Summary: The purpose of this review is first to describe the importance of early detection of vasopressin receptor mutations responsible for X-linked nephrogenic diabetes insipidus (NDI). We have proposed that all families with hereditary diabetes insipidus should have their molecular defect identified because early diagnosis and treatment of affected infants can avert the physical and mental retardation that results from repeated episodes of dehydration. Secondly, 95 published missense mutations responsible for X-linked NDI are likely to result in misfolded arginine-vasopressin V2 receptors that are trapped in the endoplasmic reticulum. These misfolded receptors are unable to reach the plasma membrane in principal collecting duct cells and to engage the circulating antidiuretic hormone, arginine-vasopressin. These misfolded proteins potentially could be rescued with pharmacologic chaperones, an active area of research pertinent to other hereditary protein misfolding diseases such as cystic fibrosis, phenylketonuria, and Anderson-Fabry disease among many others. Finally, a long-term careful surveillance of all patients with hereditary NDI should be performed to prevent chronic renal failure likely caused by the long-term functional tract obstruction with reflux.

Keywords: Nephrogenic diabetes insipidus, AVPR2 mutations, mutational analysis, misfolded mutants, pharmacological chaperones

Diabetes insipidus is a disorder characterized by the excretion of abnormally large volumes (>30 mL/kg/d of body weight for adults) of dilute urine (<250 mmol/kg). This definition excludes osmotic diuresis, which occurs when excess solute is being excreted, for example, glucose in the polyuria of diabetes mellitus. Four basic defects can be involved: (1) deficient secretion of the antidiuretic hormone arginine vasopressin (AVP), which is the most common and is referred to as neurohypophyseal (also known as neurogenic, central, or hypothalamic) diabetes insipidus; (2) renal insensitivity to the antidiuretic effect of AVP, which is known as nephrogenic diabetes insipidus (NDI); (3) excessive water intake that can result in polyuria, which is referred to as primary polydipsia; and (4) increased metabolism of vasopressin during pregnancy, which is referred to as gestational diabetes insipidus. The hereditary forms of diabetes insipidus account for less than 10% of the cases of diabetes insipidus seen in clinical practice.

The basolateral AVPR2 receptor and the luminal aquaporin-2 proteins are required for the transepithelial water permeability of the principal cells of the collecting duct

AVPR2 is a GPCR

Loss-of-function of either of these proteins will result in NDI. The V2 receptor is one of 701 members of the rhodopsin family within the superfamily of guanine-nucleotide (G) protein-coupled receptors (GPCRs) (Fig. 1).1,2 GPCRs represent the largest family of membrane proteins in the human genome. They are remark-
ably versatile signaling molecules that are responsible for the majority of transmembrane signal transduction in response to hormones and neurotransmitters. GPCRs share a common structural signature of 7 membrane-spanning helices with an extracellular N terminus and an intracellular C terminus (Fig. 2).^3

Understanding how AVPR2 receptor, the GPCR with loss-of-function responsible for X-linked NDI, operates is a major goal to provide understanding and treatment of this rare disease.

A high-resolution crystal structure of an engineered human β2 adrenergic GPCR recently was published^1–5^ and it is hoped that structure of other GPCRs, including the human AVPR2 receptor, can guide the development of specific drugs, accelerating drug discovery to treat X-linked NDI.

The cAMP-PKA Pathway

The human gene that codes for the V2 receptor (AVPR2) is located in chromosome region
Xq28 and has 3 exons and 2 small introns. The sequence of the complementary DNA predicts a polypeptide of 371 amino acids with 7 transmembrane, 4 extracellular, and 4 cytoplasmic domains (Fig. 2). The activation of V2 receptor on renal collecting tubules stimulates adenylyl cyclase via the stimulatory G protein (Gs) and promotes the cyclic adenosine monophosphate (cAMP)-mediated incorporation of water channels into the luminal surface of these cells. This process is the molecular basis of the vasopressin-induced increase of the osmotic water permeability of the apical membrane of the collecting tubule.\(^6\) In the collecting duct, the first step in the antidiuretic action of AVP is its binding to the vasopressin V2 receptor (Fig. 1) located on the basolateral membrane of collecting duct cells. This step initiates a cascade of events—receptor-linked activation of the cholera toxin-sensitive G-protein (Gs), activation of adenylyl cyclase, production of cAMP, and stimulation of protein kinase A (PKA)—that leads to the final step in the antidiuretic action of AVP; that is, the exocytic insertion of specific water channels, aquaporin-2 (AQP2), into the luminal membrane, thereby increasing the permeability of the luminal membrane.

