school, coming to the profession for timeless reasons — because of a physician they admire, or because they want to serve, or because they have suffered or witnessed suffering. Perhaps some lucky ones even today have been called to medicine through the medium of a book. If they have a love of literature, reading may well help them to discover a way to understand and identify with the ambitions, sorrows, and joys of the people whose lives are put in their hands. In medicine, we often separate life events from their meaning for those who live them. In literature, the two are united. That is reason enough to keep reading. And writing.


Hyperactive Vasopressin Receptors and Disturbed Water Homeostasis

Nine V.A.M. Knoers, M.D., Ph.D.

Regulation of water homeostasis is of vital importance for all terrestrial organisms. In most mammals, including humans, the maintenance of water balance is critically dependent on water intake, the sensation of thirst, and the regulation of water excretion in the kidney, which is under the control of the antidiuretic hormone arginine vasopressin (AVP).1 In the past decade, our insight into the AVP-mediated renal concentration mechanism has substantially improved with the elucidation of the roles of crucial molecular players in this process. The identification of disease-causing genes in hereditary disorders of water balance has been extremely helpful in identifying these pivotal molecules.

In normal physiology, AVP is secreted into the circulation by the posterior pituitary gland, in response to an increase in serum osmolality or a decrease in effective circulating volume (see diagram). In the kidney, AVP binds to the V2 vasopressin receptor in the basolateral membranes of collecting-duct cells in the last portion of the nephron (see diagram). Occupancy of this receptor results, by means of signaling through a guanine nucleotide–binding regulatory protein (G protein), in mediated activation of adenylate cyclase and the formation of cyclic adenosine monophosphate (cAMP). cAMP, in turn, activates protein kinase A, which promotes the fusion of cytoplasmic vesicles containing aquaporin-2 water-channel proteins with the apical membrane. As a result, this normally water-tight membrane becomes water-permeable. Driven by the osmotic gradient of sodium, water is then transcellularly reabsorbed, entering the cells through aquaporin-2 in the apical membrane and leaving the cells for the interstitium through aquaporin-3 and aquaporin-4, which reside in the basolateral membrane. The withdrawal of AVP results in endocytosis of the water channels, restoring the water-impermeable state of the apical membrane.

Proof of principle for the critical roles of both the V2 receptor and aquaporin-2 in orchestrating the AVP-mediated renal concentration mechanism came from the identification of mutations in the genes encoding these proteins in patients with congenital nephrogenic diabetes insipidus.2 This rare genetic disorder is characterized by a failure to concentrate urine despite normal or elevated levels of AVP. Polyuria and polydipsia, usually associated with hypernatremia, are typical features of this disorder. The disease is particularly serious in infants, in whom recurrent episodes of dehydration may result in severe neurologic deficits, growth retardation, or even death. Mutations in the gene encod-
Physiology of Water Homeostasis in Humans (Panel A) and Pathway of AVP Signaling in Renal Collecting-Duct Cells Involved in Regulating Water Excretion (Panel B).

The cells of the collecting duct are polarized into an apical surface that faces the lumen of the collecting duct, through which the tubular fluid flows, and a basolateral surface that faces the circulating blood. The relevant disorders of water balance include the syndrome of inappropriate antidiuretic hormone secretion (SIADH), congenital nephrogenic diabetes insipidus (CNDI), and nephrogenic syndrome of inappropriate antidiuresis (NSIAD). The aquaporin-2 water channel is regulated by vasopressin, and the aquaporin-3 and aquaporin-4 water channels are constitutively expressed. AVP denotes arginine vasopressin, and cAMP cyclic adenosine monophosphate.
Activating (gain-of-function) mutations in the gene encoding aquaporin-2 are responsible for congenital nephrogenic diabetes insipidus, and mutations in the gene encoding the V2 receptor (AVPR2) cause the X-linked form of congenital nephrogenic diabetes insipidus, and mutations in the gene encoding aquaporin-2 are responsible for both the autosomal recessive and autosomal dominant forms.

