Pathophysiology, diagnosis and management of nephrogenic diabetes insipidus

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Key points

- Nephrogenic diabetes insipidus (NDI) is caused by inability of the kidneys to concentrate urine by reabsorption of water in the collecting duct

- NDI can be inherited (X-linked or autosomal) or acquired, most commonly as a result of lithium treatment

- Management of primary forms of NDI focuses on dietary modification to reduce osmotic load and pharmacological treatment with prostaglandin synthesis inhibitors and thiazide diuretics

- With appropriate treatment, complications of NDI, such as failure to thrive and mental retardation resulting from repeated hypernatraemic dehydration, can be avoided

- New treatment approaches for congenital NDI have been tested in animal models, but efficacy in patients has not yet been confirmed

Abstract

Healthy kidneys maintain fluid and electrolyte homoeostasis by adjusting urine volume and composition according to physiological needs. The final urine composition is determined in the last tubular segment: the collecting duct. Water permeability in the collecting duct is regulated by arginine vasopressin (AVP). Secretion of AVP from the neurohypophysis is regulated by a complex signalling network that involves osmosensors, barosensors and volume sensors. AVP facilitates aquaporin (AQP)-mediated water reabsorption via activation of the vasopressin V2 receptor (AVPR2) in the collecting duct, thus enabling concentration of urine. In nephrogenic diabetes insipidus (NDI), inability of the kidneys to respond to AVP results in functional AQP deficiency. Consequently, affected patients have constant diuresis, resulting in large volumes of dilute urine. Primary forms of NDI result from mutations in the genes that encode the key proteins AVPR2 and AQP2, whereas secondary forms are associated with biochemical abnormalities, obstructive uropathy or the use of certain medications, particularly lithium. Treatment of the disease is informed by identification of the underlying cause. Here we review the clinical and diagnostic aspects of NDI, the various aetiologies, current treatment options and potential future developments.

[H1] Introduction

Nephrogenic diabetes insipidus (NDI) results from failure of the kidneys to concentrate urine. Patients with this disease typically produce around 12 l of urine per day. Assuming a bladder volume of 500 ml, this volume of urine will necessitate voiding approximately once per hour throughout the day and night.

Moreover urinary fluid losses need to be replenished by constant fluid intake. The disease has a substantial impact on quality of life as sleep is frequently interrupted and patients must plan their daily activities with logistics for frequent voiding and access to drinking water.

Primary and secondary forms of NDI exist. Paediatricians typically see patients with primary inherited forms of the disease, mostly boys presenting in the first year of life with failure-to-thrive and vomiting. By contrast, in adults acquired NDI is much more common than primary NDI. Medical treatment can ameliorate the symptoms of NDI and as understanding of the underlying pathophysiology improves, new potential treatment options arise. In this Review we consider the clinical, diagnostic and therapeutic aspects of NDI in the context of the physiology of renal water handling to enable a molecular understanding of the disease. We also discuss recent insights into the regulation of water permeability and their consequences with respect to diagnosis and new treatment options.

[H1] Physiology of urine concentration

Healthy adult kidneys produce approximately 180 l of primary glomerular filtrate per day.[1] The vast majority of this filtrate is reabsorbed in the proximal tubule, which is freely permeable to water owing to the constitutive expression of aquaporin (AQP)-1 water channels.[2] As solutes are reabsorbed in the proximal tubule, water follows passively along the osmotic gradient. The remaining urine is thus still isotonic when it enters the loop of Henle, the key segment for counter-current concentration (Figure 1). Urine concentration begins in the thin descending limb (TDL). Mechanisms of concentration include AQP1-mediated

exit of water into the medullary interstitium.[3] However, data from several rodent models show that Aqp1 expression is mainly restricted to the first 60% of the TDL rather than to the deeper papillary parts in which the steepest part of the osmotic gradient is generated.[4] Concentration of the tubular fluid in these innermost parts of the TDL might occur via passive sodium influx,[5] but no substantial sodium permeability has been observed.[6] Thus, the exact mechanism of urinary concentration in the innermost part of the medulla remains to be elucidated.

Urine subsequently enters the thick ascending limb (TAL, also known as the diluting segment), which is impermeable to water but actively removes sodium chloride via the co-transporter solute carrier family 12 member 1 (SLC12A1, also known as NKCC2), thereby diluting the urine.[7] The accumulation of solutes in the interstitium generates the driving force for the removal of water from the TDL (in long-looped nephrons) and the entry of sodium chloride (in short-looped nephrons), completing the counter-current multiplier.

Further removal of sodium chloride occurs in the distal convoluted tubule via SLC12A3 (also known as NCC). At entry into the arginine vasopressin (AVP)-sensitive connecting tubules and collecting ducts, urine osmolality is typically around 50–100 mOsm/kg. The final osmolality of the urine is solely dependent on the availability of water channels. If these channels are present, water exits the tubule following the interstitial concentration gradient and the urine is concentrated. If no water channels are present, dilute urine will be excreted.