The cAMP-PKA pathway is one of the most common and versatile signal pathways in eukaryotic cells and is involved in the regulation of cellular functions in almost all tissues in mammals, including regulation of cell cycle, proliferation, differentiation, and regulation of microtubule dynamics, chromatin condensation and decondensation, nuclear envelope disassembly and reassembly, as well as regulation of intracellular transport mechanisms and ion fluxes.\(^7\) Because this single second messenger (the cAMP-PKA pathway) is involved in the regulation of so many diverse cellular processes, it must be highly regulated at several levels to maintain specificity. AVP-induced changes in

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**Figure 2.** V\(_2\) receptor and identification of 193 putative disease-causing AVPR2 mutations. Predicted amino acids are given as the one-letter amino acid code. Solid symbols indicate missense or nonsense mutations; a number indicates more than one mutation in the same codon; other types of mutations are not indicated on the figure. The extracellular, transmembrane, and cytoplasmic domains are defined according to Mouillac et al.\(^30\) There are 95 missense, 18 nonsense, 46 frameshift deletion or insertion, 7 inframe deletion or insertion, 4 splice-site, and 22 large deletion mutations, and one complex mutation. See [http://www.medicine.mcgill.ca/nephros](http://www.medicine.mcgill.ca/nephros) for a list of mutations.
cAMP concentration may vary in duration, amplitude, and extension in the principal cells.

A-kinase anchoring proteins (AKAPs) contribute to the specificity of cAMP signaling by targeting PKA in proximity to cAMP gradients generated by the counterbalancing activities of adenylyl cyclases and phosphodiesterases. The activation of AKAP-anchored PKA by cAMP in discrete microdomains has been visualized in neonatal cardiomyocytes, and AKAP 18δ (a splice variant of AKAP 18) has been found in principal cells of the inner medullary collecting duct in a distribution closely resembling the distribution of AQP2, which could imply a role for AKAP 18δ in the AQP2 shuttle exocytic process. Chen et al recently showed that an inherited AKAP9 mutation caused long-QT syndrome but there is no description of AKAP mutations responsible for hereditary NDI.

AVP also increases the water reabsorptive capacity of the kidney by regulating the urea transporter A1/3, which is present in the inner medullary collecting duct, predominantly in its terminal part. AVP also increases the permeability of principal collecting duct cells to sodium. Vasopressin regulates the apical expression of the epithelial sodium channel by preventing ubiquitin-dependent endocytosis from the cell surface. In summary, in the absence of AVP stimulation, collecting duct epithelia show very low permeabilities to sodium, urea and water. These specialized permeability properties permit the excretion of large volumes of hypotonic urine formed during intervals of water diuresis. In contrast, AVP stimulation of the principal cells of the collecting ducts leads to selective increases in the permeability of the apical membrane to water, urea, and Na.

**X-LINKED NDI (OMIM 304800): LOSS-OF-FUNCTION OF AVPR2, MISFOLDING, AND FUNCTIONAL RESCUE WITH PHARMACOLOGIC CHAPERONES**