The V2 receptor is a typical member of the large superfamily of G protein–coupled receptors, which includes receptors for hormones, neurotransmitters, light, odorants, lipids, and ions. Once an agonist binds to such a receptor, an intracellular signal cascade is induced through the coupling and activation of a G protein. G protein–coupled receptors play key roles in almost all physiological functions, and mutations in the genes encoding these receptors have been identified in patients with a variety of inherited and acquired disorders, such as retinitis pigmentosa, hypothyroidism and hyperthyroidism, dwarfism, fertility disorders, obesity, and even cancer. Most such mutations that are associated with disease are inactivating mutations, resulting in reduced or total lack of response to the agonist. These loss-of-function mutations are almost exclusively germ-line mutations.

Activating (gain-of-function) mutations in G protein–coupled receptors, which induce constitutive receptor activation in the absence of the agonist, occur less frequently and consist primarily of somatic mutations that have been identified in certain adenomas and malignant tumors. Such activating mutations with germ-line transmission have been identified in only a few inherited disorders (see table).

At present, more than 180 different mutations in AVPR2 have been described. These mutations all cause congenital nephrogenic diabetes insipidus and, consistently, are inactivating mutations, resulting in receptor malfunction at different levels, such as reduced receptor expression at the cell surface or disturbances in hormone binding and G protein coupling.

In this issue of the *Journal* (pages 1884-1890), Feldman and coauthors describe two unrelated male infants with euvoletic hyponatremia and serum hypo-osmolality, along with inappropriately elevated urine osmolality and urine sodium concentrations. At first glance, the disorder in these boys resembles the syndrome of inappropriate antidiuretic hormone secretion (SIADH), a relatively common disorder characterized by insufficient suppression of AVP secretion in relation to the degree of hypo-osmolality, which leads to inappropriate urine concentration. Since serum AVP levels were undetectably low in both boys, Feldman et al. ruled out SIADH and hypothesized that a hyperactive V2 receptor could be the underlying cause of the disorder. Sequencing of AVPR2 in the two patients revealed a hemizygous point mutation in each. In an in vitro functional assay, both mutations were shown to lead to the production of a constitutively active V2 receptor. The consequence was AVP-independent, and therefore inappropriate, activation of V2 receptor–mediated renal urine concentration. Remarkably, and for reasons that remain unexplained, neither patient showed any clinical or biochemical sign of constitutive activation of extrarenal V2 receptors, which are known to mediate increases in circulating coagulation and fibrinolytic factors and a decrease in diastolic blood pressure after AVP stimulation.

The two identified activating AVPR2 mutations result in substitutions of the same amino acid, located in a part of the receptor protein that is highly conserved among G protein–coupled receptors and that is involved in G protein coupling. Elucidation of the exact mechanism underlying the activation of these mutant V2 receptors will undoubtedly be
Nephrogenic Syndrome of Inappropriate Antidiuresis

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FLUID HOMEOSTASIS DEPENDS ON PROPER WATER INTAKE, GOVERNED BY
an intact thirst mechanism, and on urinary excretion of free water, mediated by appropriate secretion of arginine vasopressin (AVP) (also known as antidiuretic hormone). AVP exerts its antidiuretic action by binding to the V2 vasopressin receptor (V2R), a G protein–coupled receptor, on the basolateral membrane of epithelial cells in the collecting duct of the kidney. Ligand binding activates the V2R, stimulating adenylate cyclase by means of Gs proteins. The resulting increase in intracellular cyclic AMP (cAMP) promotes shuffling of intracellular vesicles containing the water channel aquaporin-2 to the apical membrane of the collecting-duct cells, thereby increasing water permeability and inducing antidiuresis.