[H1] Vasopressin-regulated water permeability

Vasopressin secreted by hypothalamic neurons in response to changes in soma volume (Figure 2) is the key regulator of the water permeability of the collecting ducts. The key cell type that mediates this regulated water permeability is the principal cell, which constitutively expresses AQP3 and AQP4 on the basolateral side.[2] The water permeability of the apical membranes of the principal cell is determined by the availability of AQP2 water channels. In the absence of AVP signalling, these channels are located in intracellular vesicles and no apical water permeability exists.[8] Binding of AVP to the vasopressin V2 receptor (AVPR2) initiates a signalling cascade that ultimately leads to the insertion of AQP2 channels into the apical membrane(Figure 3). AVPR2 is a G-protein-coupled receptor and binding of AVP leads to the release of stimulatory G-protein, which in turn activates the neighbouring adenylyl cyclase.[9] The resulting increase in levels of cAMP activates protein kinase A (PKA).

The cytoplasmic carboxy-terminal tail of AQP2 has five canonical PKA-phosphorylation sites: Thr244, Ser256, Ser261, Ser264 and Thr269 (Ser269 in mice).[10] Upon administration of the vasopressin analogue 1-desamino-8-D-arginine vasopressin (DDAVP), phosphorylation of AQP2 at Ser256, Ser264 and Ser269 is abundantly increased, whereas phosphorylation at Ser261 is decreased;[10] specific phosphorylation of these residues might serve distinct physiological roles. In a mammalian cell line (LLC-PK1) transiently transfected with AQP2, phosphorylation at Ser256 was essential for trafficking of the water channel to the plasma membrane,[11] whereas in Xenopus oocytes, phosphorylation of at least three subunits of the AQP2 tetramer at Ser256 was

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required for redistribution from storage vesicles to the apical plasma membrane.[12] The critical role of Ser256 in AQP2 function was confirmed by the identification of mutations that impair phosphorylation at this site in patients with NDI.[13, 14] *In vitro* studies demonstrated the failure of such mutants to reach the apical membrane.[13, 14]

AVP also induces phosphorylation of AQP2 at Ser269, but this action is delayed and requires prior phosphorylation at Ser265.[15] Phosphorylation at Ser269 might increase retention of AQP2 at the apical plasma membrane and interactions of AQP2 with proteins participating in the endocytic machinery.[16] By contrast, AQP2 phosphorylated at Ser261 is found mainly in intracellular vesicles; phosphorylation at this site is thought to stabilize ubiquitination of AQP2[17] at Lys270.[18] Ubiquitination might, therefore, mediate the endocytosis of AQP2 from the plasma membrane in the absence of AVP stimulation. This mechanism leads to a limited tenure of AQP2 in the apical membrane of principal cells; endocytosis of this water channel enables the cells to return to their water-impermeable state. The role of AQP2 phosphorylation at Thr244 is unknown.

The actions of cAMP might not only be mediated via the classical PKA/cAMP-dependent protein kinase pathway, but also by guanine nucleotide-exchange factors such as exchange protein directly activated by cAMP (Epac).[19] Like PKA, Epac contains an evolutionally conserved cAMP-binding domain that senses intracellular cAMP levels and acts as a molecular switch to control diverse biological functions.[20] Epac might be involved in the long-term regulation of AQP2 abundance, whereas PKA has independent short-term effects.[21]

In addition to an acute role in AQP2 localization, AVP-mediated increases in cAMP promote AQP2 expression via PKA-mediated phosphorylation of the cAMP responsive element binding protein, which subsequently stimulates transcription from the AQP2 promoter.[22-24] The change in localization of AQP2 occurs within minutes, whereas the increase in transcription requires hours to take effect.[25,26]

[H1] Congenital NDI

In 1992 the *AVPR2* gene that encodes the AVP2 receptor was cloned and mutations in this gene were identified in patients with X-linked NDI (Figure 4).[27-30] The *AQP2* gene was cloned in 1993[31, 32] and in 1994 mutations in *AQP2* were found to underlie autosomal recessive NDI.[33] The discovery of these two key genes—*AVPR2* and *AQP2*—has enabled genetic testing of affected patients. Mutations in either of these genes are identified in almost all patients with a clear clinical phenotype of congenital NDI.[34, 35]

[H2] *AVPR2* mutations

Approximately 90% of patients with inherited NDI have a mutation in *AVPR2*.[36, 37] As this gene is located on the X-chromosome, *AVPR2* mutations have an X-linked pattern of inheritance; consequently the majority of patients with NDI are male. Occasionally, female patients with NDI due to *AVPR2* mutations have been described.[34, 35, 38-41] In one study, 16 of 64 (25%) female carriers of *AVPR2* mutations showed polyuric symptoms and four of these patients (6%) were diagnosed with complete NDI rather than the milder partial NDI phenotype described below.[34] Skewed X-inactivation is thought to cause symptoms in some female carriers of *AVPR2* mutations, although these symptoms do not necessarily

correlate with the X-inactivation patterns observed in leukocytes.[42] Presumably, X-inactivation patterns can differ between tissues, so the pattern in leukocytes might not reflect that in the kidney. In our experience at Great Ormond Street Hospital, London, UK, two female relatives in 20 families with *AVPR2* mutations have been diagnosed with complete NDI (unpublished data). One of these women was the index case in her family, but not all female carriers have been systematically investigated. Partial NDI symptoms were observed in two other female carriers in one family.[43]

