Pharmacologic Chaperones as a Potential Treatment for X-Linked Nephrogenic Diabetes Insipidus

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In many mendelian diseases, some mutations result in the synthesis of misfolded proteins that cannot reach a transport-competent conformation. In X-linked nephrogenic diabetes insipidus, most of the mutant vasopressin 2 (V2) receptors are trapped in the endoplasmic reticulum and degraded. They are unable to reach the plasma membrane and promote water reabsorption through the principal cells of the collecting ducts. Herein is reported two types of experiments: In vivo studies to assess clinically a short-term treatment with a nonpeptide V1a receptor antagonist (SR49059) and in vitro studies in cultured cell systems. In patients, SR49059 decreased 24-h urine volume (11.9 ± 2.3 to 8.2 ± 2.0 L; P = 0.005) and water intake (10.7 ± 1.9 to 7.2 ± 1.6 L; P < 0.05). Maximum increase in urine osmolality was observed on day 3 (98 ± 22 to 170 ± 52 mOsm/kg; P = 0.05). Sodium, potassium, and creatinine excretions and plasma sodium were constant throughout the study. In vitro studies indicate that the nonpeptide V1a receptor antagonist SR49059 and the V1a/V2 receptor antagonist YM087 (Conivaptan) rescued cell surface expression and function of mutant V2 receptors. Mutant V2 receptors with nonsense mutations were not affected by the treatment. Misfolded V2 receptor mutants were rescued in vitro and also in vivo by nonpeptide antagonists. This therapeutic approach could be applied to the treatment of several hereditary diseases that result from errors in protein folding and kinesis.


Although the activities of the protein synthesis quality control systems are generally advantageous to the cell, on occasion this stringent monitoring process can lead to intracellular retention of salvageable proteins. In recent years, it has been observed that a group of diseases stem from mutations that promote such retention and are collectively referred to as conformational or protein-misfolding diseases (1,2). Nephrogenic diabetes insipidus (NDI) (3,4), which is characterized by a loss of arginine vasopressin (AVP)-mediated antidiuresis, is one of these diseases. In congenital NDI that results from mutations in the gene that encodes the V2 receptor, most missense mutations are misfolded, trapped in the endoplasmic reticulum, and unable to reach the basolateral cell surface to engage the circulating antidiuretic hormone, AVP (5–14).

The natural history of untreated X-linked NDI includes hypernatremia, hyperthermia, mental retardation, and repeated episodes of dehydration in early infancy (15,16). In five new patients who were younger than 1 year and were from North America and in whom we provided molecular testing over the past 12 mo, plasma sodium was in every case >155 mEq/L at the time of diagnosis. We and others initially thought that close monitoring of infants whose AVPR2 mutations were diagnosed pre- or perinatally not only would prevent episodes of dehydration but also would permit close to normal growth and development. Although a low-sodium diet and distal tubule diuretics prescribed to these patients may achieve a 20 to 30% decrease in urine output (17), the low-sodium diet is difficult to follow, and affected children continue to drink large amounts of water. As a result of a physiologic gastroesophageal reflux and to the large amount of water in their stomach, these children often vomit, and, as a consequence, their nutritional intake is not optimal. There is a need, therefore, for a safe further reduction in urine output. We recently used pharmacologic compounds to rescue misfolded mutant V2 receptors by demonstrating in cultured cells that the nonpeptide V2-specific antagonists SR121463A and VPA-985 increased cell surface expression and rescued the signaling activity of seven naturally occurring AVPR2 mutations (185_193del, L59P, L83Q, Y128S, S167L, A294P, and P322H) that are responsible for NDI by promoting their proper folding and maturation (18). These results that suggested that such chaperoning of the receptor could represent a pharmacologic treatment for conformational diseases such as NDI have been confirmed by other investigators (19,20).
Here, we report a short-term trial that was conducted to test the effect of a nonpeptide V1a receptor antagonist on decreasing urine output and increasing urine osmolality in patients with X-linked NDI. We used SR49059, a V1a receptor antagonist that was tested previously in normal volunteers and patients with putative vasopressin excess–related disorders (21–24). In addition, in vitro studies were done to rescue plasma membrane and signaling of a number of mutant V2 receptors with SR49059 and YM087 (Conivaptan), a mixed V1-V2 antagonist (25–29).

