biologically active hormone. AVP release is regulated by changes in plasma osmolality (by >2%) but can also occur in response to non-osmotic stimuli. These non-osmotic stimuli are generally related to changes in either total blood volume or the distribution of extracellular fluid. Patients with depleted effective circulating volume may secrete AVP even in the presence of low plasma osmolality. In addition, hypoxia, physical pain, emotional stress, and certain drugs (e.g., nicotine) influence the release of AVP.

There are also vasopressin-expressing neurons in the suprachiasmatic nucleus (SCN), and the circadian regulation of thirst and arginine vasopressin secretion protects against overnight dehydration. This is called anticipatory thirst. SCN vasopressin neurons project to thirst neurons in the organum vasculosum of the lamina terminalis (OVLT), where vasopressin is released as a neurotransmitter [36]. For example, the SCN opposes overnight adipsia by driving water intake before sleep and by driving the secretion of AVP and lowering body temperature to reduce water loss during sleep [37]. The anticipatory signals regulating thirst, sodium appetite, and hunger have now been described in experimental animals with optogenetic tools [4]. Thirst and AVP release are regulated by the classical homeostatic, interosensory plasma osmolality negative feedback as well as by novel, exterosensory, anticipatory signals. Decreases in blood vasopressin levels and thirst have been observed within minutes following water consumption, preceding and hence "anticipating" changes in plasma osmolality. These anticipatory signals for thirst and vasopressin release concentrate on the same homeostatic neurons and circumventricular organs monitoring the composition of blood. The lamina terminalis which is the median portion of the rostral wall of the third ventricle of the cerebrum is composed of three structures: the subfornical organ (SFO), the organum vasculosum of the lamina terminalis (OVLT), and the median preoptic nucleus (MnPO) (Fig. 1). Of these, SFO and OVLT lack the normal blood-brain barrier and, therefore, have direct access to circulating components including angiotensin II. The anticipatory signals from the oropharynx and gut are now well described: MnPO inhibitory neurons that coexpress glucagon-like peptide 1 receptor ($MnPO^{GLP1r}$) transmit oropharyngeal-induced satiation and send monosynaptic inhibition to SFO^{nNOS} neurons (Fig. 1) [3]. Sensory stimuli from the oropharyngeal area are generally transported through cranial nerves V, VII, IX, and X to the central pattern generator within the nucleus tractus solitarius (NTS), which elicits swallowing action.

In its effector organ, the kidney, AVP binds to arginine vasopressin type 2 receptors (AVPR2) on the basolateral membrane of the principal inner medullary collecting duct cells and of the arcade cells. The arcades are long, highly branched renal tubule segments that connect distal convoluted tubules of several deep and mid-cortical nephrons to the origin of cortical collecting ducts. Upon binding of AVP, AVPR2 is activated and then stimulates GTP loading of the small GTPase αG_S – subunit of its coupled trimeric G-protein, eventually leading to dissociation of the G-protein

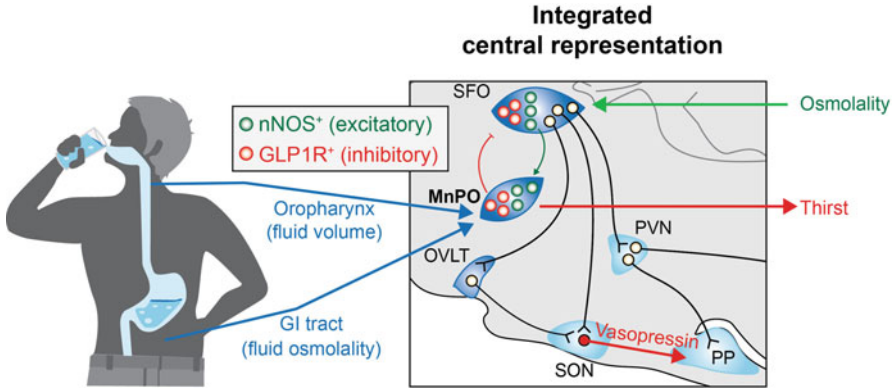

Fig. 1 Anticipation for thirst and vasopressin regulation. Following the detection of water in the mouth and its swallowing, there is a gut-to-brain signal: the osmolarity of the ingested fluid is perceived early in the digestive tract, probably in the small intestine or hepatic portal circulation, relayed by the vagus nerve and inhibits SFO^{Nos1} neurons well before any change in blood osmolality perceived by osmoreceptor cells. $MnPO^{GLP1R} \rightarrow SFO^{nNOS}$ forms an inhibitory circuit where $MnPO^{GLP1R}$ neurons are sensing volume in the oropharyngeal area and osmolarity in the gastrointestinal tract (represented schematically by blue arrows; modified from [126]). There is an integrated central representation of fluid balance at the level of individual MnPO neurons, which use this information to dynamically control drinking behavior and vasopressin secretion in real time

from the receptor (Fig. 2). GTP-αG_S can then bind to the membrane-associated adenylate cyclase 6 (AC6), activating it, primarily by stabilizing a catalytically competent form of the enzyme, which results in an increase in intracellular cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP) [118]. The elevated cAMP levels stimulate protein kinase A (PKA), leading to phosphorylation of AQP2 which in turn initiates a redistribution of aquaporin-2 (AQP2) water channels from intracellular vesicles to the apical plasma membrane, rendering this membrane water permeable. The increase in apical membrane permeability allows water to flow from the tubule lumen to the hypertonic medullary interstitium, via AQP2 in the apical membrane and via AQP3 and AQP4, constitutive water channels in the basolateral membrane. This then leads to the formation of concentrated urine. This process restores plasma osmolality and volume and is regulated by a negative feedback; upon restoration of the water balance, AVP release into the blood decreases; AQP2 is internalized, thus reducing water permeability of the collecting ducts and water reabsorption. AQP2 is retrieved to intracellular vesicles, resulting in either lysosomal degradation or intracellular storage and relocation to the plasma membrane upon restimulation with AVP. At the basolateral side of the collecting duct, AVP binding to AVPR2 can also stimulate mechanisms that lead to termination of signaling. Desensitization and internalization of AVPR2, regulated by β-arrestin, decrease the amount of receptors in the plasma membrane [87].

In recent years, our knowledge of the AQP2 dynamics in the cell has increased significantly. For further details, the reader is also referred to several excellent reviews on this subject (reviews in [11, 29, 47, 78, 80, 121]). AQP2 is one of the 13 members of the aquaporin family of water channels. After transcription, AQP2 is folded into its native monomeric conformation in the endoplasmic reticulum, and homotetramerization takes place [38]. The tetramers are forwarded to the Golgi apparatus, where two out of four monomers are complex N-glycosylated. These functional water channels are then stored in intracellular vesicles to be transported to the apical membrane [84].