A few *AVPR2* mutations have been identified recurrently owing to a founder effect. For example, the Hopewell mutation (Trp71X)—derived from an Ulster Scot immigrant who arrived in Halifax, Canada on the ship Hopewell in 1761—has been identified in >40 North American patients.[44, 45] Another recurrent mutation is the Cannon mutation (Leu312X), which is named after a large pedigree with ≥38 affected patients in Utah.[45] Most patients, however, have individual mutations; to date >250 different *AVPR2* mutation have been described in >300 families ([46] and our unpublished data). Interestingly, most of these mutations are missense and, when assessed *in vitro*, seem to encode functional but misfolded receptors, which are retained and degraded in the endoplasmic reticulum (ER).[47]

[H3] *AQP2* mutations

Approximately 10% of cases of congenital NDI are due to loss-of-function mutations in the *AQP2* gene located on chromosome 12.[45,34, 48] These mutations are typically inherited in an autosomal recessive fashion, although a few mutations have been described to cause autosomal dominant disease.[13, 49-51]

AQP2 is a homotetramer; four subunits assemble to form the water channel. Dominant mutations typically affect amino acids in the C-terminus, leading to aberrant trafficking; mutant AQP2 subunits are able to oligomerize with wild-type protein to form tetramers, but exert a dominant-negative effect on trafficking of these assembled tetramers.[52] Interestingly, specific mutations direct AQP2 trafficking to distinct cellular compartments, such as the Golgi complex,[13] late endosomes and lysosomes[49] or the basolateral membrane.[51, 53] These findings have provided insights into the intracellular trafficking motifs of AQP2.

[H2] Epidemiology

Few population-based data on the incidence of congenital NDI exist, but estimates can be made. In 2000 we assumed that we had complete ascertainment of all the patients with *AVPR2* mutations who were born in the province of Quebec, Canada between 1988 and 1997.[35] Indeed, in the subsequent 18 years, no patients with *AVPR2* mutations born in the province during this period have emerged. Based on these data, we estimated the incidence of X-linked NDI in the general population of Quebec to be four in 454,629 or approximately 8.8 per million (SD 4.4 per million) male live births. This estimate might be representative of the general world population. However, owing to chance population genetic events, such as a founder effect, the incidence of NDI is elevated in certain regions. For example, in the Canadian maritime provinces of Nova Scotia and New Brunswick, we estimate the incidence of the Hopewell mutation to be 58 per million male live births.[35] A higher incidence of NDI is also found in Utah due to the prevalence of the Cannon mutation.[44]

Based on the families referred to us for genetic testing, we estimate that the incidence of NDI secondary to *AQP2* mutations is five to ten times lower than that of NDI secondary to *AVPR2* mutations. The incidence of this type of congenital NDI might, however, be increased in populations with a high degree of consanguinity; we have found an over-representation of the Val71Met *AQP2* allele in patients of Pakistani descent.[54]

[H1] Secondary inherited forms of NDI

In addition to primary forms of congenital NDI a few cases of secondary inherited NDI have been reported.[55, 56] 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.[55, 57] Most of the primary diseases associated with secondary NDI, such as Bartter syndrome and apparent mineralocorticoid excess, are associated with hypokalaemia and hypercalciuria. These associations might provide clues to the aetiology of NDI, as both hypokalaemia and hypercalciuria are associated with decreased AQP2 expression.[58, 59] Hypercalciuria has been suggested to cause a urinary concentration defect mediated by the calcium-sensing receptor, which is expressed on the luminal side of collecting duct cells and is thought to affect AQP2 trafficking by altering cAMP levels.[60, 61]

[H1] Acquired NDI

In adult nephrology practice the majority of patients who present with NDI have acquired this disease, with lithium treatment being the predominant cause.[62-64] Reports of NDI among patients treated with lithium vary widely but the incidence of this adverse effect has been reported to be as high as 85%.[63, 65] As

0.25–0.77% of the general population aged >65 years are prescribed lithium, it is not surprising that lithium-induced NDI is fairly commonly.[66, 67] Indications for lithium treatment include bipolar disorder, schizoaffective disorder, depression, alcoholism and cluster headaches.[64] Cessation of lithium therapy can resolve the symptoms of NDI, but this approach is not an option in most cases because the beneficial effects of the drug on the psychiatric disorder outweighs the negative impact of the polyuric complications on quality of life.