Materials and Methods

Study Participants

We tested five adult male patients (weight 83.5 ± 3.9 kg) who had X-linked NDI and bore the following AVPR2 mutations that were identified previously by sequencing: Three patients, who were 20, 42, and 41 yr of age and had R137H (30) (patients 1, 2, and 3); one 21-yr-old with W1645 (31) (patient 4); and one 20-yr-old with 185_193del (31) (patient 5). All had a documented lifelong history of polyuria and polydipsia, and extensive previous testing demonstrated a lack of urinary osmolality response to AVP or dDAVP. Specifically, maximal urine osmolalities (mOsm/kg) that were obtained during dDAVP infusions were, respectively, 104, 145, and 248 (patients 1, 2, and 3); 85 (patient 4); and 65 (patient 5) (32). Two patients were being treated with hydrochlorothiazide, which was discontinued for 1 wk before the study. None of the patients described here were considered to have a mild phenotype (9). The following short-term trial conformed to the Declaration of Helsinki and was approved by the Ethics Committee at Hôpital du Sacré-Cœur de Montréal, and all participants approved and signed a detailed informed consent.

SR49059 Administration for 2 D. Patients were tested at the Clinical Research Unit of the Hôpital du Sacré-Cœur de Montréal and received a constant Na⁺, K⁺, osmotic and caloric diet for the 3 d of the study. A dietician met the patients before the study and made a detailed evaluation of their usual diet during the previous month. This diet was reproduced for the 3 d of the study. None of these patients followed a sodium-restricted diet. The dietician met the patients every day during testing and enforced the same diet throughout the study. Water intake was not restricted and was recorded during the 3 d of the study. After 24-h control measurements (day 1, no medication), SR49059 was administered orally for the next 2 d; on day 2, the patients received 150 mg at 8 a.m. and 300 mg at 1:00 p.m. and 6:00 p.m.; on day 3, the patients received 300 mg at 8:00 a.m., 1:00 p.m., and 6:00 p.m. BP and pulse were measured every 30 min from 8:00 a.m. to 12:00 a.m. for the first two patients and every 2 h for the last three patients. Urine volume was obtained by spontaneous voiding every 30 min from 8:00 a.m. to 10:30 p.m. From 10:30 p.m. to 8:00 a.m., depending on individual patients, all urine excretion was measured at unspecific times; urine flow was calculated; and urine osmolality, Na⁺, K⁺, and creatinine were measured on these samples. Plasma Na⁺ and plasma AVP were measured at 7:30 a.m. and 1:30 p.m. each day. Urinary Na⁺, K⁺, creatinine, osmolality, and AVP were obtained on each urine sample.

SR49059 Administration for 7 Days. Two patients who bore the R137H mutation (patients 1 and 3) were also treated 6 wk after the 2-d administration of SR49059 for 7 d with the following dosages of SR49059: 750 mg on day 1 (150 mg at 8:00 a.m., 300 mg at 1:00 p.m., and 300 mg at 6:00 p.m.) and 900 mg (300 mg three times daily) for the following 7 d. Urine and plasma measurements were obtained on days 1, 6, and 9 (postdosing). On days 6 (on SR49059) and 9 (off SR49059), basal plasma samples were obtained for sodium at 8:00 a.m., and urine was obtained every 30 min from 8:30 a.m. to 12:00 p.m. for volume, osmolality, Na⁺, K⁺, creatinine, and AVP.

Cell Culture Studies

V2 Receptor Mutant Expression. Mammalian expression plasmids encoding the wild-type, 12 missense mutations (L59P, L83Q, Y128S, R113W, R137H, W164S, A165D, S167L, I209F, A294P, S315R, and R322H) two in-frame deletions (185_193del and V279del) or five nonsense mutations (W71X, S167X, Q180X, W284X, and R337X) were transiently or stably transfected in COS-1 or HEK 293 cells, as described previously (18,33).