AQP2 plays a key role in short-term regulation and long term-adaptation of collecting duct water permeability. Short-term regulation (within minutes) is mediated by PKA-dependent phosphorylation and intracellular translocation of AQP2 from intracellular vesicles to the apical

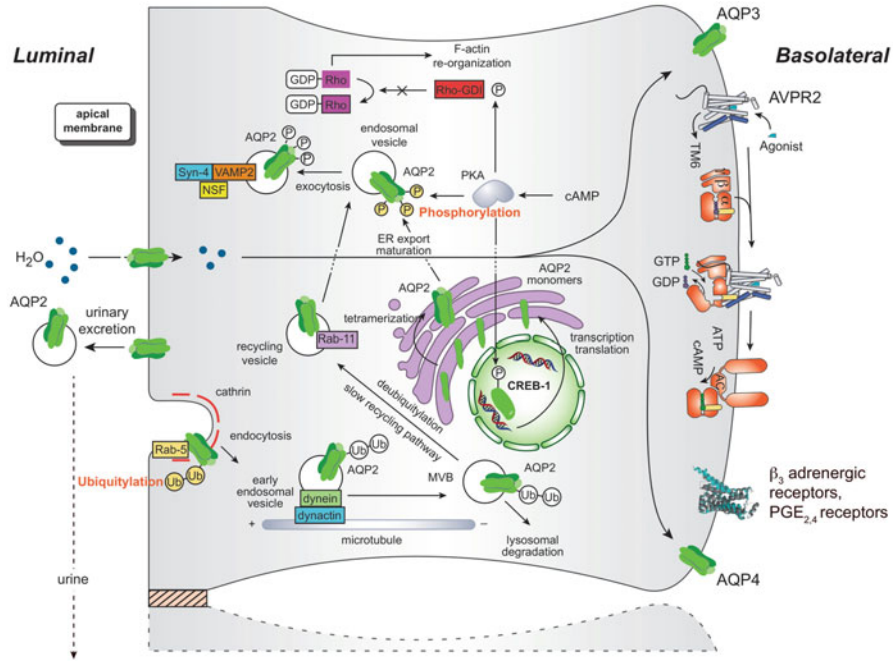

Fig. 2 Intracellular signal transduction pathway initiated by AVP binding to AVPR2. Upon binding of AVP, AVPR2 is activated and then stimulates GTP loading of the small GTPase αG_S – subunit of its coupled trimeric G-protein, eventually leading to dissociation of the G-protein from the receptor. Following the publication of Kobilka and colleagues [39], we have represented here the hallmark of the G-protein-coupled receptor (GPCR) activation as the outward movement of the cytoplasmic end of transmembrane domain 6 (TM6) that opens up a large intracellular cavity to accommodate the G-alpha 5 subunit from G_s, leading to nucleotide exchange and activation of the G-protein. GTP-αG_S can then bind to the membrane-associated adenylate cyclase 6 (AC6), activating it. The resulting cAMP-production stimulation activates PKA, which in turn phosphorylates its target proteins AQP2, Rho-GDI, and CREB1. The transcription factor CREB-1-p stimulates AQP2 transcription, Rho-GDI-p initiates actin reorganization required for AQP2 transport, and AQP2-p homotetramers are transported to the apical membrane. Syn-4 and VAMP2 are involved in the insertion of AQP2 tetramers in the apical membrane. There, they render the membrane permeable for water, which is reabsorbed from the passing pro-urine and transported back into the blood stream by AQP3 and AQP4. Rab5-mediated AQP2 endocytosis by clathrin-coated vesicles is triggered by short-chain ubiquitylation and leads to termination of the response. Internalized AQP2 vesicles are transported to early and late endosomes as well as multivesicular bodies (MVBs) for storage. From MVBs, they can then either be lysosomally degraded or recycled via the Rab-11-dependent slow recycling pathway. The cAMP pathway can also be stimulated independent of AVPR2 signaling, for instance, by activation of ß3 adrenergic receptors or PGE2,4 receptors

plasma membrane. Long-term adaptation is seen when circulating AVP levels are increased over a period of hours and involves a change in the total abundance of the AQP2 protein in collecting duct cells as a result of regulatory actions at the transcriptional or posttranscriptional level. Both short- and long-term modulations of AQP2 are extremely complex and still not fully understood processes.

Short-Term Regulation of Water Reabsorption in Collecting Duct

PKA-dependent phosphorylation at the cytoplasmic carboxy-terminal serine 256 residue (pS256-AQP2) is essential and sufficient to cause trafficking of AQP2 to the apical membrane [34]. Phosphorylation at other canonical phosphorylation

sites in the carboxy-terminus of AQP2 (i.e., Ser264, Ser269, Ser261) is involved also but apparently less critical (review in [29]). Phosphorylation at Ser269 inhibits AQP2 endocytosis, resulting in increased retention of AQP2 at the apical plasma membrane. Phosphorylation at Ser261 plays an important role in sorting AQP2 to degradation pathways. Studies using oocytes as a model system indicated that for plasma membrane localization, three out of four monomers in an AQP2 tetramer need to be phosphorylated [50]. PKA is the main kinase for AQP2 phosphorylation, but other kinases may potentially participate in the regulation of AQP2 trafficking. Besides PKA sites, putative phosphorylation sites for Protein kinase G (PKG), Protein kinase C (PKC), and casein kinase II are also present in the AQP2 sequence. In addition, it has been shown that anchoring of PKA to PKA-anchoring proteins (AKAPs), which ensures targeting of PKA to AQP2-bearing vesicles, is another prerequisite for AVP-mediated AQP2 translocation [57].

Reorganization of the actin cytoskeleton is also required for AQP2 transport to and accumulation in the apical membrane. The actin cytoskeleton most likely provides a network that anchors the AQP2-bearing vesicles in the unstimulated cell. Vasopressin has been shown to depolymerize apical F-actin in rat inner medullary collecting duct, resulting in the fusion of AQP2-carrying vesicles with the apical membrane [111], indicating that reorganization of the apical actin network may be critical in promoting the trafficking of AQP2-bearing vesicles. Activation of small GTPase RhoA induces F-actin-containing stress fibers and tonically inhibits AQP2 trafficking. Rho inhibition through PKA-mediated phosphorylation of Rho-GDP dissociation inhibitor (Rho-GDI) is shown to be a key event for actin reorganization inducing AQP2 translocation [58].

The plasma membrane insertion of AQP2-containing vesicles in the apical membrane is similar to the process of synaptic vesicle fusion with the presynaptic membrane and involves vesicle (v) soluble NSF attachment protein receptors (SNAREs) and target membrane (t) SNAREs. The apical membrane-specific t-SNARE is syntaxin 4 (Syn-4), which interacts specifically with the v-SNARE protein VAMP2 located on the cytoplasmic side of AQP2-containing endosomal vesicles [66, 71, 85]. V- and t-SNARES are recycled by the AAA-type ATPase NSF.

AQP2 is internalized after removal of AVP stimulation when the water balance is restored. During this endocytotic process, AQP2 accumulates in clathrin-coated pits and is internalized via a clathrin-mediated process [114]. Endocytosis is regulated by short-chain ubiquitylation at lysine 270 (K270) in the AQP2 terminal tail [51]. The process of ubiquitylation of AQP2 involves several ubiquitin E3 ligases, including NEDD4, and the heat-shock protein 70 (Hsp70)-interacting protein CHIP [76, 119, 122]. Specificity of the endocytotic AQP2 internalization is mediated by Rab5 protein, an effector-binding factor involved in plasma membrane to early endosome transport [90]. To be available for recycling, AQP2-containing endosomes need to be redistributed to the perinuclear region. This process is mediated by dynein-dependent transport along microtubules [73]. From the endosomal system – early/late endosomes and/or multivesicular bodies (MVBs) – AQP2 is either recycled by the Rab11-dependent slow recycling pathway or marked for lysosomal degradation [113]. Prolonged K270 ubiquitylation induces MVB trafficking and localization to internal vesicles of MVBs followed by lysosomal degradation, while deubiquitylation increases localization to early endosomes and the limiting membrane of MVBs and enables AQP2 recycling [51, 102, 113].

