Human osmoregulation

Maintaining the water homeostasis of the body is crucial for its proper functioning. Accordingly, water and electrolytes are balanced by a carefully orchestrated interplay between volume- and osmoregulation that is regulated by multiple hormones. A key hormone in this process is the antidiuretic hormone arginine-vasopressin (AVP), which is released from the pituitary in states of hypernatremia or hypovolaemia (1, 2).

The main target organ of AVP in the process of osmoregulation is the kidney, which is composed of approximately one million nephrons, the functional units in kidney. In these nephrons, ultrafiltration results in the formation of a daily volume of approximately 180 l of pro-urine. Of this large volume, approximately 99% of the water is reabsorbed by the tubular epithelial cells of the nephron, whereas only 1.5–2 l of water is excreted via the urine. Approximately 90% of the tubular water reabsorption occurs in the proximal tubules and descending loop of Henle, where water is reabsorbed iso-osmotically via the water channel aquaporin-1. The remaining 9% can be reabsorbed in the principal cells of the collecting duct, the final part of the nephron, and is regulated by AVP. Binding of AVP to its type 2 receptor on the basolateral (interstitial) side of the principal cells induces a cAMP cascade that increases protein levels of the water channel aquaporin-2 (AQP2) and triggers the protein kinase A-induced translocation of aquaporin-2 to the apical membrane. This makes V2R-specific cell-permeable agonists very promising therapeutics for NDI as a result of misfolded V2R receptors.
different types of NDI are known. First, acquired NDI can originate as a side-effect of drugs, with the most prominent being the anti-bipolar drug lithium. Second and third, autosomal recessive and dominant inheritable NDI is caused by gene mutations in the AQP2 gene (3, 4). Finally, mutations in the AVPR2 gene, which encodes V2R, is the cause of the X-linked inheritable form of NDI (5, 6). Over recent years, much progress has been made with respect to resolving the molecular defect that causes X-linked NDI and, because promising approaches have been undertaken to identify potential cures for this most prominent form of NDI, these will be reviewed here. The other types of NDI have recently been reviewed elsewhere (1, 7, 8).

Clinical phenotype and current treatment

NDI is characterised by the kidney’s inability to concentrate the pro-urine in response to AVP. As a consequence, untreated adult patients may have a daily output of 15–20 l of highly dilute (usually < 100 mOsmol/kg) urine. In newborn infants, NDI may manifest itself as irritability, poor feeding, poor weight gain and dehydration symptoms, such as dryness of the skin, loss of skin turgor, reseeded eyeballs depression of the anterior fontanel and a scaphoid abdomen. When left untreated, young patients show failure to thrive and reduced growth, most likely as a result of feeding problems, ingestion of high amounts of water or repeated episodes of dehydration. In addition, repeated periods of hypernatraemic dehydration may result in permanent brain damage, mental retardation and/or developmental delay (7, 9).

Classically, the diagnosis NDI was made after a dehydration test, followed by administration of the synthetic AVP analogue 1-desamino-8-o-AVP (dDAVP). All patients suffering from NDI will not be able to concentrate their urine to values beyond 200 mOsm, whereas patients suffering from central DI will show a response to dDAVP administration. In patients with acquired NDI, often as a result of chronic lithium treatment for bipolar disorder, the urine concentrating defect is caused by a down-regulation of AQP2 (10) and a loss of the water-transporting principal cells of the collecting duct (11). However, these patients have some residual concentrating capacity, allowing them to concentrate their urine > 200 mOsm when challenged in a dehydration test or upon administration of dDAVP.

Fig. 1. Transcellular water transport in renal collecting principal duct cells and molecular cause of X-linked nephrogenic diabetes insipidus (NDI) (a) Vasopressin binding to its type 2 receptor (V2R) triggers a cAMP cascade that leads to the insertion of aquaporin-2 (AQP2) water channels in the apical membrane. This allows water to pass through this membrane and transcellular water transport to realise concentration of the pro-urine and thereby antidiuresis. (a) In the X-linked form, NDI is often caused by V2R class II mutants trapped in the endoplasmic reticulum as a result of their misfolding, making them unavailable for binding arginine-vasopressin (AVP) at the basolateral plasma membrane. As a result, no transcellular water transport takes place, leading to polyuria. PKA, protein kinase A.

Besides their distinct patterns of inheritance, X-linked and autosomal inheritable NDI can be discriminated by the presence or absence of an extrarenal response. Further to its role in the kidney, the V2R also triggers the release of von Willebrand factor, factor VIII and tissue-type plasminogen factor. Therefore, in patients with mutations in the AQP2 gene, plasma levels of the above factors can be measured when a dDAVP challenge is performed, whereas patients suffering from the X-linked form, in which the V2R is not functional, will fail to show this extrarenal response (7, 9). Currently, if a family history of NDI is known, gene sequencing is the method of choice to determine the presence of a mutation in the AVPR2 or AQP2 genes in a fast, reliable and stress-free manner.