Immunofluorescence Microscopy and Flow Cytometry. All immunofluorescence and flow cytometry studies were carried out in COS-1 cells or HEK 293 as described previously (18,33). Briefly, V2 receptors were detected using antibodies directed against the myc- or

Table 1. Water, Na⁺, and K⁺ intake; 24-h urine osmolar excretion; Na⁺, K⁺, and creatinine excretion; and plasma sodium during day 1 (control) or SR49059 administration (days 2 and 3)

<table>
<thead>
<tr>
<th></th>
<th>Day 1 (Control)</th>
<th>Day 2</th>
<th>Day 3</th>
<th>P Value, Paired t Test (Day 1 versus Day 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water intake (L/d)</td>
<td>10.7 ± 1.9</td>
<td>10.7 ± 2.2</td>
<td>7.2 ± 1.6</td>
<td>0.028</td>
</tr>
<tr>
<td>Na intake (mmol/d)</td>
<td>169.8 ± 3.4</td>
<td>179.4 ± 0.6</td>
<td>194.0 ± 4.3</td>
<td>NS</td>
</tr>
<tr>
<td>K intake (mmol/d)</td>
<td>101.2 ± 6.8</td>
<td>105.8 ± 4.2</td>
<td>107.0 ± 3.0</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma sodium (mEq/L)</td>
<td>141.0 ± 0.6</td>
<td>140.8 ± 1.8</td>
<td>140.2 ± 2.2</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma potassium (mEq/L)</td>
<td>4.02 ± 0.11</td>
<td>3.82 ± 0.05</td>
<td>3.70 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma creatinine (µmol/L)</td>
<td>87 ± 13</td>
<td>87 ± 15</td>
<td>88 ± 17</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min per 1.73 m²)</td>
<td>122 ± 19</td>
<td>122 ± 17</td>
<td>113 ± 20</td>
<td>NS</td>
</tr>
<tr>
<td>Urine volume (L/d)</td>
<td>11.9 ± 2.3</td>
<td>10.7 ± 2.5</td>
<td>8.2 ± 2.0</td>
<td>0.005</td>
</tr>
<tr>
<td>Osmolar excretion (Osm/d)</td>
<td>1068.4 ± 112.0</td>
<td>996.0 ± 55.0</td>
<td>930.7 ± 72.0</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine excretion (mol/d)</td>
<td>16.8 ± 1.6</td>
<td>15.5 ± 2.0</td>
<td>15.1 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Na excretion (mEq/d)</td>
<td>199.3 ± 20.4</td>
<td>163.9 ± 17.0</td>
<td>140.9 ± 13.2</td>
<td>NS</td>
</tr>
<tr>
<td>K excretion (mEq/d)</td>
<td>71.8 ± 16.5</td>
<td>65.5 ± 6.9</td>
<td>71.0 ± 7.5</td>
<td>NS</td>
</tr>
<tr>
<td>Na + K excretion (mEq/d)</td>
<td>271.1 ± 32.4</td>
<td>229.4 ± 16.4</td>
<td>212.0 ± 20.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Na excretion decreased, although nonsignificantly, during the 3 d, probably indicating that these patients were taking more Na⁺ before the study than they revealed to the dietician.*
Ha-epitope that was fused at the N-terminus of the constructs. For microscopy, immunoreactivity was assessed using secondary Oregon-green–conjugated anti-mouse antibodies. For flow cytometry, the phycoerythrin-conjugated anti-mouse antibody was used.

**cAMP Accumulation.** Total cAMP accumulation was measured in COS-1 cells or HEK 293 by assessing the transformation of [3H]ATP into [3H]cAMP as described previously (34).

**Plasma AVP Measurements.** Plasma AVP was measured by RIA as described previously (35).

**Statistical Analyses**

Simple statistics were performed for the major variables of interest. Overall results were analyzed by two-way ANOVA. Comparison between time points was performed using paired t test. A two-tailed $P < 0.05$ was considered statistically significant. Values are reported as mean ± SEM. All analyses were performed with the statistical package SAS (SAS Institute Inc., Cary, NC).