Long-Term Regulation of Water Reabsorption in Collecting Duct

Long-term adaptation of collecting duct water permeability following AVP stimulation is mediated by changing the expression of AQP2 mRNA and protein (review in [48]). An increase in cAMP levels result in PKA-mediated phosphorylation and activation of a cAMP-responsive element-

binding protein 1 (CREB-1), which increases AQP2 expression by enhancing its gene transcription. Evidence is accumulating that nuclear receptor transcription factors (i.e., PPARγ, FXR, GR, MR, LXRs) are also involved in the long-term regulation of water transport in renal collecting ducts by either up- or downregulating AQP2 gene transcription and expression (review in [29, 125]). In addition, micro-RNAs (i.e., miR-32 and miR-137) appear to be important posttranscriptional regulators of AQP2 protein abundance [53, 96].

Alternative Signaling Pathways

The AVP-dependent cAMP-PKA pathway is the canonical pathway to regulate AQP2 expression and trafficking. In recent years, it has been demonstrated that alternative pathways bypassing AVPR2 signaling may also influence AQP2 regulation and trafficking (reviews in [48, 78]). These alternative pathways are interesting since they may be targets for therapy of NDI. The cAMP pathway can be stimulated by the activation of other G-protein-coupled receptors (GPCRs), such as the prostaglandin receptors (EP2/EP4), the calcitonin receptor, the secretin receptor, and the β3-adrenergic receptor (reviews in [48, 78]). In addition, AQP2 trafficking and abundance are affected by the cAMP-exchange protein directly activated by cAMP (Epac) pathway. The latter pathway is suggested to be involved in the long-term regulation of AQP2, whereas the cAMP-PKA pathway has independent short-term effects. The AVPR2-elicited pathway can also be bypassed in a cAMP-independent way. For instance, activation the Cyclic guanosine monophosphate (cGMP) pathway by nitric oxide donors or atrial natriuretic peptide promotes AQP2 trafficking and membrane insertion, and activation of the Wnt5-frizzled receptor promotes AVP-independent AQP2 phosphorylation via intracellular calcium/calmodulin/calcineurin signaling [2].

Congenital Nephrogenic Diabetes Insipidus

Congenital nephrogenic diabetes insipidus (NDI) is a rare inherited disorder, characterized by insensitivity of the distal nephron to the antidiuretic effects of AVP. As a consequence, the kidney loses its ability to concentrate urine, which may lead to severe dehydration and electrolyte imbalance (hypernatremia and hyperchloremia).

Clinical Presentation

Patients with NDI have normal birth weight, and pregnancies are not complicated by polyhydramnios. The urine-concentrating defect in NDI is present from birth, and manifestations of the disorder generally emerge within the first weeks of life. With breast milk feedings, infants usually thrive and do not develop signs of dehydration. This is because human milk has a low salt and protein content and therefore a low renal osmole load. With cows' milk formula feedings, the osmole load to the kidney increases, resulting in an increased demand for free water. This is usually not provided by oral feeding, and therefore, hypernatremic dehydration appears. Polyuria and polydipsia, irritability, poor feeding, recurrent vomiting, and poor weight gain are usually the initial symptoms [21, 107]. Patients are eager to suck but may vomit during or shortly after the feeding. Dehydration is evidenced by dryness of the skin, loss of normal skin turgor, recessed eyeballs, increased periorbital folding, depression of the anterior fontanel, and a scaphoid abdomen. Intermittent high fever is a common complication of the dehydrated state, predominantly in very young children. Body temperature can be normalized by rehydration. Seizures can occur but are rare and most often seen during therapy, particularly if rehydration proceeds too rapidly. Constipation is a common symptom in children with NDI. Nocturia and nocturnal enuresis are common complaints later in childhood.

If untreated, most patients fail to grow normally. In a retrospective study of 30 male NDI patients, most children grew below the 50th percentile, most of them having standard deviation (SD) scores lower than −1 [68]. Some well-treated patients, however, may achieve normal adult height. Catch-up growth occurs at least in some patients after normalization of water and electrolyte balance, especially in those with adherence to treatment. Bone maturation is generally not delayed.

In a more recent European report on the long-term follow-up of patients with NDI, growth was improved compared with historic cohorts with median height SDS being −1.1 at presentation and − 0.9 SD at last follow-up and median weight SDS being −2.1 and 0.2, respectively [107]. Initial feeding problems and the ingestion of large amounts of low-calorie fluid resulting in a decreased appetite may play roles in failure to thrive seen in NDI. Furthermore, it is possible that repeated episodes of dehydration have some as yet undetermined negative effects on growth.

Intellectual disability has long been considered an important complication of untreated NDI and assumed to be a sequel of recurrent episodes of severe brain dehydration and cerebral edema caused by overzealous attempts at rehydration. Additional evidence underscoring the assumption that NDI has adverse effects on the cerebrum is provided by several reports describing intracranial calcifications in NDI patients [109]. Such lesions are generally considered to be the result of hemorrhage or necrosis. Most of the reported patients with cerebral calcifications were intellectually disabled. Nowadays, intellectual disability is rare due to earlier recognition and treatment of NDI. Exact estimates of the current frequency of intellectual disability under modern treatment are unknown. One psychometric study reported 2 of the 17 male NDI patients (aged 3–30 years) have a total intelligence quotient of more than 2 SD below the norm. Fourteen patients had an intelligence score within or above the normal range, and one patient had a general index score between −1 and − 2 SD [40].

The psychological development of NDI patients is influenced by a persistent desire for drinking and the need for frequent voiding, which compete with playing and learning. Therefore, many NDI patients are characterized by hyperactivity, distractibility, short attention span, and restlessness. In the psychometric study mentioned earlier, the criteria for attention deficit hyperactivity disorder were met in 8 of 17 tested NDI patients [40].

In a recent cohort of NDI patients, one of 36 patients had severe global developmental delay which was already noted at his presentation at 7 months, and 5 of 28 children in whom information on learning and behavior information was available had attention deficit hyperactivity disorder (ADHD) and/or learning difficulties and/or impaired concentration, pointing to overall improvement in mental and psychological development of individuals with NDI over the last decades [21, 107].

Urological complications are noted in 40%–50% NDI patients [20, 107]. Persistent polyuria can result in the development of megacystis, trabeculated bladder wall, hydroureter, and hydronephrosis [21, 68, 107]. Urinary tract distension may be seen on ultrasound examination even in infants and young children. Potential complications of urinary tract dilatation are rupture of the urinary tract, infection, intractable pain, improper bladder function, and/or kidney failure. These complications may occur as early as the second decade of life. Large-capacity hypotonic bladder dysfunction might require clean intermittent catheterization.

Patients should be trained to void regularly in order to assure that maximal urinary bladder capacity remains within normal range. Both patient groups with *AVPR2* and *AQP2* mutations can develop urinary tract dilatation and bladder dysfunction. Nocturnal enuresis usually persists longer than in healthy children due to pronounced polyuria. One study reported the median age at resolution being around 11 (range 5–16) years [107].

Chronic kidney disease stage 2 or higher is reported in about 20% of the patients; however, no case of end-stage kidney disease has been reported so far [21, 107].