X-linked NDI is generally a rare disease in which the affected male patients do not concentrate their urine after administration of AVP. Because this form is a rare, recessive, X-linked disease, female individuals are unlikely to be affected, but heterozygous females can show variable degrees of polyuria and polydipsia because of skewed X-chromosome inactivation. In Quebec, the incidence of this disease among male individuals was estimated to be approximately 8.8 in 1,000,000 male live births. To date, 193 putative disease-causing AVPR2 mutations have been published in 307 NDI families (Fig. 2). Ninety-five of these 193 mutations are missense mutations likely to be misfolded, trapped in the endoplasmic reticulum, and unable to reach the basolateral cell surface to engage the circulating antidiuretic hormone, AVP. Most of the AVPR2 mutants that we and other investigators have tested are type 2 mutant receptors. They did not reach the cell membrane and were trapped in the interior of the cell. Other mutant G-protein-coupled receptors and gene products causing genetic disorders also are characterized by protein misfolding. Mutations that affect the folding of secretory proteins; integral plasma membrane proteins; or enzymes destined to the endoplasmic reticulum, Golgi complex, and lysosomes result in loss-of-function phenotypes irrespective of their direct impact on protein function because these mutant proteins are prevented from reaching their final destination. Folding in the endoplasmic reticulum is the limiting step: mutant proteins that fail to fold correctly are retained initially in the endoplasmic reticulum and subsequently often degraded. For example, cystic fibrosis is caused mainly by point mutations in the gene encoding an apical membrane adenosine triphosphate (ATP)-regulated chloride channel, which is known as the cystic fibrosis transmembrane conductance regulator (CFTR). The main disease-associated mutation, ΔF508 (deletion of the phenylalanine residue at position 508 of the wild-type protein), disrupts the folding of CFTR in the endoplasmic reticulum, leading to almost complete degradation of this channel. Interestingly, however, properly folded CFTR with this mutation can traffic to the plasma membrane, where it forms a functional chloride channel. These findings suggest that rescuing the folding of ΔF508-CFTR could be used eventually to treat patients with cystic fibrosis. Wang et al used the shotgun liquid chromatography mass spectrometry (LC-MC) method of multidimensional protein-identification technology to analyze cells
expressing the gene encoding the wild-type CFTR and ΔF508-CFTR. Multidimensional protein-identification technology analysis of wild-type CFTR and ΔF508-CFTR immunoprecipitates identified nearly 200 CFTR-associated proteins (compared with controls in which nonspecific antibodies or cells lacking CFTR were used). Collectively, these proteins have been named the CFTR interactome. These proteins included known CFTR-binding chaperones, such as calnexin, HSP40-HSP-70, and HSP90, as well as many previously unknown interactors.

RNA interference (RNAi)-mediated knockdown of the HSP90 co-chaperone present in wild-type and ΔF508-CFTR immunoprecipitates, AHA1, corrected the amount of both endoplasmic reticulum-associated and cell surface-associated ΔF508-CFTR. These data suggest that disruption of AHA1 facilitates a folding pathway that favors not only the stability of the channel, but also coupling to the endoplasmic export machinery.

If the misfolded protein/traffic problem that is responsible for so many human genetic diseases can be overcome and the mutant protein transported out of the endoplasmic reticulum to its final destination, then these mutant proteins could be sufficiently functional. Therefore, using pharmacologic chaperones or pharmacoperones to promote escape from the endoplasmic reticulum is a possible therapeutic approach. We used selective nonpeptide V₂ and V₁-receptor antagonists to rescue the cell-

**Figure 3.** (A) Urine volume, urine osmolality, and water intake on day 1 and after SR49059 administration (day 3) in 5 adult male patients with X-linked NDI. (B) The same values are described for the afternoon period (2:00 pm to 8:00 pm) when the effect of SR49059 was suspected to be maximal. Mean values (±SEM) are presented. *P < .05, paired t test. Data reprinted with permission from Bernier et al. 249
surface expression and function of naturally occurring misfolded human V2 receptors. Because the beneficial effect of nonpeptide V2 antagonists could be secondary to prevention and interference with endocytosis, we studied the R137H mutant previously reported to lead to constitutive endocytosis. We found that the antagonist did not prevent the constitutive /HARRESTIN-promoted endocytosis. These results indicate that as for other AVPR2 mutants, the beneficial effects of the treatment result from the action of the pharmacologic chaperones. In clinical studies, we administered a nonpeptide V1a vasopressin antagonist SR49059 to 5 adult patients who have NDI and bear the del62-64, R137H, and W164S mutations. SR49059 significantly decreased urine volume and water intake and increased urine osmolality (Fig. 3), whereas sodium, potassium, creatinine excretion, and plasma sodium were constant throughout the study. This new therapeutic approach could be applied to the treatment of several hereditary diseases resulting from errors in protein folding and kinesis.

LARGE DILATATION OF THE URINARY TRACT AND BLADDER COULD LEAD TO REFLUX AND CHRONIC RENAL FAILURE

All polyuric states (whether neurogenic, nephrogenic, or psychogenic) can induce large dilatations of the urinary tract and bladder, and bladder function impairment has been well documented in patients who bear AVPR2 or AQP2 mutations (Fig. 4). Of interest, an inducible mouse model of NDI was produced recently by floxed Aqp2 gene deletion, which also showed evidence of structural damage from the sustained polyuria. Chronic renal failure secondary to bilateral hydronephrosis has been observed as a long-term complication in some rare patients. Renal and abdominal ultrasound should be performed annually, and simple recommendations, including frequent urination and double voiding, could be important to prevent these consequences.

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