**Results**

**Patient Studies**

**Safety and Efficacy of SR49059 Administration in Adult Patients Who Bear the R137H, W164S, or 185_193del Mutations.** No significant changes in BP and pulse were encountered throughout the study, and no untoward clinical or biochemical abnormalities were observed. Intake and output; plasma sodium and potassium and 24-h urine osmolar excretion; and Na$^+$, K$^+$, and creatinine excretion are presented in Table 1 and Figure 1. SR49059 significantly decreased 24-h urine volume and 24-h water intake from day 1 to day 3. A maximal increase in urine osmolality was observed from 2:00 p.m. to 8:00 p.m. on day 3 (98 ± 22 to 170 ± 52 mOsm/kg; $P = 0.05$; Figure 1). Plasma Na$^+$ was constant, indicating that the changes in urine volume and water intake were secondary to the SR49059 administration and not to voluntary decrease in water intake that would have led to increased plasma Na$^+$. Individual urine volume and urine osmolality responses are presented for three patients, each bearing a different mutation (Figure 2, A, B, and C). The maximal urine osmolality during treatment was observed for patient 3 (Figure 2), who had a urine osmolality of 248 during a diagnostic dDAVP infusion (32). To various extent, the treatment significantly decreased urinary output and increased urine osmolality in all patients. Plasma AVP levels were measured on day 1 (control), two

Figure 1. (A) Urine volume, urine osmolality, and water intake on day 1 and after SR49059 administration (day 3) in five adult male patients with X-linked nephrogenic diabetes insipidus (NDI). (B) The same values are described for the afternoon period (2:00 p.m. to 8:00 p.m.) when the effect of SR49059 was suspected to be maximal. Mean values (± SEM) are presented. *$P < 0.05$, paired $t$ test.
times on day 2 (8:00 a.m. and 2:00 p.m.), and four times on day 3 (8:00 a.m., 2:00 p.m., 8:00 p.m., and 12:00 a.m.). Plasma AVP values were significantly different among patients (from 1.97 to 6.24 pg/ml; ANOVA, \( P < 0.05 \)), but no significant differences among days or time effects were seen, and plasma values tended to decrease during the study (day 1, 5.39; day 2, 3.01, day 3, 3.34 pg/ml).

Longer treatment was tested for two patients who bore the R137H mutations. Reduced urine volumes and increased urine osmolalities were maintained for the 7 d of the treatment with SR49059 (Figure 3).

**Cell Culture Studies**

**SR49059.** The effect of the nonpeptide V1a antagonist SR49059 was assessed on cell surface expression and function of two missense (R137H and W164S), one in-frame deletion
(185_193del), and one nonsense (W284X) mutation. Immunofluorescence microscopy showed that under basal conditions, all V2 mutants were poorly expressed at the cell surface of transfected COS-1 cells (Figure 4A). A 16-h treatment with SR49059 increased the plasma membrane targeting of three of the V2 receptor mutants but not the nonsense mutant receptor. Figure 4B shows the quantitative assessment of the increase in cell surface expression for the 185_193del V2 receptor mutant using flow cytometry.

The increased cell surface expression of R137H, W164S, and 185_193del V2 receptor mutants after treatment with SR49059 resulted in a significant potentiation of the AVP-mediated cAMP production, suggesting that SR49059 restored function by acting as a pharmacologic chaperone. In contrast, the SR49059 treatment had no effect on AVP-stimulated cAMP accumulation in cells that expressed W284X V2 receptors (Figure 5A).

Kinetic analysis of the effect of SR49059 on the function of one of the V2 mutant receptors (185_193del) in HEK 293 cells is shown in Figure 5B. The potentiation effect peaked at 2 h after treatment but was maintained for at least 12 h after the antagonist. The relatively slow onset of the effect is consistent with the notion that the drug acts by favoring folding and cell surface trafficking of newly synthesized receptors (36). The modest increase in AVP-stimulated cAMP production that was observed in cells during the course of the experiment most
likely reflects accumulation of a small number of 185_193del V2 mutant receptors at the cell surface.