Diagnostic Procedures

The observation of polyuria in a dehydrated infant, together with the finding of a high serum sodium concentration and inappropriately dilute urine (Uosm/Posm), provides presumptive evidence for a renal concentrating defect. To confirm the concentrating defect and to distinguish the renal form of diabetes insipidus from the central form, a vasopressin test is performed with 1-deamino-8-D-arginine vasopressin (DDAVP), a synthetic analogue of the natural arginine vasopressin that produces a high and prolonged antidiuretic effect. In the test, DDAVP (10 μg for infants <1 year old, 20 μg for children >1 year old) is administered intranasally. Urine is collected during the subsequent 5.5 hours. The first collected portion of the urine should be discarded. The maximal urine osmolality in any collected aliquot is chosen as a measure of the concentrating capacity. After DDAVP administration, NDI patients are unable to increase urinary osmolality, which remains below 200 mOsm/kg H_2O (normal values: <1 year old >600, between 1 and 2 years old between 600 and 800, >2 years old >800 mOsm/kg H_2O) and cannot reduce urine volume or free-water clearance.

Performing a water restriction test is not recommended for the diagnosis of NDI as it can lead to pronounced dehydration and unneeded suffering of the patients.

Plasma vasopressin levels are normal or only slightly increased in affected children. Other laboratory findings have been described, which mainly result from chronic dehydration. Serum sodium concentration is generally elevated and may be above 170 mmol/L. There is also an increase in serum chloride concentration and retention of urea and creatinine. All values normalize by adequate rehydration. In addition, reduced glomerular filtration rate (GFR) and renal blood flow can return to normal when a normal hydration state has been achieved.

The primary congenital form of NDI has to be differentiated from central diabetes insipidus (due to lack of AVP) and from the secondary or acquired forms, which are much more common. In our experience, the urinary osmolality obtained after DDAVP administration in secondary disorders is always higher than in NDI.

Secondary Nephrogenic Diabetes Insipidus

In addition to primary forms of congenital NDI, a few cases of secondary inherited NDI have been reported [11] (Table 1). These patients have Mendelian diseases that affect tubular function with NDI as a secondary complication. In some such patients, the NDI symptoms dominate the clinical picture, leading to an initial misdiagnosis, with the true underlying cause of disease subsequently identified. Most of the primary diseases associated with secondary NDI, such as Bartter syndrome and apparent mineralocorticoid excess, are associated with hypokalemia and hypercalciuria. Patients with polyhydramnios, hypercalciuria, and isosthenuria have been found to bear *KCNJ1* (ROMK), *SLC12A1* (NKCC2), and *MAGED2* mutations. Patients with polyhydramnios, profound polyuria, hyponatremia, hypochloremia, metabolic alkalosis, and sensorineural deafness were found to bear *BSND* (Barttin) mutations. These studies demonstrate the critical importance of the proteins ROMK, NKCC2, and barttin in transferring NaCl to the medullary interstitium and thereby generating, together with urea, a hypertonic milieu.

Acquired Nephrogenic Diabetes Insipidus

Although the hereditary forms of NDI are relatively rare, a wide range of pathologic conditions and drug treatments can lead to acquired NDI (Table 1). In our experience, the urine osmolality obtained after DDAVP administration in these acquired disorders is always higher than in congenital NDI. All these disorders have been shown to coincide with decreased expression of AQP2 or deregulated AQP2 trafficking to the apical membrane [32, 63, 72, 105]. For instance, prolonged

Table 1 Causes of secondary or acquired nephrogenic diabetes insipidus

Monogenetic diseases associated with secondary NDI
Renal Fanconi syndromes
Bartter syndrome (type 1 or type 2)
Familial hypomagnesemia with hypercalciuria and nephrocalcinosis
Distal renal tubular acidosis (dRTA)
Apparent mineralocorticoid excess (AME)
Ciliopathies (nephronophthisis, Bardet-Biedl syndrome, etc.)
Other renal diseases
Obstructive uropathy
Renal dysplasia
Postischemic damage
Amyloidosis
Sarcoidosis
Chronic renal failure
Renal impairment in sickle cell disease or trait
Drug-induced
Lithium
Ifosfamide
Amphotericin B
Tetracyclines
Biochemical abnormalities
Hypercalcemia and hypercalciuria and nephrocalcinosis
Hypokalemia

treatment with lithium, the drug of choice for treating bipolar disorders and prescribed to 1 in 1000 of the population, results in the development of NDI in at least 20% of treated individuals [120]. The development of lithium-NDI is believed to occur in two phases. In the first short-time phase, lithium causes a decrease in AQP2 expression (review in [55]). Lithium enters the cells via epithelial sodium channel (ENaC) and accumulates in principal cells. How lithium downregulates AQP2 is not clear but likely involves glycogen synthase kinase 3 (GSK3)-β, which is important in AVP-regulated antidiuresis and is inhibited by lithium. GSK3 inhibitors other than lithium also reduce AQP2 expression in collecting duct cell cultures, and ablation of GSK alpha or beta in mice causes an inability to concentrate urine by dehydration or DDAVP [19]. Lithium also influences AQP2-mediated water reabsorption by increased tubular release of prostaglandin E2. In the second phase, lithium reduces the percentage of principal cells in the collecting duct to the advantage of intercalated cells, involved in acid-base homeostasis [18]. The exact contribution of this collecting duct remodeling in the lithium-induced resistance to vasopressin remains to be elucidated. Autophagic degradation of aquaporin-2 has been implicated in hypokalemia and hypercalcemia-induced NDI [19].

Nephrogenic Diabetes Insipidus: Genetics

Three different inheritance patterns of congenital NDI have been recognized. In most cases (about 90%), NDI is transmitted as an X-linked recessive trait (MIM304800). In these families, female carriers who are usually unaffected, transmit the disease to their sons, who display the complete clinical picture. In 1992, mutations in *AVPR2* were shown to underlie X-linked NDI [89, 103]. In a minority of families (about 10%), the transmission and phenotypic characteristics of NDI are not compatible with an X-linked trait. In these families, females display the complete clinical picture of NDI and are clinically undistinguishable from affected male family members. Family pedigrees suggested the existence of both an autosomal recessive (MIM 222000) and an autosomal dominant form (MIM 125800) of NDI. It was subsequently demonstrated that both autosomal forms of NDI are caused by mutations in *AQP2* [22, 81]. The prevalence of NDI is not exactly known, but the disease is assumed to be rare. The estimate of the prevalence of NDI in Quebec, Canada, is 8.8:1,000,000 males. However, owing to chance population genetic events, such as a founder effect, the incidence of NDI is elevated in certain regions, for example, in Utah and Nova Scotia [11].

X-Linked Nephrogenic Diabetes Insipidus: Mutations in AVPR2

The X-linked form of NDI is caused by loss-of-function mutations in *AVPR2* (MIM 300538) (reviews in [8, 11, 46, 59, 80, 121]). *AVPR2* is a relatively small gene, consisting of three exons

separated by two short intervening sequences (introns); two isoforms are known that are generated by alternative splicing [30]. *AVPR2* is localized on the X chromosome on locus Xq28. The cDNA encodes a receptor protein of 371 amino acids, has a predicted molecular mass of approximately 40 kDa, and shares the general structure of a G-protein-coupled receptor consisting of seven hydrophobic transmembrane helices, connected by extracellular and intracellular loops. The receptor contains one unique consensus sequence site for N-linked glycosylation in the extracellular amino-terminus [42] and phosphorylation sites for G-protein-coupled receptor kinases (GRK) represented by a serine cluster in the carboxy-terminus [43]. The amino-terminal part of the protein including the first transmembrane domain and the positively charged first intracellular loop are important for proper insertion and orientation in the membrane [110]. A conserved glutamate-dileucine motif in the intracellular carboxy-terminal part of the receptor is essential for receptor transport from the endoplasmic reticulum (ER) to the Golgi apparatus [61]. Two conserved adjacent cysteines in the C-terminus are palmitoylated, thereby anchoring the carboxy-tail to the plasma membrane and controlling the tertiary structure of this region of the receptor [110].