In rats, chronic lithium administration (>4 weeks) led to epithelial remodelling in the collecting duct and a dramatic reduction in the number of principal cells.[68] Moreover, in the mouse collecting duct cell line mpkCCD, lithium exposure decreased Aqp2 abundance independent of cAMP levels.[69] Consistent with these data, decreased urinary excretion of AQP2 has been observed in patients with lithium-induced NDI.[70] The exact mechanism of lithium toxicity on the principal cell remains to be elucidated, but robust data suggest that lithium exerts its effect after entering the cell through epithelial sodium channels (ENaCs), which have a high permeability for lithium.[10] Consequently, treatment with ENaC blockers, such as amiloride, can increase urine osmolality and ameliorate polyuria in lithium-induced NDI.[71-73]

Other acquired causes of typically transient NDI include hypercalcaemia, hypercalciuria and obstructive uropathy. In patients with obstructive uropathy, NDI is thought to result from direct suppression of AQP2 expression, which might be mediated by hydrostatic pressure. Animal studies have shown decreased Aqp2 expression in the setting of bilateral ureteric obstruction,[74]

whereas in unilateral obstruction a marked decrease in Aqp2 is seen only in the obstructed kidney.[75] This down-regulation of Aqp2 persists up to 30 days after release of obstruction, perhaps explaining the diuresis often observed after release of severe obstruction in patients.[9]

[H1] Initial presentation

Most patients with congenital NDI present with failure to thrive during the first few months of life, whereas those with acquired NDI typically present later in life with polyuria and or polydipsia. In patients with congenital NDI the antenatal history is typically normal with no polyhydramnios, which helps to distinguish NDI from other polyuric disorders, such as Bartter syndrome. Parents often report that the baby sucks vigorously but vomits shortly afterwards. This vomiting is thought to occur because intake of large fluid volumes causes gastro-oesophageal reflux.

NDI is a rare disease so does not feature prominently on the diagnostic radar of frontline medical staff. Families seeking medical attention for their yet undiagnosed baby might, therefore, be sent home without further investigations because the attending professionals are falsely reassured by the large urine output. Laboratory investigations in such instances would show the typical picture of hypernatraemia with inappropriately dilute urine, the biochemical "fingerprint" of diabetes insipidus. If blood tests are obtained and hypernatraemia is noted without consideration of a urinary concentration defect, inappropriate treatment with 0.9% saline might be instigated because of concerns regarding too rapid correction of the hypernatraemia. Unfortunately,

given the imbalance between the sodium concentration of the administered fluid (154 mmol/l) and the urine (usually <10 mmol/l), this approach will result in even higher plasma sodium concentrations.

[H1] Diagnosis

In patients who present with hypernatraemic dehydration and dilute urine, a DDAVP test can help to distinguish between cranial diabetes insipidus (which is normally the result of disease of the hypothalamus or surrounding tissues) and NDI. In patients with suspected diabetes insipidus who present with polyuria and normal plasma biochemistries, a water deprivation test might first be performed to challenge urinary concentration.

[H2] DDAVP test

As DDAVP has high specificity for AVPR2 it can be used to assess the renal response whilst avoiding AVPR1-mediated vasoconstriction. DDAVP is available in formulations suitable for oral, intranasal, subcutaneous or intravenous (IV) administration. After DDAVP administration a urine osmolality >800 mOsm/kg is usually considered normal and excludes a diagnosis of NDI, whereas a urine osmolality below plasma osmolality indicates AQP2 deficiency and is consistent with the diagnosis. Care needs to be taken in the interpretation of intermediate results, especially in infants, as full urinary concentrating ability develops during the first year of life.[76] Other causes for submaximal values include interstitial renal disease, use of loop diuretics, partial NDI, washout of the interstitial medullary concentration gradient or technical failure of the test, for example as a result of incomplete DDAVP absorption.

Various protocols for the DDAVP test exist, but we prefer to use IV administration to enable the most reliable absorption of the polypeptide.[77] The DDAVP dose (0.3 mcg/kg) used in this test is the same as that used to boost factor VIII concentration in patients with mild haemophilia or von Willebrand disease.[78, 79] The key risk of the test is the development of hyponatraemia in patients who respond to DDAVP and keep drinking. The physiological suppression of thirst from reduced plasma osmolality prevents this complication in the vast majority of responders, but patients with habitual polydipsia and babies who keep receiving feeds from their carer are at risk. Strict observation of fluid balance is required to prevent this potentially serious complication and patients should only be allowed to receive a fluid volume equivalent to their ongoing losses.

A key advantage of IV administration is the short half-life of DDAVP in blood, which necessitates an observation time of only 2 h, thus minimizing the burden of the test. Other advantages include a low risk of test failure owing to incomplete absorption and the ability to distinguish between X-linked and autosomal-recessive forms of NDI, as AVPR2 mediates haemodynamic and coagulation factor changes in response to high-dose DDAVP.[77] This response is abrogated in patients with defective AVPR2 function, yet retained in those with mutations in *AQP2*. The low plasma levels of DDAVP achieved with other forms of administration make detection of these subtle changes virtually impossible.