Clinical disorders of water balance are common, and alterations in many steps of this pathway have been described. Urinary concentrating defects associated with diabetes insipidus may result from a deficiency of AVP or from nephrogenic causes, such as X-linked, inactivating mutations in the V2R or autosomal recessive or autosomal dominant lesions in aquaporin-2. Conversely, the syndrome of inappropriate antidiuretic hormone secretion (SIADH) manifests as an inability to excrete a free water load, with inappropriately concentrated urine and resultant hyponatremia, hypo-osmolarity, and natriuresis. SIADH occurs in the setting of euvolementa, without evidence of renal disease or thyroxine or cortisol deficiency. Though usually transient, SIADH may be chronic; it is often associated with drug use or a lesion in the central nervous system or lung. When the cardinal features of SIADH were defined by Barter and Schwartz, AVP levels could not be measured. Subsequently, radioimmunoassays have revealed...
that SIADH is usually associated with measurably elevated serum levels of AVP.

We describe two unrelated male infants whose clinical presentation was consistent with the presence of chronic SIADH but who had undetectable AVP levels. We postulated that novel activating mutations of the V2R might account for their unique presentation. Evaluation revealed novel activating mutations of the V2R leading to what we term “nephrogenic syndrome of inappropriate antidiuresis” (NSIAD).

CASE REPORTS

Patient 1 presented at 3 months of age with irritability, and Patient 2 presented at 2.5 months of age with two generalized seizures. Both children had had unremarkable early neonatal courses. Both were exclusively bottle-fed formula (7 mmol of sodium per liter). Both infants had mild systolic hypertension with otherwise normal physical examinations. Initial laboratory evaluations demonstrated hyponatremia with normal serum levels of potassium and bicarbonate (Table 1). Both children had serum hypo-osmolality with inappropriately elevated urinary osmolality and urinary sodium levels. Both had low blood urea nitrogen and low or low-normal serum creatinine levels, low or suppressed plasma renin activity, and normal aldosterone levels, indicating euvolemma. Serum cortisol, thyroid-function tests, and coagulation studies were all normal. Imaging studies of the head and chest were unremarkable, with the exception of a small pars intermedia cyst in Patient 2. Despite clinical and laboratory presentations consistent with the presence of SIADH, serum AVP levels were undetectable in both patients (Quest Diagnostics Nichols Institute). Both children were initially treated with fluid restriction, followed by the administration of an osmotic agent (urea), resulting in increased urinary output and normalization of the serum sodium level.

Neither child had a family history of hyponatremia or of an SIADH-like syndrome. Both patients had no siblings. The mother of Patient 1, subsequently found to be heterozygous for an activating mutation of AVPR2 (see below), had normal simultaneous serum sodium levels (140 mmol per liter) and serum and urine osmolality (293 and 795 mOsm per kilogram of water, respectively).

The parents of the patients provided written informed consent for the publication of the case reports through a protocol approved by the institutional review board of the University of California at San Francisco.

METHODS

MUTATION ANALYSIS

Genomic DNA from the two patients and their mothers was isolated from whole blood with the use of the Puregene Blood Kit (Gentra Systems). The entire coding region of the V2R gene, AVPR2, was amplified as described previously. The resulting amplicons were sequenced with multiple forward and reverse primers with the use of Big Dye (version 3.1) sequencing chemistry and an ABI Prism sequencer (model 3100, Applied Biosystems) according to the manufacturer’s protocols. SeqScape software (Applied Biosystems) was used to assemble the sequence data and compare the results with the AVPR2 reference sequence (GenBank accession number NT 025965).

CONSTRUCTION OF VASOPRESSIN EXPRESSION CONSTRUCTS

pCDNA3 (Invitrogen) was used as the control plasmid. Human wild-type V2R complementary DNA (cDNA), subcloned into pCDNA3, was used as a normal control and template for mutagenesis. Site-directed mutagenesis was performed with the use of a QuickChange II site-directed mutagenesis kit (Stratagene). Mutations were confirmed by direct sequencing.