To date, there are more than 280 distinct disease-causing mutations in *AVPR2*, and the number is constantly increasing (reviews in [7, 59, 80, 121], and www.hgmd.org). The mutations are not clustered in one domain of the AVPR2 but are scattered throughout the protein, except for the part coding for the N- and C-terminal tails of the receptor. More than 50% of the mutations are missense mutations; nonsense mutations (8%), splice site mutations (1%), small deletions (18%), small insertions/duplications (7%), small indels (2%), large gene deletions (8%), large insertions/duplications (1%), and complex rearrangements (2%) account for the remainder of mutations (reviews in [7, 46, 59, 80, 121], and www.hgmd.org). Several mutations (p.Asp85Asn, p.Val88Met, p.Arg113Trp, p.Tyr128Ser, p.Arg137His, p.Ser167Leu, p.Arg181Cys, p.Arg202Cys, p.Ala294Pro, and p.Ser315Arg) are recurrent as evidenced by the fact that these mutations were found on different haplotypes in ancestrally independent families. The most frequent of these recurrent mutations (p.Asp85Asn, p.Val88Met, p.Arg113Trp, p.Arg137His, p.Ser167Leu, p.Arg181Cys, p.Arg202Cys) occur at potential mutational hotspots [8].

AVPR2 mutations seem to be present in all ethnical groups tested with no preference of one mutation for any ethnic group over others. However, two founder mutations, the p.Trp71* "Hopewell" mutation in Ulster Scott immigrants and the p.Leu312* in a large Utah pedigree, result in an increased prevalence of X-linked congenital NDI in their descendants in certain communities in Nova Scotia, Canada, and Utah [33].

The molecular mechanism underlying the renal insensitivity for AVP differs between mutants. As upcoming pharmacological treatments for NDI likely depend on the underlying mechanism, GPCR mutations in general and *AVPR2* mutations in particular have been divided in five different classes according to their cellular fate ([98], review in [78]).

Class I comprises all mutations that interfere with proper transcription, mRNA processing, and translation, resulting in truncated proteins that are often rapidly degraded, such as splice site, frameshift, and nonsense mutations [98]. Class II mutations are missense or insertions/deletions of one or more nucleotide triplets, resulting in fully translated proteins. Due to the mutation, however, mutant receptors are misfolded and retained in the endoplasmic reticulum (ER), as the ER is the organelle that has the cellular quality control over proper folding and maturation of synthesized proteins. Misfolded proteins are subsequently mostly targeted for proteasomal degradation [26]. Intracellular entrapment of missense AVPR2 mutants and their rapid degradation likely represent the most important cause of NDI, as more than 50% of the *AVPR2* mutations are missense mutations and cellular expression revealed that most of these result in ER-retained proteins. Class III mutations result in plasma membrane-expressed receptors with reduced affinity for the stimulatory G_s protein. Mutations in this group are missense mutations and in-frame deletions, mostly located in transmembrane and intracellular domains [99]. Class IV mutations have low affinity for

AVP [99]. Finally, class V mutations are missorted to incorrect cellular compartments, like arrestin-positive endocytotic vesicles [5]. Sometimes, mutants do not exert a full phenotype of a particular class and then often also show features of another class. For example, some AVPR2 missense mutants are partially ER-retained (class II) but are also partially expressed in the plasma membrane, where they might show a reduced G-protein coupling (class III) or AVP binding (class IV). As such, it provides an explanation for the observed small antidiuretic response to high doses of DDAVP in NDI patients harboring such mutations [92].

Nephrogenic Syndrome of Inappropriate Antidiuresis

NSAID, with four identified *AVPR2* gain of function mutations: R137C, R137L, F229V, and I130N, is the mirror image of NDI. [7]. In NDI, the kidneys cannot concentrate the urine, whereas in NSIAD, urinary dilution is impaired, independent of the presence or absence of vasopressin. Consequently, patients with NDI are at risk of hypernatremic dehydration, whereas hyponatremia is a typical manifestation of NSIAD, mimicking the syndrome of inappropriate antidiuresis (SIADH); unlike SIADH, AVP levels are usually low or undetectable in NSIAD [28]. The diagnostic pathways also mirror: in NDI, an agonist for the AVPR2, such as synthetic V_2-vasopressin analogue 1-deamino-8-D-arginine vasopressin (DDAVP), is given to assess the ability of the kidneys to concentrate urine. Conversely, administration of an AVPR2 antagonist, such as tolvaptan, provides an assessment of urinary dilution capacity in patients suspected of NSIAD.

Recently, it was shown that NSIAD can also be caused by mutations in Gsα-coding *GNAS* gene. Miyado et al. reported two families with a dominantly inherited form of NSIAD segregating with the GNAS variants p.F68_G70del and p.M255V [79]. The *GNAS* mutation p.F376V was reported in two unrelated patients with hyponatremia, and it was associated with additional clinical symptoms, suggesting gain of function not only of AVPR2 but also of other GPCR, including the lutropin (LHGR) and parathyroid hormone (PTH1R) receptors [10]. The severity of the phenotype in the patients with the dominantly inherited mutations was quite variable: some patients presented with seizures in early childhood associated with euvolemic hyponatremia, inappropriately elevated urine osmolality, and suppressed vasopressin levels. Other patients had no apparent symptoms and were normonatremic when investigated, but they had elevated urine osmolality despite suppressed vasopressin levels and a history of spontaneously low fluid intake. Symptomatic family members were treated with fluid restriction with normalization of hyponatremia.

Genotype-Phenotype Correlations in X-Linked NDI?

Almost all pathogenic variants in *AVPR2* result in a uniform clinical NDI phenotype with polyuric manifestations in the first weeks of life and poor growth. There are, however, a few exceptions to this rule. Several mutations appear to be associated with a milder form of NDI, characterized by a later manifestation, not at birth but later in childhood, and without growth retardation. At present, 18 *AVPR2* pathogenic variants resulting in partial NDI have been reported (Table 2; review in [59]).

Intrafamilial variability of the X-linked NDI phenotype has also been described. A nice example is the case described by Kalenga et al., who reported a Belgian family in which the p.Arg137His pathogenic variant was associated with severe NDI in the proband but with very mild NDI in his affected brother [49]. Genetic and/or environmental modifying factors are likely to account for this intrafamilial phenotype variability.

The Autosomal Recessive and Autosomal Dominant Forms of Nephrogenic Diabetes Insipidus: Mutations in the Aquaporin-2 Water Channel

Both the autosomal recessive and the autosomal dominant types of NDI are caused by mutations in the *AQP2* water channel gene (MIM 107777;

Table 2 *AVPR2* pathogenic variants resulting in partial NDI

Pathogenic *AVPR2* variants	Effect of the variant(s)	References
p.Asn317Lys p.Asn317Ser p.Asn321Tyr p.Met311Val	Reach the cell surface with impaired ligand capacity and partial AVP/DDAVP binding	[83]
p.Asp85Asn	Decreased ligand-binding affinity and decreased coupling to G_s	[104]
p.Gly201Asn	Decreased number of cell surface AVPR2 receptors	[104]
p.Pro322Ser p.Val88Met	Reduced cell surface expression and/or decreased binding affinity for AVP	[1, 12]
p.Ser334del p.Tyr128Ser p.Thr273Met p.Ser329Arg	Impaired intracellular trafficking	[27, 70, 116]
p.Arg104Cys	Decreased AVP binding most likely due to conformational changes	[27]
p.Leu161Pro c.276A > G (splice site)	Unknown	[123], [108]