The main and most obvious treatment for NDI patients is the replacement of urinary water losses by a sufficient supply of fluid, combined with a low-solute diet to reduce the renal osmolar load and thus decrease the mandatory water excretion. The diuretic hydrochlorothiazide, combined with a second diuretic amiloride or the cyclooxygenase inhibitor indomethacin, has been proven effective to reduce the urine output of NDI patients by up to 50%. Treatment with these diuretics decreases the distal tubular sodium transport, thereby leading to hypovolaemia. To compensate for this, the renin–angiotensin–aldosterone system will be activated, resulting in increased angiotensin II-mediated sodium reabsorption in the proximal tubules of the nephron. Because water transport in this segment follows sodium uptake iso-osmotically via AQP1, this will lead to an increased reabsorption of water, thereby reducing the amount of water delivered to the distal nephron (12, 13). Treatment with diuretics, however, may affect the delicate sodium balance in NDI patients, and therefore require close monitoring of the serum osmolality when treatment is started.

**Misfolding of V2R causes X-linked nephrogenic diabetes insipidus**

To date, over 200 mutations have been described in the AVPR2 gene [http://www.medicine.mcgill.ca/nephros/avpr2.html], which can be categorised into five classes according to their cellular fate (8). Briefly, class I mutations result in premature stop codons or unstable RNA. Class II comprises the missense mutations that lead to endoplasmic reticulum (ER)-retention of full-length receptors. Class III and IV mutations are defect in Gs protein or AVP binding, respectively, whereas class V mutations result in trafficking defects beyond the early secretory pathway. These classes and examples of V2R mutations that have been categorised this way have been reviewed elsewhere (8, 14).

Of these five classes, class II is the most prevalent one, comprising approximately 45% of all mutations, and the missense mutations of this class often affect only one amino acid in the whole receptor (15). The misfolding resulting from such a class II mutation may therefore not necessarily impair the intrinsic functionality of the receptor. Indeed, rather than inhibiting receptor function, over-expression studies by us (16) and others (17–19) have shown that approximately 60–70% of class II V2R mutants are intrinsically functional as determined by over-expression experiments. Therefore, rather than resulting in a nonfunctional protein (Class III or IV), the cell biological cause by which class II leads to the NDI phenotype is their retention in the ER (16) and/or ER-Golgi intermediate compartment (20), rendering them inaccessible to AVP, which cannot penetrate the plasma membrane (Fig. 1a).

**Rescue of V2R mutants by cell-permeable antagonists**

Identification of the molecular defects underlying NDI, combined with notion that most G protein-coupled receptors (GPCRs) are normally functional at the plasma membrane, incited efforts to rescuing the plasma membrane localisation of class II V2R mutants aiming to identify a novel and superior treatment for NDI. This has been attempted using chemical compounds that affect molecular chaperones (folding proteins), which are therefore referred to as chemical chaperones (21). To reduce cellular toxicity and increase specificity, cell-penetrating peptides (22) and cell-permeable antagonists (see below) have been used. In 2000, the research groups of Dr Bichet and Dr Bouvier achieved a major breakthrough because they showed that ER-retained V2R mutants can be stabilised by incubation with the nonpeptide cell-permeable antagonist SR121463 (17). After the entry of this compound into the cell and its binding to the mutant receptor, this compound stabilised the receptor’s conformation (Fig. 2, step 1). Analogous to ER-resident molecular chaperones, these cell-permeable antagonists are called pharmacological chaperones.

As a result of their stabilisation, the receptor mutants are no longer recognised by the ER quality control mechanism as being misfolded, which allows them to exit the ER (Fig. 2, step 2), achieve mature glycosylation in the Golgi compartment and be inserted into the plasma membrane (Fig. 2, step 3). It was shown that subsequent plasma membrane rescue does not interfere with proper targeting of the newly matured receptors because these were translocated to the basolateral plasma membrane of polarised renal cells, leading to a similar localisation as found for the wild-type receptor (21, 23).