CELL CULTURE AND TRANSIENT TRANSFECTION

For functional studies, COS-7 cells were cultured in Dulbecco’s modified Eagle’s medium with 10 percent fetal-calf serum and antibiotics. Cells were plated in six-well plates (Falcon 3046, Becton Dickinson) at approximately 95 percent confluence 24 hours before transfection with the use of Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. Each well received 3 µg of plasmid DNA and 500 ng of a cAMP-responsive luciferase reporter plasmid (pCREluc) containing 16 copies of the consensus cAMP response element. To control for transfection efficiency, cells were cotransfected with 50 ng of renilla luciferase reporter plasmid (pRL-CMV, Promega) per well. Cells were incubated at 37°C in 5 percent carbon dioxide for 24 hours after transfection and were then lysed and assayed for luciferase activity with the use of the Dual Luciferase Reporter Assay System (Promega).
Table 1. Characteristics of the Two Patients. *

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Age-Matched Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at presentation (mo)</td>
<td>3.0</td>
<td>2.5</td>
<td>—</td>
</tr>
<tr>
<td>Clinically significant findings</td>
<td>Irritability</td>
<td>Generalized seizures</td>
<td>—</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>80–128</td>
<td>93–118</td>
<td>108†</td>
</tr>
<tr>
<td>Diastolic</td>
<td>34–92</td>
<td>37–57</td>
<td>66†</td>
</tr>
<tr>
<td>Serum and plasma studies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium (mmol/liter)</td>
<td>123</td>
<td>118</td>
<td>134–143</td>
</tr>
<tr>
<td>Potassium (mmol/liter)</td>
<td>4.6</td>
<td>4.7</td>
<td>3.4–4.9</td>
</tr>
<tr>
<td>Chloride (mmol/liter)</td>
<td>91</td>
<td>86</td>
<td>98–107</td>
</tr>
<tr>
<td>Bicarbonate (mmol/liter)</td>
<td>28</td>
<td>21</td>
<td>23–32</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>&lt;0.3</td>
<td>0.3</td>
<td>0.3–0.7</td>
</tr>
<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>&lt;5</td>
<td>3</td>
<td>8–23</td>
</tr>
<tr>
<td>Aldosterone (ng/dl)</td>
<td>10</td>
<td>24‡</td>
<td>6–68</td>
</tr>
<tr>
<td>Plasma renin activity (ng of angiotensin I/ml/hr)</td>
<td>3.8</td>
<td>&lt;1‡</td>
<td>&lt;15</td>
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<td>Osmolality (mOsm/kg)</td>
<td>252</td>
<td>247</td>
<td>285–293</td>
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<tr>
<td>AVP (pg/ml)</td>
<td>&lt;1</td>
<td>&lt;1‡</td>
<td>1.0–13.3</td>
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<td>Thrytropin (mlU/liter)</td>
<td>0.77</td>
<td>4.58</td>
<td>1.7–9.1</td>
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<tr>
<td>Free thyroxine (ng/dl)</td>
<td>1.09</td>
<td>0.97</td>
<td>0.8–1.8</td>
</tr>
<tr>
<td>Cortisol (µg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>13.3</td>
<td>10.3, 43§</td>
<td>4–20</td>
</tr>
<tr>
<td>60 Min after intravenous synthetic corticotropin (15µg/kg)</td>
<td>36.5</td>
<td>—</td>
<td>&gt;20</td>
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<tr>
<td>Coagulation studies</td>
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<td></td>
<td></td>
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<tr>
<td>von Willebrand factor antigen (%)</td>
<td>104</td>
<td>100</td>
<td>46–155</td>
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<tr>
<td>Ristocetin cofactor (%)</td>
<td>108</td>
<td>65</td>
<td>56–155</td>
</tr>
<tr>
<td>Factor VIII activity (%)</td>
<td>153</td>
<td>123</td>
<td>50–150</td>
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<tr>
<td>Fibrinogen (mg/dl)</td>
<td>198</td>
<td>360</td>
<td>170–435</td>
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<tr>
<td>d-Dimer fragment</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Prothrombin time (sec)</td>
<td>11.7</td>
<td>10.9</td>
<td>9.2–11.9</td>
</tr>
<tr>
<td>Partial-thromboplastin time (sec)</td>
<td>24.3</td>
<td>32.5</td>
<td>21.3–34.8</td>
</tr>
<tr>
<td>Urine studies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>284</td>
<td>390‡</td>
<td>300–900</td>
</tr>
<tr>
<td>Sodium (mmol/liter)</td>
<td>35</td>
<td>75‡</td>
<td>—</td>
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<tr>
<td>Magnetic resonance image of the head</td>
<td>Normal</td>
<td>Normal</td>
<td>—</td>
</tr>
<tr>
<td>Chest radiograph</td>
<td>Normal</td>
<td>Normal</td>
<td>—</td>
</tr>
</tbody>
</table>