GenBank accession number z29491). Human *AQP2* is a small gene consisting of four exons, comprising 5 kb genomic DNA. The 15 kb mRNA encodes a protein of 271 amino acids which has a predicted molecular weight of 29 kDa [35]. AQP2 belongs to a family of integral membrane proteins, aquaporins, which function as selective water transporters throughout the plant and animal kingdom. In mammals, 13 different aquaporins have been identified to date, eight of which (aquaporins 1–4, 6–8, and 11) are highly expressed in the kidney. In the plasma membrane, AQP2 exists as a homotetramer, with each monomer containing a water pore. Each AQP2 monomer has six membranes spanning α-helical domains and intracellular N- and C-termini. The six transmembrane helices are connected via three intracellular loops (A, C, and E) and two extracellular loops (B and D), the latter containing highly conserved Asn-Pro-Ala (NPA) sequence motifs. The six transmembrane helices surround a single, narrow water-conducting channel, while the two extracellular loops fold into the membrane from opposite sites with their NPA motifs forming a constriction in the middle of the permeation channel. Another conserved structural feature, the aromatic/arginine (ar/R) constriction site located at the extracellular side of the channel, acts as a selectivity filter, preventing permeation of all molecules bigger than water ([31], review in [29]). AQP2 is exclusively localized in the apical membrane and a subapical compartment of collecting duct cells. It is upregulated by dehydration or AVP, indicating that it is the AVP-regulated water channel.

To date, 52 putative disease-causing mutations in *AQP2* have been identified in families with autosomal recessive NDI (reviews in [8, 59, 78, 121]). These include 42 missense mutations, two nonsense mutations, two small deletions, and four splice site variants. Most mutations are found between the first and last transmembrane domain of AQP2. Expression studies in *Xenopus laevis* oocytes have revealed that most *AQP2* missense mutations that cause recessive NDI are class II mutations. Thus, these mutations lead to misfolding of the mutant protein, retention in the endoplasmic reticulum (ER), and rapid degradation of *AQP2* (review in [121]). When overexpressed in oocytes and Chinese hamster (CHO) cells, six of these AQP2 mutants (A147T, T126M, G64R, L22V, A47V, and T125M) confer water permeability [74, 117]. This indicates that at high expression levels, these AQP2 mutant proteins escape from the ER and are routed to the plasma membrane, where they are functional. In terms of possible treatment strategies, these results are of high importance, since they suggest

that functional channels may be stimulated to reach the plasma membrane by restoring mutant trafficking.

One AQP2 missense mutant, P262L, located in the AQP2 C-terminal tail, a region until then believed to result in dominant NDI, surprisingly was found to be involved in recessive NDI [23]. In cell biological experiments, it was shown that the P262L mutant is a functional water channel that forms hetero-oligomers with wt-AQP2. These wt-AQP2/AQP2-P262L heterotetramers are located in the apical membrane, indicating that the apical sorting of wild-type AQP2 is dominant over the missorting signal of AQP2-P262L. This is different from dominant NDI, because in this form mutants retain wt-AQP2 in intracellular locations (see below). The recessive inheritance in the two patients encountered (patients were heterozygous for a R187C or A190T mutation on one allele, combined with a P262L mutation on the other allele) can be explained as follows: AQP2-R187C and AQP2-A190T are retained in the ER and do not interact with AQP2-P262L. AQP2-P262L folds properly and assembles in homotetramers but will be retained mainly in intracellular vesicles. The consequent lack of sufficient AQP2 proteins in the apical membrane of the patients' collecting duct cells explains their NDI phenotype. In the parents coding for wt-AQP2 and AQP2-R187C or AQP2-A190T, wt-AQP2 will not interact with either mutant but will form homotetrameric complexes, of which the insertion into the apical membrane will be regulated properly by vasopressin and will give a healthy phenotype. In the parents coding for wt-AQP2 and AQP2-P262L, both proteins likely assemble into heterotetramers. The dominancy of wt-AQP2 sorting on the localization of AQP2-P262L will result in proper AVP-regulated trafficking of the heterotetrameric complexes to the apical membrane and will also give a healthy phenotype [23].

More than ten families have been described with autosomal dominant NDI, initially uncovered due to father-to-son transmission of the disease. In these families, subsequent sequencing of *AQP2* revealed putative disease-causing mutations in one *AQP2* allele. At least 13 pathogenic variants that give rise to autosomal dominant NDI have been described. These are six missense variants, one 1-bp insertion, and six small deletions [59, 78, 121]. All mutations causing dominant NDI are located in coding region of the C-terminal tail of AQP2, which is not part of the pore-forming segment, but contains important sorting signals that govern intracellular transport of the protein [99]. Indeed, all functionally tested mutant AQP2 proteins found in dominant NDI appeared to be folded functional water channels that were sorted to other subcellular locations in the cell than wt-AQP2, e.g., late endosomes/lysosomes and the basolateral membrane. Because none of these mutants were misfolded, they were, in contrast to AQP2 mutants in recessive NDI, able to interact and form heterotetramers with wt-AQP2. Due to this wt-mutant interaction and the dominancy of the missorting signals in the mutant protein, the wt-mutant complexes are also missorted. Formation of heterotetramers with wt-AQP2 has been shown for most of the dominant AQP2 mutants. For instance, expression studies in polarized cell lines have revealed the dominant AQP2- E258K mutant is routed to the Golgi complex or late endosomes/lysosomes [81]. In co-expression studies with wild-type AQP2, a dominant-negative effect was observed, caused by impaired routing of wild-type AQP2 to the plasma membrane after hetero-oligomerization with the E258K mutant [52]. Mistargeting to the basolateral membrane has been reported for the AQP2-721delG, AQP2–763-772del, AQP2–812-818del, and AQP2–779-780insA mutants [62, 75]. TheAQP2-727delG mutant was shown to interfere with the routing of wild-type AQP2 to the apical membrane by its mistargeting to the basolateral membrane and late endosomes/lysosomes [75]. The loss of appropriate AQP2 heterotetramer trafficking in dominant NDI is caused by several mechanisms. The phosphorylation site at Ser256, serving as an introducible apical sorting signal, may be inactivated, overruled by basolateral sorting signals, or reprogrammed to induce basolateral sorting, all causing intracellular misrouting (review in [121]).

One-sixteenth of all tetramers formed are wt-AQP2-only tetramers explaining the relatively

milder phenotype in autosomal dominant NDI compared to autosomal recessive NDI [24, 52, 75].

Recently, Calvanese et al. using an in silico automated method for modeling point mutations in protein structures, classified *AQP2* mutations into four main groups according to their molecular effect: mutations affecting (1) the pore, (2) tetramer assembly, (3) monomer folding, and (4) signal loss for protein phosphorylation [16]. The majority of the *AQP2* mutations they modelled are known to cause autosomal recessive NDI (12/14), while two mutations cause autosomal dominant NDI. Their findings were in line with the results of in vitro functional studies mentioned above. Remarkably, they also revealed a clear correlation between the type of mutation-induced structural defect and the experimentally observed phenotypes; mutations affecting the monomer folding cause the most severe phenotypes. These mutants are nonfunctional due to their misfolding and retention in the ER. The functionality of the mutants affecting the NPA motifs and the ar/R selectivity filter is also seriously impeded. However, mutations affecting other features of the pore, such as its dimension, or mutations affecting the tetramer assembly are associated with milder phenotypes, with resulting mutant AQP2 proteins partially retaining their water channel functionality.