[H1] Spectrum of clinical disease

[H2] Mental impairment

As with most inherited diseases a wide spectrum of severity of clinical disease is observed in NDI. The most severe consequences are seen in patients who have not received adequate treatment and have experienced repeated episodes of hypernatraemic dehydration, leading to brain damage and impaired mental development.[39, 80, 81] In some such patients, intracranial calcifications can be observed. These calcifications are thought to result from endothelial cell necrosis during severe dehydration and thus might represent a radiological correlate to brain damage from recurrent hyperosmolar stress associated with hypernatraemic dehydration.[82]

Fortunately, cases of severe mental impairment in patients with NDI are now virtually non-existent in countries where modern health care is available. Nevertheless, impaired school performance and behavioural abnormalities are often reported among patients with NDI. In a Dutch study, 8 of 17 participants were diagnosed with attention deficit hyperactivity disorder.[83] This diagnosis might be partly related to the constant craving for water and need to void. Some data, however, suggest that behavioural abnormalities might be an intrinsic aspect of the disease. Vasopressin (and oxytocin) act as neurotransmitters that modulate the autonomic fear response[84] and vasopressin has been described to enhance aggressiveness, anxiety and stress levels in an animal model.[85] Raised vasopressin levels might also potentially affect long-term behaviour in patients with NDI.

[H2] Flow uropathy

Dilatation of the urinary tract is a recurrently noted complication of NDI and patients with poor voiding habits are especially at risk.[39, 86, 87] In those with

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hydronephrosis, anatomic causes of obstruction should also be considered. Such obstructions are potentially remediable and even minor impediments to urine flow can cause severe dilatation in this polyuric disorder.[88] Obstructive nephropathy can be seen in cranial diabetes insipidus and NDI, and if untreated can lead to obstructive end-stage renal disease.

Partial NDI

Some patients with congenital NDI have a mild phenotype; they present after infancy with normal development, often for the assessment of polyuria or enuresis, and typically show intermediate urine osmolality after DDAVP administration (greater than plasma osmolality but <800 mosm/kg). Patients with partial NDI typically carry mutations that result in partial function of either AVPR2 [43, 89] or AQP2.[90, 91] The first mutation to be associated with partial NDI was the Asp85Asn mutation in *AVPR2*.[89] Interestingly, another missense mutation, Val88Met, has been associated with partial NDI in some patients and complete NDI in others.[43, 92, 93] The underlying disease mechanism in patients with these missense mutations is ER retention of the mutant protein, but degree to which the mutant protein can escape from this retention and traffic to the cell membrane seems to differ between individual patients.

Treatment

Current treatment approaches for congenital NDI focus on amelioration of the symptoms rather than curing the disease. If possible, treatment of acquired NDI should target the underlying cause, such as relief of urinary obstruction or amiloride therapy in lithium-associated NDI.[73] If such approaches are not possible, treatment of congenital NDI is comparable to that of primary NDI.

Management of hypernatraemic dehydration

Most emergency protocols for any patient with hypernatraemic dehydration

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suggest initial treatment with 0.9% saline, owing to concerns about lowering plasma sodium levels too rapidly. The situation differs, however, in patients with NDI because of the ongoing losses of essentially pure water with the urine; infusion of 0.9% saline will result in excess sodium chloride administration and thus worsen the hypernatraemia. In these patients, isotonic fluids should be reserved only for acute intravascular volume expansion in hypovolaemic shock, which is an exceedingly rare complication, as extracellular fluid volume is usually sufficiently preserved in hypernatraemia. Thus, patients with NDI should be treated with hypotonic fluids, either enterally (using water or milk) or if necessary intravenously (using 5% dextrose in water). Hypotonic fluids must never be administered as an intravenous bolus; instead, the infusion rate should only slightly exceed the urine output. The aim is to provide just enough water to safely normalize plasma sodium concentration at a rate of <0.5 mmol/l per h (<10–12 mmol/l per day). The main risk of a rapid decrease in plasma sodium is cerebral oedema and potentially death.[94-97] Consequently, careful fluid balance and frequent monitoring of clinical state and biochemistries is key to safe treatment and requires a clinical environment with sufficient experience in the treatment of complicated electrolyte disorders. Fluids should be taken orally as soon as feasible, to enable the thirst physiology to properly regulate fluid intake. As dextrose in water provides no osmotic load (Box 1), urine output can decrease substantially, highlighting the importance of ongoing monitoring and fluid balance to avoid rapid swings in plasma sodium concentrations.

[H2] Diet

Dieticians have a key role in the management of patients with NDI, especially in the first year of life when intake of fluid and calories is coupled. Minimizing the

osmotic load (Box 1), whilst providing the recommended caloric and protein intake to enable normal growth and development is a cornerstone of paediatric NDI management.

[H2] Thiazide diuretics

The use of diuretics in polyuric disorders seems counterintuitive. The therapeutic potential of these agents in NDI was discovered more or less serendipitously, but reported in patients as early as 1905.[98] This observation was confirmed in the 1950s when administration of hydrochlorothiazide was shown to reduce urine output in experimental animals with diabetes insipidus by as much as 50%.[99] An early study in patients with NDI showed a small reduction in urine volume with concomitant increases in urine osmolality and sodium shortly after commencing hydrochlorothiazide treatment.[100] After 3 days of treatment, sodium excretion had decreased to less than baseline, whereas urine output had decreased to ~50% of baseline. These changes were associated with reductions in plasma volume and body weight. The researchers astutely concluded that the initial increase in urine osmolality was related to inhibition of urinary dilution, mediated by decreased salt reabsorption through the thiazide-sensitive co-transporter SLC12A3 in the distal tubule, and that the loss of sodium led to a reduction in plasma volume with subsequent enhanced proximal reabsorption of the glomerular filtrate, so that less water was presented to the collecting duct and lost in the urine.