* To convert values for creatinine to micromoles per liter, multiply by 88.4. To convert values for urea nitrogen to millimoles per liter, multiply by 0.357. To convert values for aldosterone to nanomoles per liter, multiply by 0.0277. To convert values for AVP to picomoles per liter, multiply by 0.923. To convert values for free thyroxine to picomoles per liter, multiply by 12.87. To convert values for cortisol to nanomoles per liter, multiply by 27.59. To convert values for fibrinogen to micro-moles per liter, multiply by 0.0294.

† Measurement is given for the 95th percentile.

‡ The value was obtained during an episode of hyponatremia after initial presentation.

§ Two baseline levels were obtained on separate occasions.
as described previously.7,8 Data are presented as mean (±SE) luciferase activity expressed in arbitrary units and adjusted for renilla luciferase activity in three experiments, each performed in triplicate. All five types of samples (pCDNA3 vector alone and vector with wild-type V2R, the R137C mutant, the R137L mutant, or the R137H mutant) were run simultaneously under the same conditions during each of the three experiments.

RESULTS

Genomic DNA was isolated from both boys and their mothers, and the V2R gene, AVPR2, was sequenced directly. AVPR2 is X-linked, and therefore, paternal DNA is not informative. Each patient carried a mutation in codon 137 of AVPR2. In Patient 1, nucleotide 770 was mutated from cytosine to thymine, changing arginine to cysteine at codon 137 (R137C); in Patient 2, nucleotide 771 was mutated from guanine to thymine, changing arginine to leucine at codon 137 (R137L) (Fig. 1A). Arginine 137 maps to the predicted second cytoplasmic loop, near the cytoplasmic boundary of the third transmembrane domain; this same amino acid is mutated to histidine (R137H) in a form of familial nephrogenic diabetes insipidus, R137L and R137C mutations in G protein–coupled receptors, the highly conserved motif of physiological processes.10 Many diseases are caused by mutations in G protein–coupled receptors.11,12 For some G protein–coupled receptors, a particular disease state has been ascribed to inactivating mutations that render the receptors unresponsive to ligand, whereas a converse condition has been linked to gain-of-function mutations, resulting in constitutive activation.

Although many different inactivating mutations of the X-linked V2R have been described that cause nephrogenic diabetes insipidus,2 to our knowledge, no naturally occurring activating mutations of the V2R have been reported previously. The two cases described here are characterized by chronic SIADH but with undetectable AVP levels, constituting a novel example of gain-of-function mutations caused by a hemizygous V2R mutation. In the light of these findings, we suggest referring to all SIADH-like conditions as syndromes of inappropriate antidiuresis (SIAD) and that these two case reports constitute a subtype, NSIAD. To our knowledge, these cases are the only reported examples in which mutations affecting the same amino acid cause two different genetic diseases: R137H causes nephrogenic diabetes insipidus, and R137L and R137C cause NSIAD (Fig. 1B).