To determine which antagonist is the most ideal compound to restore NDI in patients, other V2R-binding antagonists were characterised to optimise the plasma membrane rescue and subsequent activation of the receptor. Rescue of specific mutants may depend on the compound used because it was shown that the V1aR antagonist SR49059 (which acts as a weak antagonist on the V2R) is able to rescue the V2R mutants K100D, C319Y (19) and R137H (24), whereas it could not rescue the mutants del62-64, D136A, P322S, W323H and F328H, which are all close to the cytoplasmic ends of the respective transmembrane domains in which they are present (19). By contrast, the V2R antagonist SR121463 was able to efficiently rescue the plasma membrane expression of all of these mutants. Moreover, four mutants at the interface of transmembrane domains II and IV were insensitive to rescue by either of these antagonists. Thus, the location of the mutation in the receptor determines whether it can be rescued (19). An overview of the above data is shown in Table 1.

A second determinant for the efficiency of rescue is the affinity of the antagonist for the receptor. From low to high affinity, the V1aR antagonist SR49059, or the V2R antagonists OPC31260, OPC41061 and SR121463, showed an increased ability to restore
the plasma membrane localisation of eight ER-retained mutants. Furthermore, plasma membrane rescue and its corresponding maturation demonstrated a positive correlation with the concentration in which the compounds were applied (25). On the basis of the latter two determinants, applying a high concentration of a high-affinity antagonist would be the method of choice for the treatment of patients. However, once the antagonist-bound receptor reaches the plasma membrane, it requires displacement by AVP or its synthetic analogue dDAVP to allow activation of the receptor (Fig. 2, step 4). At high concentrations, the low-affinity antagonist SR49059 showed optimal rescue, most likely as a result of its easy displacement by dDAVP, whereas high-affinity antagonists failed to show efficient displacement, despite recruiting high amounts of mutant receptor to the plasma membrane. However, at clinically feasible concentrations (< 30 nM) (18), the V2R antagonists OPC31260 and OPC41061 could be displaced by dDAVP, allowing functional rescue (25). Therefore, we consider that the most efficient overall functional rescue is a balance between the ability of a compound to promote the mutant receptor’s trafficking to the plasma membrane, and its ability to be displaced by a natural or synthetic agonist there.

Nonpeptide antagonists as a treatment for NDI

Currently, only one clinical trial has been conducted to test the efficiency of cell-permeable antagonists, in which five patients with mutations in the AVPR2 gene (three encoding the mutation R137H, one encoding W164S and one the del62-64 mutation) were treated with SR49059 (Relcovaptan). Patients were administered three oral doses daily for two consecutive days. Although there was some variation in the responses between patients, an average reduction of the 24-h urine volume (from 11.9 to 8.2 l/day), combined with an increased 24-h urine osmolality (from approximately 100 to 150 mOsm/kg) and reduced water intake (10.7–7.2 l/day), was observed (18). In a subsequent 7-day trial, one patient showed a maximum urine osmolality of approximately 450 mOsm, approximately two-fold higher than his baseline levels (18). Although this increased level is still far below the maximum concentrating ability of a healthy individual, it resulted in a 50% reduction in urine output and water consumption in this patient, clearly demonstrating that cell-permeable antagonists are promising compounds to relieve NDI in patients and improve their quality of life.

Because of potential interference with the cytochrome P450 metabolic pathway, however, the trial with SR49059 had to be discontinued. Recently, the cell-permeable antagonist OPC41061 (Tolvaptan) has been approved in the USA and Europe for the treatment of hyponatraemia in the syndrome of inappropriate antidiuretic hormone secretion and congestive heart failure (26, 27). This positive safety assessment, together with OPC41061 being an efficient pharmacological chaperone at clinically feasible concentrations, paves the road for clinical trials to test the potential of Tolvaptan for treating NDI in the future.

Potential of nonpeptide agonists for the treatment of NDI

The pharmaceutical industry has recently made efforts to develop nonpeptide V2R-specific agonists for oral administration. Likely, their small-molecule composition and relatively high hydrophobicity allows them to pass cell membranes, thereby facilitating an improved uptake by the intestinal tract compared to ‘classical’ peptide-based agonists such as dDAVP. Recently, we demonstrated that a recently-developed nonpeptide V2R agonist, OPC51803 and the two novel nonpeptide agonists, VA999088 and VA999089, were able...
Table 1. Overview of Plasma Membrane and/or Functional Rescue of Type 2 Vasopressin Receptor Mutants in Nephrogenic Diabetes Insipidus.