A SLC12A3-independent mechanism of thiazide-mediated reductions in urine output was proposed based on studies in *Slc12a3* knock-out mice with lithium-induced NDI.[101] A marked reduction in urine volume with unchanged urine

osmolality was observed in these mice after hydrochlorothiazide administration. The researchers speculated that this reduction might be mediated by inhibition of carbonic anhydrase in the proximal tubule, resulting in reduced proximal sodium uptake and consequently—via tubuloglomerular feedback—to decreased glomerular filtration. They also found that in mpkCCD cells treated with lithium, hydrochlorothiazide administration led to an increase in Aqp2 abundance at the apical membrane.[101] However, the finding that lithium-treated and control *SLC12A3* knock-out mice had similar urine osmolality after hydrochlorothiazide administration suggests that this effect is negligible *in vivo*.

The hypothesis of impaired proximal sodium reabsorption as a mechanism for reduced urine volume in NDI[101] is surprising and in direct contrast to the earlier hypothesis that enhanced proximal sodium uptake leads to enhanced proximal water reabsorption.[100] To what degree these apparently opposing mechanisms occur in patients remains to be determined.

[H2] **Prostaglandin synthesis inhibitors**

An effect of prostaglandins on epithelial water permeability was noted soon after their discovery.[102] With the advent of inhibitors of prostaglandin synthesis, such as indomethacin, experiments showed a synergistic effect of these new drugs with AVP.[103] An early study in an animal model of diabetes insipidus demonstrated that indomethacin reduced water diuresis independent of AVP.[104] A similar effect of prostaglandin synthesis inhibitors was noted in patients with NDI.[105-108]

Prostaglandin synthesis inhibitors have become essential components in the treatment of NDI, particularly in the first years of life when management is the most complicated. The effect of these drugs can be quite marked when first initiated. Indeed, hyponatraemic seizures associated with rapid lowering of plasma sodium levels as a result of commencement of indomethacin and hydrochlorothiazide have been reported.[109] In our experience, urine osmolality is unchanged after indomethacin treatment, suggesting that the drug acts mainly by enhancing the proximal reabsorption of salt and water.[110]

[H1] Future outlook

[H2] Gene discovery

Since the discovery of the two known NDI disease genes, *AVPR2* and *AQP2*, in the 1990s (Figure 4) no additional causative genes have been reported. Mutations in AVPR2 or AQP2 are not, however, found in all patients. Between 2003 and 2013, the Montreal laboratory performed genetic testing for NDI in 208 families and identified causative mutations in *AQP2* in 18 families and in *AVPR2* in 119 families (D. Bichet unpublished data). In some of the samples, mutations in genes causing renal disorders with secondary forms of NDI, such as Bartter syndrome were found, whereas in others, the phenotype was not detailed enough to clearly establish a diagnosis of NDI.[56] In a Japanese study no mutations were identified in 7 of 62 (11%) families with clinical NDI,[34] suggesting that genes other than *AQP2* and *AVPR2* might be involved. However the phenotypes of these patients were poorly described and not further investigated by the referring physicians.

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In our clinical experience very few patients with a convincing NDI phenotype have no identified mutation. In our Great Ormond Street cohort, we have identified three such patients from two families (unpublished data). The results of our linkage analyses in these families were, however, consistent with the presence of mutations in *AVPR2* or *AQP*2. Thus, if additional NDI disease genes are identified, they are likely to be causative only in a small subset of patients.

[H2] Novel treatments

The promise of molecular medicine is that through improved understanding of specific molecular defects in individual patients, we can develop targeted, rational treatments.[111] Unfortunately, progress has been slow at best. Although several novel treatment approaches for patients with congenital NDI have been suggested, little clinical data are available. These novel approaches focus on restoring AVP signalling upstream of AQP2 so are not applicable to patients with *AQP2* mutations.

[H3] *AVPR2 antagonists*

A new treatment strategy for NDI focuses on the fact that most patients carry missense mutations in *AVPR2* that result in misfolding of AVPR2 and its retention in the ER.[47] If proper folding is induced using a molecular chaperone, the mutant receptor can escape the ER, traffic to the cell membrane and provide normal AVP signalling.

Cell-permeable AVPR2 antagonists are molecular chaperones that fit into the AVP binding pocket of AVPR2 and induce proper folding of the receptor. In patients with suitable mutations, these antagonists might provide a new treatment approach for NDI. The problem with this strategy is that the tighter

the AVPR2 antagonists fit into the binding pocket, the better they promote surface expression of the receptor, but the less likely they are to diffuse off the receptor and enable normal AVP signalling. Conversely, compounds with low affinity for AVPR2 are less efficient at promoting surface expression, but are more likely to diffuse off the receptors that traffic to the cell surface. Antagonists with intermediate affinity have the best overall efficacy, but one such agent, the orally active, nonpeptide AVPR1A antagonist SR49059, only moderately reduced urine volume in five patients with X-linked NDI.[112] SR49059 is no longer provided by the pharmaceutical company because of the possibility of idiosyncratic increases in liver enzymes (these increases were not observed in patients with NDI who received the drug) and no further clinical data on this treatment approach have been published.