The mechanism by which these missense mutations constitutively activate the V2R requires further investigation. V2R, a class 1b G protein–coupled receptor,12,13 exists in the plasma membrane in equilibrium with inactive and active conformations.10 The binding of ligand shifts the equilibrium to the active state, permitting coupling with intracellular G proteins and activation of intracellular effectors. With activation, V2R is desensitized through phosphorylation by specific G protein–coupled receptor kinases. Subsequent recruitment of β arrestin to the phosphorylated receptor terminates the signal by blocking further interaction with G proteins and also initiates receptor internalization through its ability to bind clathrin and other endocytic adapters.14

By comparison with other class 1 G protein–coupled receptors, the highly conserved motif of aspartic acid, arginine, and tyrosine or histidine (DRY/H) in V2R at the junction of the third transmembrane domain and second intracellular loop

DISCUSSION

G protein–coupled receptors constitute the largest gene family of receptors involved in signal transduction and are responsible for regulating many physiological processes.10 Many diseases are caused by mutations in G protein–coupled receptors.11,12 For some G protein–coupled receptors, a particular disease state has been ascribed to inactivating mutations that render the receptors unresponsive to ligand, whereas a converse condition has been linked to gain-of-function mutations, resulting in constitutive activation.
Figure 1. Nucleotide Sequence of the Wild-Type and Two Mutant AVPR2 Genes in the Affected Region (Panel A) and Diagram of V2R (Panel B).

In Panel A, the normal CGC sequence encoding R137 is changed to TGC (R137C) in Patient 1 and to CTC (R137L) in Patient 2. In Panel B, R137 is indicated. The inset highlights changes in R137 that result in either congenital nephrogenic diabetes insipidus or NSIAD.
appears to be critical for receptor function. The arginine residue in the DRY/H motif appears to be invariant, though the aspartic acid and tyrosine may be replaced by other amino acids without altering the function (e.g., from aspartic acid to glutamic acid). The arginine residue in the DRY/H motif corresponds to R137, the site of the inactivating R137H mutation in nephrogenic diabetes insipidus and the activating R137C and R137L mutations in NSIAD. The R137H mutant behaves as a constitutively desensitized receptor, since it is phosphorylated, binds to β arrestin (thereby blocking its ability to activate G proteins), and is sequestered in intracellular vesicles.

Constitutively activated mutant G protein–coupled receptors have also been created by altering this DRY/H motif — for example, through in vitro mutation of aspartate to alanine at codon 136 in the V2R. The NSIAD mutations found in this domain may stabilize the receptor in an active conformation, activating G proteins and downstream signaling events in the absence of ligand. In theory, such a gain of function could also affect many other aspects of V2R biology, including phosphorylation, internalization, down-regulation, and recycling to the cell membrane.

The effects of the constitutive activation of V2R may not be limited to the kidney. V2R is also expressed in endothelial cells, where it appears to mediate vasodilation after the administration of the vasopressin analogue desmopressin and to mediate the rise in circulating levels of von Willebrand factor and tissue plasminogen activator. We thought that these responses, which are absent in patients with nephrogenic diabetes insipidus, might be constitutively activated in our patients with NSIAD. However, we found no clinical or laboratory evidence of coagulopathy in either patient (Table 1).

The frequency of NSIAD is not known, but it may not be rare. Previous studies of patients with SIADH have noted variable patterns of AVP secretion, particularly in response to water loading and water restriction. As many as 10 to 20 percent of affected patients have AVP levels at or below the limits of detection by radioimmunoassay. Thus, some of these patients may in fact have NSIAD due to V2R-activating mutations. Such patients would probably have clinical presentations similar to those in our patients, although the severity and clinical course may depend on the nature of the mutation.

Patients with NSIAD would be expected to have low AVP levels. However, since the AVP assay is not optimized to identify low values, we recommend sequencing the V2R gene to identify specific mutations before the diagnosis of NSIAD is finalized. Other patients may be identified who have the NSIAD phenotype but without V2R mutations, suggesting the presence of additional defects in this signaling cascade. One such possibility would be an activating mutation in aquaporin-2.