Differential Diagnosis between the X-Linked and the Autosomal Forms of NDI

With a few exceptions, no differences in clinical symptoms between X-linked and autosomal recessive forms of NDI can be observed nor in the time of onset of the disease. Only in a minority of patients with the X-linked form of NDI, namely, those individuals carrying *AVPR2* mutations with partial insensitivity to AVP (Table 2), the disease onset is not directly after birth but later in childhood. In general, the initial symptoms in most autosomal dominant cases also appear later in childhood.

Male patients with X-linked NDI can be discriminated from patients with autosomal recessive NDI on the basis of their extrarenal reaction to the intravenous administration of the synthetic AVPR2-vasopressin analogue 1-deamino-8-D-arginine vasopressin (DDAVP). Patients with autosomal recessive NDI show a decrease in blood pressure, accelerated heart rate, and increases in von Willebrand factor, factor VIII, and tissue-type plasminogen activator levels, whereas in all studies of patients with X-linked NDI, these extrarenal responses are absent as a result of an extrarenal mutant AVPR2 [67]. In female patients, the interpretation of this intravenous DDAVP test is more complicated. Although absence of the extrarenal responses to intravenous administration of DDAVP in females clearly points to the presence of an AVPR2 defect, a normal response cannot be interpreted as indicative of a defect beyond AVPR2 and thus an AQP2 defect. The discrepancy between the renal and extrarenal response to DDAVP in these female *AVPR2* mutation carriers might be explained by variability in the pattern of X-inactivation between different tissues.

Nephrogenic Diabetes Insipidus in Females

Several families have been described in which females show classical clinical and laboratory features of NDI. After the identification of *AQP2* mutations as a cause for autosomal recessive NDI and in some cases for autosomal dominant NDI, a satisfying explanation for the complete manifestation of the disease in some females had been found. However, several families have been reported in which symptomatic females do not have an AQP2 defect but are heterozygous for an AVPR2 defect [27, 97]. In some of these women, maximal urinary osmolality after DDAVP administration does not exceed 200 mosmol/L. Of interest, in some of the reported families, asymptomatic female family members shared the same *AVPR2* mutation with the manifesting females. The most likely explanation for the existence of different phenotypes in carriers of an *AVPR2* mutation, varying from no symptoms to complete manifestation of the disorder, is skewed X-inactivation [77]. This

hypothesis was underlined by studies investigating the X-inactivation patterns in peripheral blood leukocytes of female carriers via the detection of a methylated trinucleotide repeat in the human androgen receptor gene [106]. In asymptomatic females, random X-inactivation was found, while in most female carriers who showed clinical NDI symptoms, skewed X-inactivation patterns occurring preferentially to normal X alleles were recognized. In a few females with overt clinical NDI, however, random X-inactivation was identified [82].

In conclusion, clinical NDI phenotypes may correlate with the X-inactivation patterns in females with heterozygote *AVPR2* mutations. In some female carriers, however, the clinical phenotype cannot be predicted by evaluation of X-inactivation patterns in peripheral blood cells, probably due to the fact that X-inactivation ratios within an individual may vary between different tissues.

Nephrogenic Diabetes Insipidus: Treatment

Conventional Treatment

Symptomatic treatment of NDI is aimed to achieve normovolemia by replacing urinary water losses and reducing urinary volume. Adequate supply of fluid to prevent dehydration is the most important component of the therapy. For reducing urine output, a low-solute diet is applied to diminish the renal osmole load and decrease obligatory water excretion. Initially, a diet low in sodium (1 mmol/kg per day) as well as protein (2 g/kg per day) was recommended. However, severe limitations of dietary protein may introduce serious nutritional deficiencies. Therefore, it is preferable to prescribe dietary restriction of sodium only.

Diuretics such as hydrochlorothiazide (2–4 mg/kg per 24 hours) were the first class of drugs shown to be effective in lowering the urine volume in NDI. When combined with a reduction of salt intake, hydrochlorothiazide reduces urine volume by 20%–50% of baseline values. However, thiazide-induced hypokalemia may cause further impairment or urine concentrating ability in patients with NDI. Another possible risk associated with hypokalemia is cardiac arrhythmia. Simultaneous administration of potassium salt is therefore advised in patients with hypokalemia. Very low daily sodium intake in combination with thiazide diuretics should be avoided to prevent the development of hyponatremia.

There is ample evidence that the combined administration of hydrochlorothiazide with either a prostaglandin-synthesis inhibitor such as indomethacin (2 mg/kg per 24 hours) or the potassium-sparing diuretic amiloride is much more effective in reducing urine volume than the thiazide diuretics alone [44, 60]. Prolonged use of prostaglandin-synthesis inhibitors, however, might be complicated by gastrointestinal and hematopoietic side effects. Gastrointestinal complaints and complications include anorexia, nausea, vomiting, abdominal pain, ulceration, perforation, and hemorrhage. Hematopoietic reactions include neutropenia, thrombocytopenia, and, rarely, aplastic anemia. In addition, renal dysfunction has been described during indomethacin therapy, most often consisting of a slight reduction in GFR.

In patients who are not tolerating indomethacin, selective inhibitors of cyclooxygenase-2 (COX-2) might be helpful [91]. Caution in using indomethacin and selective COX-2 inhibitors in NDI is warranted as their administration can potentially lead to the acute deterioration of renal function in dehydrated patients.

Amiloride counterbalances the potassium loss from prolonged use of thiazides and thus prevents hypokalemia. Since amiloride appears to have only minor long-term side effects, the combination of hydrochlorothiazide (2–4 mg/kg/24 hours) with amiloride (0.3 mg/kg/24 hours) is the first choice of treatment. Our personal experience of more than 20 years with the amiloride-hydrochlorothiazide combination, however, indicates that amiloride is less well tolerated in young children below the age of 4–6 years because of persistent nausea. Therefore, we advise the temporary use of the combination of indomethacin-hydrochlorothiazide in these young children.

For a long time, the following mechanism for the antidiuretic effect of thiazides in NDI has been proposed: thiazides reduce sodium reabsorption in the distal tubule by inhibition of the NaCl cotransporter (NCC). This subsequently results in increased sodium excretion, extracellular volume contraction, decreased glomerular filtration rate, and increased proximal sodium and water reabsorption. Consequently, less water and sodium reach the collecting tubules, and less water is excreted. This long-standing hypothesis has been challenged by Magaldi, who reported new insights into the possible mechanism of action, based on microperfusion studies in rat inner medullary collecting duct (IMCD) [69]. In these studies, it was shown that in the absence of vasopressin, hydrochlorothiazide, when added to the luminal side, increased osmotic and diffusional water permeabilities, thus decreasing water excretion. When prostaglandins were added, the effect of thiazides decreased. This finding may offer one explanation why indomethacin potentiates the effect of thiazides in NDI [69]. Antidiuretic effect of thiazides is associated with an increase in AQP2 expression in collecting duct cells [54]. Long-term side effects of chronic thiazide administration such as hyperuricemia, alterations in serum lipid spectrum, and glucose intolerance should be monitored.

Although the drugs mentioned above reduce urine excretion, they are unable to achieve urine volumes produced in healthy individuals. Therefore, many young patients with NDI require nasogastric tube or gastrostomy to provide adequate nutrition and fluid supply [20]. Consequently, current research focuses on methods to treat NDI on a more causative level than solely try to fight the symptoms.