[H3] AVPR2 agonists

Cell-permeable agonists that stimulate AVPR2 independent of AVP are another potential treatment approach for patients with NDI. As these agents do not block AVPR2 signalling they could potentially be a more effective treatment approach than AVPR2 antagonists.

[H3] Vasopressin analogues

A mutation-specific treatment strategy has also been suggested for patients with the Asn321Lys mutation in *AVPR2*.[113] The mutant receptor seems to have normal cell surface expression *in vitro*, but severely decreased affinity for vasopressin and DDAVP. AVP signalling via this receptor could reportedly be rescued using the vasopressin analogue Val[4]-desmopressin. Clinical data on the use of this compound in patients with NDI bearing this mutation have not yet been reported.

[H3] Prostaglandin receptor agonists

Another novel treatment approach for patients with *AVPR*2 mutations focuses on enhancing cAMP production in the principal cells independent of AVPR2, for example via stimulation of prostaglandin E2 receptors coupled to adenylyl cyclase (Figure 3). Such a strategy would not benefit patients with the autosomal form of NDI, as *AQP2* mutations result in a defect downstream of cAMP production.

In a mouse model of X-linked diabetes insipidus with conditional deletion of the *Avpr2* gene, administration of a prostaglandin E2 receptor EP4 subtype (EP4 receptor) agonist resulted in an increase in urine concentration.[114] The peak increase in urine concentration (from 150 mOsm/kg to a maximum of 500 mOsm/kg) was short lived (3 h), possibly due to down regulation of EP4 receptors in response to the high osmolality, but with mini-pump constant infusions a sustained increase in urine osmolality from 200 mOsmol/kg to 300 mOsmol/kg was observed.

Doubling of urine osmolality in patients with NDI would halve urine output (to ~6 l daily in adults) so would constitute successful treatment. In a rat model of NDI secondary to the administration of the AVPR2 antagonist, butaprost, an EP2 receptor agonist approximately doubled urine osmolality.[115] To date, no clinical data on the use of prostaglandin receptor agonists in patients with NDI have been reported. Moreover, given the proven efficacy of prostaglandin synthesis inhibitors in NDI treatment, doubts remain as to whether this approach would be a viable treatment option.[116]

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[H3] Secretin receptor agonists

The secretin receptor is a G-protein coupled receptor expressed in principal cells. Stimulation of this receptor can increase cAMP levels.[117] Infusion of secretin and fluvastatin resulted in doubling in urine osmolality in mice with conditional deletion of the *Avpr2* gene.[118] If appropriate scientific and ethical permission was obtained, short-term therapy with a combination of secretin and fluvastatin could potentially be tested in humans with X-linked NDI. Long-term secretin treatment might be limited by adverse effects of the drug.

[H3] cGMP phosphodiesterase inhibitors

Increased levels of cyclic guanosine monophosphate (cGMP) have been shown to enhance trafficking of AQP2 to the luminal membrane and induce a short-lived increase in urine osmolality in Brattleboro rats (a model of cerebral diabetes insipidus with deficient vasopressin production by the hypothalamus).[119] This finding suggests a potential role of cGMP phosphodiesterase inhibitors, such as sildenafil, in the treatment of patients with NDI resulting from *AVPR2* mutations.

[H3] Gene therapy

Genome editing of somatic tissue or embryos is of considerable recent interest to correct defective genes in humans but the ethics, especially of embryo editing is controversial 120 121.. Currently, no such endeavours have reached the clinical arena. We anticipate that the ethical governance, safety and long term effects of such editing therapies will be determined possibly within the next two decades so that patients with hereditary disorders, such as NDI will benefit from them.

[H1] Conclusions

Our understanding of the molecular physiology of urine concentration has advanced tremendously in the past 25 years. The study of patients with inherited

NDI has identified AVPR2 and AQP2 as key proteins required for the regulation of water permeability in the collecting duct. Other tubular disorders, such as Bartter syndrome, can present with a secondary form of NDI, which might cause diagnostic confusion. In adult nephrology practice, the majority of patients with NDI have an acquired form of the disease owing to lithium treatment. Current treatment of congenital NDI focuses on dietary modification, thiazides and prostaglandin synthesis inhibitors. Novel therapies, such as mutation-specific treatment using molecular chaperones, have been investigated in animal models, but few data from clinical studies are currently available. The aim of these treatment approaches is amelioration of the clinical phenotype of NDI. In the future, gene therapy approaches involving kidney-specific delivery of wild-type *AVPR2* or *AQP2* could potentially enable long-term cure of congenital NDI.