Treatment of NSIAD poses a challenge. Water restriction improved serum sodium levels and osmolality in both infants but limited calorie intake in these formula-fed infants. Agents that act downstream from the V2R, such as demeclocycline or lithium, might antagonize the constitutively activated receptors, but they have potentially limiting adverse effects. AVP antagonists are under clinical development but would probably be ineffective, given the ligand-independent nature of the lesion. Ideally, one might be able to use an inverse agonist.

The ability of the mutant V2R from each patient to induce cAMP, as measured by a cAMP-inducible luciferase reporter, was evaluated. Each bar represents cells transfected with the renilla luciferase expression vector, the cAMP-inducible luciferase reporter plasmid, and pCDNA3 either alone (control), reflecting basal cAMP levels in the cells, or with the following inserts subcloned into the pCDNA3 plasmid: wild-type V2R; R137H mutant V2R, found in Patient 1; or R137L mutant V2R, found in Patient 2. Each value represents the mean (±SE) of three independent experiments with all five samples run simultaneously, each performed in triplicate.
that would suppress receptor activity in the absence of agonist; two potential nonpeptide V2R inverse agonists have been studied in vitro. In the absence of a definitive therapy, we have successfully treated our patients with urea to induce an osmotic diuresis. This approach has occasionally been used for the treatment of chronic SIADH in adults.

Study of the V2R has been important for understanding the physiology of water balance and has served as a prototype for G protein–coupled receptor biology. Further characterization of NSIAD may offer additional insights into fluid homeostasis and clinical disease, as well as expand our understanding of G protein–coupled receptor signaling.

Supported in part by a training grant (T32DK07161, to Drs. Feldman and Huang) and a grant (M01RR01271, to the Pediatric Clinical Research Center) from the National Institutes of Health.

REFERENCES


Dr. Rosenthal reports having received grant support and speaking honoraria from Pfizer and having served on an advisory board for Tereica and Pfizer. Dr. Vargas reports having received grant support from Wyeth and Janssen. Dr. Lustig reports having received lecture fees from Novo Nordisk and grant support from Novartis. Dr. Mathis reports having equity interest in AstraZeneca, Pfizer, Merck, and SIRNA Therapeutics; receiving grant support from Satellite Healthcare and Genentech; and receiving speaking honoraria from Shire. Dr. Fenwick is employed by Quest Diagnostics, owns stock in the company, and developed the clinical assay used for sequencing AVPR2 in this study.

We are indebted to Jan Ocrant and Kamer Tezcan for referring the patients; to Mariel Birnbaumer (National Institute of Environmental Health Sciences, Division of Intramural Research) for sharing the V2R cDNA; to Christian Vaisse (University of California at San Francisco) for the cAMP-responsive luciferase reporter plasmid; to Mark von Zastrow (University of California at San Francisco), Jon Nakamoto (Quest Diagnostics), and Hillel Gitelman for thoughtful discussions and review of the manuscript; and to Isabella Damm (University of California at San Francisco) for technical support.
of great help in clarifying the relationships between the structure and function of the V2 receptor and possibly other G protein–coupled receptors; it may even guide the design of rational drugs for V2 receptor–associated disease.

This report adds a new example to the currently short list of gain-of-function mutations as causes of genetic disorders of tubular transport. The identification and functional analysis of such disease-associated gain-of-function mutations, however, have proved important to our understanding of the physiology of renal tubular transport. For instance, functional analysis of activating mutations in the renal epithelial sodium channel identified in Liddle’s syndrome, an autosomal dominant form of salt-sensitive hypertension, provided further insight into normal regulation of this channel. The activating mutations were shown to disrupt the interaction of the renal epithelial sodium channel with an important regulatory protein called Nedd4; failure of this interaction prevents the removal of the renal epithelial sodium channel from the cell surface, leading to constitutive expression at the cell membrane and increased sodium reabsorption. This mechanism accounts for the development of hypertension. An increased awareness of the possibility of gain-of-function mutations, triggered by the report of Feldman et al., may well lead, in the near future, to the elucidation of other unexplained genetic disorders of tubular transport.