<table>
<thead>
<tr>
<th>Compound</th>
<th>(Ant)agonists</th>
<th>Specificity</th>
<th>Plasma membrane rescue</th>
<th>Functional rescue</th>
<th>Reference</th>
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<tr>
<td>SR49059</td>
<td>Antagonist</td>
<td>V1R</td>
<td>R137H, S167T, C319Y</td>
<td>R137H</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>L44P, Δ62-64, R113W, I130F, S167T, G201D, T204N, V206D</td>
<td>C319Y, S167T</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ΔV278, L922P, R337X</td>
<td>Not determined</td>
<td>23</td>
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<td></td>
<td>L44P, Δ62-64, R113W, I130F, S167T, G201D, T204N, V206D</td>
<td>None</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>VA999068</td>
<td>Agonist</td>
<td>V2R</td>
<td>None</td>
<td>L44P, Y128S, I130F, S167T, V280C, P322S</td>
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</tr>
<tr>
<td>VA999069</td>
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The compound name, nature and highest specificity for a subtype of vasopressin receptor are indicated. Furthermore, those mutants positively tested to be rescued from the endoplasmic reticulum to the plasma membrane, together with whether their function was restored, are shown. *R337X could not directly be activated by the MCF compounds, but only after plasma membrane rescue and subsequent stimulation with 1-desamino-8-D-AVP.

Fig. 3. Proposed mechanism of intracellular activation of class II vasopressin type 2 receptor (V2R) mutants. Similar to cell-permeable antagonists, cell-permeable agonists are able to bind V2R mutants in the endoplasmic reticulum. Rather than stabilising their conformation, these agonists directly activate these intracellularly-located receptors by signaling to pre-formed receptor-G protein-adenylate cyclase (AC) complexes. The resulting cAMP response will then activate protein kinase A (PKA) to induce trafficking of aquaporin-2 (AQP2) storage vesicles to, and their fusion with, the apical membrane, thereby restoring the NDI phenotype.
to functionally rescue ER-retained V2R mutants in polarised renal cells. The agonists were able to induce a cAMP response, eventually leading to an increase in translocation of AQP2 to the apical cell membrane (Fig. 3) in six out of seven V2R mutants (L44P, Y128S, I130F, S167T, Y280C and P322S, but not S167L). Importantly, the non-cell-permeable peptide agonist dDAVP did not induce signalling for any of these mutants, indicating that entry into the cell is critical for receptor activation. Moreover, incubation with the non-peptide agonists did not affect ER localisation for mutants, failed to induce receptor maturation, but resulted in a rapid cAMP response, indicating that the observed signal was derived from ER-retained receptors. In addition, nonpeptide agonists did not increase degradation of intracellularly retained V2R mutants (28). In addition, Jean-Alphonse et al. (29) revealed that three structurally related nonpeptide agonists (MCF14, MCF18 and MCF57) increased cAMP levels of intracellularly retained V2R mutants L44P and A294P, coinciding with an increased membrane expression and improved maturation of the V2R mutants. Moreover, membrane rescued mutants were sensitive to AVP, and thus functional.

These latter MCF agonists also exhibited antagonistic effects on V2R-dependent β-arrestin recruitment, internalisation and subsequent ERK activation, indicating an additional beneficial effect on V2R signalling (29) Thus, the signalling and trafficking properties of the MCF agonists on the wild-type and mutant V2R are different from the VA999088, VA999089 and OPC51803, as summarised in Table 1. This indicates that the compounds studied by Jean-Alphonse et al. (29) and ourselves may bind to different residues of the AVP binding pocket of V2R, which may explain the different mechanism of rescue. Alternatively, differences regarding the mechanism could also lie in the use of stably transfected nonpolarised renal cells (tsA201 cells).

In conclusion, the effect of nonpeptide agonists on V2R mutants is mutation- and agonist-dependent, and studies clearly demonstrate the potential of cell-permeable V2R agonists as future therapies for NDI as a result of misfolded V2R mutants.

**Future perspectives**

The broad applicability of nonpeptide antagonists as pharmacological chaperones has been demonstrated because specific antagonists or allosteric modulators have been shown to rescue the plasma membrane expression of mutants of the GPCRs rhodopsin (30), calcium-sensing receptor (31) and gonadotrophin-releasing hormone receptor (32, 33). Future clinical trials will determine whether these promising in vitro experiments can be extrapolated to a positive effect in patients and whether pharmacological chaperones are promising treatments for the 'conformational diseases' resulting from misfolded receptors.

Treatment with nonpeptide V2R agonists, however, is likely superior over nonpeptide antagonists because rescue of cell surface expression and subsequent displacement by endogenous AVP is circumvented. In addition, the assembly of a complete β2-adrenergic receptor signalosome in the ER (34, 35), activation of the ER-localised GPR30 by its naturally cell permeable agonist oestrogen (36) and intracellular activation of V2R mutants by nonpeptide agonists (28) clearly show that GPCRs normally functioning in the plasma membrane can generate a signalling cascade from an intracellular location. Thus, nonpeptide agonists appear to represent ideal therapeutics to treat not only NDI as a result of misfolded V2R mutants, but also many other diseases as a result of misfolded and ER-retained GPCRs.

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