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Box 1: Osmotic load

The osmotic load consists of osmotically active substances that need to be excreted in the urine. The key contributors to the osmotic load are proteins, which are metabolized to urea (approximately 4 mmol per g of protein) and salts. A typical western diet contains about 800 m0sm per day. An individual with a urine osmolality of 800 m0sm/kg only needs 1 l of water to excrete this osmotic load, whereas a patient with maximal urine osmolality of 100 m0sm/kg will need at least 8 l of water for excretion. 1 g of table salt providing an osmolar load of ~36 mOsm (18 mOsm Na and 18 mOsm Cl). Consequently, in a patient with a urine osmolality of 100 mOsm/kg, each gram of salt ingested will increase the obligatory urine output by 360 ml. The osmolar load of a diet can be roughly estimated by multiplying the millimolar amounts of sodium and potassium by two (to account for the accompanying anions) and adding the amount of protein in g multiplied by four.[122] As metabolism of lipids and sugars does not produce byproducts that require renal excretion, they do not contribute to the osmotic load. Diet is the key contributor to the osmotic load, but metabolism of own proteins during catabolic states also generate urea.

Figure 1: The renal concentration and dilution mechanism. The loop of Henle forms a counter-current multiplier system that concentrates the urine. Urine is isotonic when it enters the loop of Henle and hypotonic when it exits into the collecting duct. The concentration gradient generated in the loop of Henle is driven by the active reabsorption of NaCl in the thick ascending limb by the transporter solute carrier family 12 member 1. The mechanism of concentration in the thin descending limb is not completely resolved, but likely

involves passive water efflux and/or NaCl influx. Final concentration of urine occurs in the collecting duct and depends on the availability of aquaporin 2 water channels. The approximate osmolalities of the tubular fluid (pink boxes) and interstitial fluid (green boxes) are indicated.

Figure 2: Regulation of vasopressin secretion. a | The concentration of various ions in the blood is continuously monitored by sensory neurons located in brain regions such as the subfornical organ, median preoptic nucleus and organum vasculosum lamina terminus.[123] These sensory neurons track osmotic conditions and signal any perturbations to magnocellular neurosecretory cells in the supraoptic nuclei and paraventricular nuclei in the hypothalamus. The magnocellular neurosecretory cells then go on to produce and release vasopressin into the circulation through the posterior pituitary. The autonomic nervous system also regulates kidney function. b | Cell autonomous osmoreception in vasopressin neurons. Changes in osmolality cause inversely proportional changes in soma volume. Shrinkage activates TRPV1 channels and the ensuing depolarization increases action potential firing rate and vasopressin release from axon terminals in the neurohypophysis. Increased vasopressin levels in blood enhance water reabsorption by the kidney (antidiuresis) to restore extracellular fluid osmolality toward the set point. Hypotonic stimuli inhibit TRPV1. The resulting hyperpolarization and inhibition of firing reduces vasopressin release and promotes diuresis. Abbreviation: TRPV1, transient receptor vanilloid-type 1. Part a modified with permission from Nature Publishing Group © Colwell, C. S. Preventing dehydration during sleep. *Nat. Neurosci*. **13**, 403–404 (2010). Part b modified with permission from John Wiley & Sons Ltd © Prager-Khoutorsky, M. & Bourque, C. W. Mechanical basis of osmosensory transduction in magnocellular neurosecretory neurons of the rat supraoptic nucleus. *J. Neuroendocrinol*. http://dx.doi.org/10.1111/jne.12270.

Figure 3: Water transport in the principal cell. Binding of AVP to the AVPR2 receptor expressed on the basolateral side of the principal cell stimulates the

release of GαS, which in turn activates adenylyl cylase, resulting in increased

cAMP production. PKA is activated by cAMP and phosphorylates several residues

in the C-terminus of AQP2. This phosphorylation enables trafficking of AQP2 to

the apical membrane and the formation of AQP2 water channels. AQP2 is

constantly retrieved from the membrane by endocytosis, so that ongoing water

permeability depends on delivery of AQP2 to the membrane, either by recycling

or generation of new channels. Water diffuses via these channels along the

concentration gradient from the tubular lumen into the principal cell and exits

into the interstitium via the constitutively expressed AQP3 and AQP4 water

channels. In addition to PKA-mediated phosphorylation ofAQP2, cAMP enhances

transcription of AQP2. Some of the changes induced by cAMP are likely mediated

by EPAC. The G-protein coupled receptors EP2, EP4 and the secretin receptor are

also expressed in principal cells. Targeting of these receptors could potentially

be used to stimulate membrane insertion of AQP2 in the absence of functional

AVPR2. Abbreviations: AQP, aquaporin; AVP, arginine vasopressin; AVPR2,

vasopressin V2 receptor; CREB, cAMP responsive element binding protein; EP2,

prostaglandin E2 receptor EP2 subtype; EP4, prostaglandin E2 receptor EP4

subtype; EPAC, exchange protein directly activated by cAMP; G, G protein; PKA,

protein kinase A.

Figure 4 | Timeline of key advances in the understanding of nephrogenic

diabetes insipidus. Abbreviations: AQP2, aquaporin 2; AVP, arginine

vasopressin; AVPR2, vasopressin V2 receptor.