YM087. Because the clinical development of SR49059 was interrupted (vide infra), we also tested the ability of YM087, a dual V1a and V2 vasopressin receptor antagonist (27), to rescue cell surface expression and function of 19 naturally occurring V2R mutants in COS-1 cells. As shown in Figure 6, YM087 promoted cell surface expression and potentiated AVP-mediated cAMP production for 10 naturally occurring mutations tested. As was the case for SR49059, YM087 had no effect on the nonsense mutations selected as negative controls: W284X (Figure 6), W71X, S167X, Q180X, and R337X (data not shown).

Discussion
To date, no specific treatment that is aimed at restoring the function of the mutant V2 receptor is available to treat patients with X-linked NDI. Volume contraction and thiazide diuretics, amiloride, and indomethacin are acting only indirectly by decreasing the amount of tubular fluid presented to the distal tubule (37,38). These indirect forms of treatment are most effective in patients who have mild to moderate forms of X-linked NDI and bear incomplete loss-of-function mutations. These patients with mild to moderate disease are rare, and most patients are completely unresponsive to AVP or dDAVP (32). Here, we present evidence that nonpeptide vasopressin antag-
onists are potential specific treatments of this disease. It has been argued that the diversity of mutations in NDI may complicate the search for a universal therapeutic strategy for these patients (39). However, because approximately 50% of all NDI mutations are missense, a pharmacologic chaperone-based therapy could represent a potential general treatment of this protein-misfolding disease.

Manning et al. (40) designed in the 1970s numerous vasopressin and oxytocin receptor agonists and antagonists. Their clinical use in humans, however, was deceptive because antagonists in rats were found to be agonists in humans. This was found later to be due to different molecular structures of the receptors that are responsible for different affinities for agonists and antagonists in human and rat species (41). The random screening of chemical compounds resulted in the development of oral nonpeptide vasopressin receptor antagonists now called “Vaptans,” Vap for vasopressin, tan for antagonists (42). The structure of these compounds imitates the structure of the native hormone AVP, and these antagonists interfere with the binding pocket of AVP (43). During the past few years, various selective, orally active AVP V1a (OPC-21268, SR49059 [Relcovaptan]), V2 (OPC-31260, OPC-41061 [Tolvaptan], VPA-985 [Lixivaptan], SR121463A and B, VP-343, and FR-161282), and

Figure 3. Urine volume and osmolality on day 6 of a 7-d treatment with SR49059 and on day 9, 2 d after treatment. Patient 5 (see Figure 2A) had a urine osmolality of approximately 400 mOsm/kg after SR49059 (300 mg) administration. Urine osmolality then decreased to 200 during the remaining of the morning observation. On day 9, 2 d after cessation of SR49059, urine osmolality values were similar to the control values obtained on day 1 (see Figure 2A).

Figure 4. SR49059 treatment on cell surface expression of four V2 receptor mutants. (A) Immunofluorescence microscopy of nonpermeabilized COS-1 cells that transiently expressed wild-type, R137H, W164S, 185_193del, or W284X mutations and were incubated or not for 16 h with 10^{-5} M SR49059. (B) Cell surface receptor expression was measured by flow cytometry analysis of cells that stably expressed the 185_193del mutant V2 receptor incubated in the absence or presence of 10^{-5} M SR49059.
SR49059 was a selective V1a antagonist with inhibition constants (K<sub>i</sub>) ranging from 1.5 to 6.5 nM (21). SR49059 displayed competitive nanomolar affinity for V1a receptors but weak affinities for human and nonhuman V2, V1b, and oxytocin receptors with K<sub>i</sub> ranging from 220 to 1080 nM (21).

The absolute bioavailability of the nonmicronized formulation F1 used in this study was low and variable (5.3 ± 4.7%) with a Tmax of approximately 3 h and a terminal half-life of approximately 23 h. A 300-mg dose of the F1 formulation was reported in the Clinical Investigator Brochure to increase plasma concentration to 18.5 ng (33.5 micromolar, MW of SR49059 is 620.5) 3 h after the administration of SR49059 to normal volunteers. In our in vitro studies, a 16-h pretreatment with 10 micromolar (10<sup>−5</sup> M) concentration of SR49059 or YM087 (MW 535.04) rescued cell surface expression and cAMP production.