Therapeutic Strategies for Treatment of X-Linked NDI

Because in vitro expression studies reveal that the majority of *AVPR2* mutations in X-linked NDI result in normal protein that is retained within the endoplasmic reticulum (ER), agents that restore plasma routing are under investigation as potential treatments. Promising agents are cell-permeable AVPR2 antagonists and agonists that in vitro rescue the intracellular retention of several AVPR2 mutants [6, 45, 101]. An important problem with the antagonists is that once the mutant AVPR2 is rescued to the basolateral membrane, the antagonist needs to be displaced by high concentrations of AVP/DDAVP to induce cAMP signaling. Therefore, low-affinity antagonists are believed to have the highest clinical value. However, their efficiency in rescuing is lower than that of high-affinity ligands, and the high concentrations required to be administered for sufficient activity by low-affinity antagonists might lead to severe complications in patients. The use of non-peptide agonists has somewhat circumvented this problem since they do not need displacement to activate AVPR2. All high-affinity agonists have been shown to induce receptor maturation as well as translocation to the plasma membrane and to elicit a cAMP response [45].

The feasibility of treatment with these so-called pharmacological chaperones has been tested in vivo. In individuals with NDI who have missense *AVPR2* mutations, Bernier et al. showed that treatment with a non-peptide V_{1a} receptor antagonist had beneficial effects on urine volume and osmolality starting a few hours after administration. However, the long-term effect of this drug could not be tested because the clinical development of this V_{1a} receptor antagonist was interrupted during the course of this study as a result of possible interference with the cytochrome P450 metabolic pathway [6]. Tolvaptan, an AVPR2 antagonist used in hyponatremia and polycystic kidney disease, was recently found in in vitro transfected cells, to rescue the function of the M272R mutation, by allowing both proper glycosylation maturation and membrane sorting and response to DDAVP, but has not been tested yet in NDI patients bearing this mutation [95].

Remarkably, certain non-peptide AVPR2 agonists such as OPC51, VA88, and VA89 were shown to be able to intracellularly stimulate AVPR2 and increase cAMP production and AQP2 translocation to the apical membrane [100]. In contrast to pharmacochaperone-assisted folding and rescue of the receptors, the

localization and maturation state of AVPR2 did not change upon activation, indicating that these compounds do not act as molecular chaperones. The mode of action by which receptors trapped intracellularly can still activate their coupled G-protein, and how this stimulates adenylate cyclase is not yet understood. Future in vivo and clinical testing has to confirm whether the pharmacological chaperones or the intracellularly acting non-peptide agonists have the desired positive effects in patients and meet the safety requirements.

In patients with X-linked NDI, bypassing AVPR2 could be an alternative way to treat the disease.

By stimulation of the E-prostanoid receptor EP4, NDI symptoms were greatly reduced in a conditional *avpr2*-deletion mouse model [64]. This was due to raised AQP2 levels, most probably as a consequence of cAMP production caused by EP4 stimulation. A similar effect was seen after stimulation of the EP2 receptor by the agonist butaprost [88]. The EP2 receptor is a more interesting candidate for treatment of NDI than the EP4 receptor since EP2 agonists have already been tested in clinical studies for other diseases and have shown promising results concerning safety issues. However, clinical trials in NDI are necessary to evaluate the effects and safety of EP2 agonists for this disorder.

Another potential therapeutic strategy bypassing AVPR2 could be an activation of the cGMP-signaling pathway. Several groups have shown that nitric oxide donors and atrial natriuretic factor stimulate the insertion of AQP2 in renal epithelial cells in vitro and in vivo via a cGMP-dependent pathway without increasing the expression of *AQP2* [13] and the selective cGMP phosphodiesterase inhibitor sildenafil citrate (Viagra) prevents degradation of cGMP resulting in increased membrane expression in AQP2 in vitro and in vivo [15]. In a small number of NDI patients subjected to clinical trials with sildenafil citrate, no decreases in urine volume or increases in urine osmolality were observed (personal communication in [80]). Alternative AVP-independent strategies are the use of calcitonin, which has a vasopressin-like effect on AQP2 trafficking and urine concentrating ability via cAMP-mediated mechanism [14] and of various statins (simvastatin, fluvastatin) that were reported to increase AQP2 expression and water reabsorption in the kidney via an as yet unknown mechanism [65, 94]. Using a systemic high-throughput chemical screening procedure, Nomura et al. identified AG-490 (an epidermal growth factor receptor (EGFR) inhibitor) as a compound that stimulates AQP2 exocytosis, induces AQP2 membrane accumulation, and stimulates urine concentration in an AVP-independent manner [86]. The EGFR inhibitor erlotinib has been shown to increase AQP2-mediated renal water reabsorption, reduce urine volume, and increase urine osmolality in mice with lithium-induced NDI [17]. The most recent approach to potentially treat X-linked NDI is based on the activation of adenosine monophosphate kinase, an energy-sensing kinase that phosphorylates the Na-K-2CL cotransporter NKCC2 in the thick ascending limb of Henle's loop, by the oral antidiabetic drug metformin. Klein et al. showed that metformin increased osmotic water permeability by increasing AQP2 phosphorylation and accumulation in the apical membrane [56]. Efe et al. demonstrated that metformin improved urine concentrating ability in *avpr2* KO mice [25]. Despite these promising results in in vitro studies and in animal models, none of these compounds have yet been translated into therapy of NDI.

Therapeutic Strategies for Treatment of Autosomal NDI

Similar to AVPR2 mutants, the majority of AQP2 mutants causing autosomal recessive NDI are missense mutations that lead to aberrant folding of AQP2 in the ER. Hence, finding substances that are able to re-establish natural AQP2 folding holds comparable promises for treatment of recessive NDI as it has been shown for the X-linked form. In CHO and MDCK cells, glycerol has proven the applicability of chemical

chaperones to AQP2 by restoring ER export in high concentrations [117].

Yang et al. described partial restoration of cellular AQP2 processing upon treatment of conditional AQP2-T126M knock-in mice with an Hsp90 inhibitor, 17-allylamino-demethoxygeldanamycin (17-AAG), eventually resulting in improved urinary concentrating ability [124]. The precise explanation underlying the beneficial effect of this Hsp inhibitor remains to be elucidated. Furthermore, it is not unlikely that Hsp90 inhibition may have severe side effects that outweigh the advantages [115]. Therefore, lengthened studies addressing safety issues of Hsp90 or other chaperone inhibitors have to be conducted in order to elucidate the applicability of these compounds in NDI therapy.

Based on the improvement of AVP-dependent cAMP signaling of collecting duct cells in a hypercalcemia-induced NDI mouse model, Sohara et al. also tested the phosphodiesterase-4 inhibitor rolipram in the knock-in dominant NDI mice [112]. Their data indicated that rolipram is able to increase cAMP levels leading to increased AQP2 phosphorylation and translocation to the apical membrane. Phosphodiesterase-4 is a common protein that also is involved in immunosuppressive and anti-inflammatory pathways, and therefore, its inhibition may have severe side effects. Rolipram has been tested in two male patients with X-linked NDI and did not cause any relief of symptoms [9], but the potential for other PDE inhibitors in the treatment of NDI needs to be examined further. The polyuria described in the patient bearing the autosomal dominant AQP2-E258K mutation [81] is now well controlled by DDAVP, 20 micrograms intranasally bid (D.G. Bichet, personal information).

Therapeutic Strategies for NSIAD

Fluid restriction is the mainstay of treatment for NSIAD [93]. It allows controlling hyponatremia and reducing the risks of neurological complications. This may be challenging, particularly in infants in which excessive fluid restriction may limit caloric intake. Oral urea in these patients may improve urine output by inducing osmotic diuresis [28, 41].

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