Because in our clinical studies the maximal urine osmolality was observed, in general, 2 to 3 h after the oral administration of 300 mg of SR49059, these in vivo and in vitro results agree with these pharmacokinetics data. We demonstrated that SR49059, a V1a receptor antagonist that shows moderate affinity for the V2 receptor (275 nM) (21), rescued plasma membranes and signaling of the R137H, W164S, and 185_193del mutants, a confirmation of previous results obtained with the selective V2 receptor antagonists SR121463A, SR121463B, and VPA-985 (18,33) and other mutant V2 receptors (19,20). It is hypothesized that these compounds will enter the cell and the endoplasmic reticulum compartment and may stabilize the misfolded mutant receptor to a conformation that will permit further maturation through the endoplasmic reticulum and Golgi compartments. These nonpeptide vasopressin antagonists, with different affinities for V1 or V2 receptors, may be seen as a mold on which the unstable mutant receptor will wrap itself, perhaps hiding its hydrophobic residues and decreasing free energy (2). In recent in vitro results (45), we demonstrated that in vitro treatment with SR121463 for 16 h led to an important decrease in the polyubiquitination immunoreactive signal, indicating that the increased receptor maturation is accompanied by a decrease in the proportion of receptor being targeted to the polyubiquitination-dependent degradation pathway.

In five patients who had X-linked NDI and harbored three different AVPR2 mutations, SR49059 had beneficial effects on urine volume and osmolality starting a few hours after administration. This lag in efficacy is compatible with our previous in vitro observations (18), demonstrating that the pharmacologic chaperones need to permeate the cell and favor folding and trafficking of functional mutant receptors to the cell surface to be active. That nonsense mutations could not be rescued by the antagonist treatment is consistent with such a proposed mode of action. In a recent study, we demonstrated that the β-arrestin–mediated constitutive endocytosis of the V2 receptor (46) is not affected by SR49059 (33). The functional rescue observed in vitro and in our clinical study thus is unlikely to result from a stabilization of the V2 receptor at the cell surface.

Urine osmolality increased by 50% on day 3 from 2:00 p.m. to 8:00 p.m., and a maximum urine osmolality of 430 mOsm/kg (Figure 2A) was documented in patient 3, who was able to

mixed V1a/V2 (YM-087 [Conivaptan], JTV-605, and CL-385004) receptor antagonists have been studied intensively in various animal models and have reached phase III clinical trials for some of them (44).

We gave SR49059, a potent and selective, orally active, nonpeptide V1a receptor antagonist to five patients with V2 receptor defects. Previous in vitro binding experiments of human V1a receptors obtained from platelets, adrenals, aortic smooth muscles, and nonpregnant myometrium demonstrated that SR49059 was a selective V1a antagonist with inhibition con-

![Figure 5](image-url) Signaling activity of COS-1 and HEK293 cells after treatment with SR49059. (A) Potentiation of arginine vasopressin (AVP)-stimulated cAMP accumulation was measured in COS-1 cells that transiently expressed wild-type, R137H, W164S, 185_193del, or W284X V2 mutant receptors after a 16-h pretreatment of 10<sup>−5</sup> M SR49059. Cells that were not treated with SR49059 (□) are compared with treated cells (■). The fold increases are given above the solid bars. The value of one obtained for the wild-type V2 receptors indicates that SR49059 does not alter its maximal efficacy. (B) Duration of the effect of SR49059 pretreatment on AVP-stimulated cAMP accumulation. HEK 293 cells that stably expressed the 185_193del V2 mutant receptor were treated with 10<sup>−5</sup> M SR49059 for 16 h. At the end of the treatment, the antagonist was removed by successive washing, and the AVP-stimulated cAMP accumulation was determined at indicated times after the washing procedures.
increase his urine osmolality only to 248 mOsm/kg during a previous dDAVP infusion (32). The urine osmolality changes were not secondary to increased endogenous plasma AVP concentrations. The effects on urine concentration occurred with no change in BP or pulse, an observation consistent with the lack of hemodynamic effect observed in hypertensive patients after the administration of 300 mg of SR49059 (22). The excretion of tonomoles being constant (Table 1), doubling urine osmolality will half the urine output. In patients with a mean urinary output of 12 L/d, a theoretical decrease of urine volume to 6 L/d could be obtained provided that a sustained drug effect could be reached through optimization of the drug regimen. A nonsignificant decrease in sodium excretion was observed, possibly indicating that these patients were not strictly in Na+/H1 balance and/or pointing to the possible restoration of an AVP antinatriuretic effect (47). The highest increase in urine osmolality and consequent decrease in urine volume was observed with patient 3 with a maximal increase in urine osmolality after dDAVP of 248 mOsm/kg. Further clinical studies will needed to test whether the ability to rescue will depend on basal receptor function.

The proof-of-principle results obtained in this study indicate that pharmacologic chaperone-based therapy could be applied to other missense mutations or in-frame deletions or insertions that are responsible for X-linked NDI. Among the 207 families who had X-linked NDI and were referred to our laboratory (48; unpublished data), 66 of the 155 different putative disease–causing mutations are missense mutations that potentially are amenable to rescue by these pharmacologic chaperones.

Unfortunately, the clinical development of SR49059 has been interrupted during the course of these studies as a result of possible interference with the cytochrome P450 metabolic pathway. We would have liked to administer SR49059 to other patients with congenital NDI caused by a noncorrectable defect such as nonsense V2 receptor mutants or aquaporin mutants, but this was not possible. We also wanted to test whether other

Figure 6. YM087 treatment on cell surface expression and AVP-stimulated cAMP accumulation of nine missense (L59P, L83Q, Y128S, R137H, W164S, A165D, S167L, A294P, P322H), one in-frame deletion (185_193del), and one nonsense (W284X) V2 receptors in COS-1 cells. cAMP units are the same as used in Figure 5.
vasopressin antagonists, which could be clinically developed, may also act as pharmacologic chaperones for NDI-causing mutations. Another nonpeptide vasopressin receptor antagonist that is in advanced clinical testing phase and has an excellent safety profile for another application (27), YM087, was found to rescue cell surface expression and function of nine missense V2 mutant receptors. This provides supporting data that will allow us and others to test this compound and additional vasopressin ligands, including nonpeptide vasopressin agonists in patients with X-linked NDI, when they become available for such trials.

In addition to be a promising avenue for the treatment of X-linked NDI, stabilization of protein conformation, using small cell–permeable ligands, may represent a generally applicable rescue strategy for different diseases resulting from improper protein folding and targeting. These diseases include cystic fibrosis, osteogenesis imperfecta, α1-antitrypsin deficiency, NDI caused by mutations in AQP2, Gitelman syndrome, Fabry disease, and many others (49–58).

Acknowledgments

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References

Pharmacochaperones post-translationally enhance cell surface expression by increasing conformational stability of wild-type and mutant vasopressin V2 receptors. J Biol Chem 279: 47254–47263, 2004


50. Burrows JA, Willis LK, Perlmutter DH: Chemical chaper-


See related editorial, “Thinking about Rare Kidney Diseases,” on pages 15–16.
Correlation


The authors regretfully report two labeling errors in the figures of this article. In Figure 2B, the cellulose acetate (CA) and ethylen-vinyl-alcohol (EVAL) labels should be reversed (see below). Also, in Figure 5 the black bars indicate CA, not AC. Please see the corrected figures below.

Correction


In this article, the authors regretfully report an error in a plasma concentration datum. The second sentence of the first full paragraph in the right-hand column on page 239 should read as follows (bold text indicates corrected information):

A 300-mg dose of the F1 formulation was reported in the Clinical Investigator Brochure to increase plasma concentration to 18.5 ng/ml (30 nanomolar, MW of SR49059 is 620.5) 3 h after the administration of SR49059 to normal volunteers.

Correction


In this article, the authors regretfully report an error in the representation of data as seen in Figure 5. The data discussed in the text is correct, as are the numbers in the figure, but the y-axis and the lines in the graph are not correct. Please see the corrected figure below.