Autosomal recessive familial neurohypophyseal diabetes insipidus with continued secretion of mutant weakly active vasopressin

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Received February 23, 1999; Revised and Accepted April 15, 1999

Familial neurohypophyseal diabetes insipidus is an autosomal dominant disorder characterized by postnatal development of arginine vasopressin (AVP) deficiency due to mutations in the AVP gene. All published mutations affect the signal peptide or the neurophysin-II carrier protein and are presumed to interfere with processing of the preprohormone, leading to neuronal damage. We studied an unusual Palestinian family consisting of asymptomatic first cousin parents and three children affected with neurohypophyseal diabetes insipidus, suggesting autosomal recessive inheritance. All three affected children were homozygous and the parents heterozygous for a single novel mutation (C301→T) in exon 1, replacing Pro7 of mature AVP with Leu (Leu-AVP). Leu-AVP was a weak agonist with ~30-fold reduced binding to the human V2 receptor. Measured by radioimmunoassay with a synthetic Leu-AVP standard, serum Leu-AVP levels were elevated in all three children and further increased during water deprivation to as high as 30 times normal. The youngest child (2 years old) was only mildly affected but had Leu-AVP levels similar to her severely affected 8-year-old brother, suggesting that unknown mechanisms may partially compensate for a deficiency of active AVP in very young children.

INTRODUCTION

Familial neurohypophyseal diabetes insipidus (FNDI) (reviewed in refs 1,2) accounts for ~1% of all cases of neurohypophyseal or ‘central’ diabetes insipidus (DI) (3). It is characterized clinically by polyuria and polydipsia, typically beginning between 1 and 6 years of age. This is a result of progressive deficiency of the antidiuretic hormone arginine vasopressin (AVP) and is often accompanied by the loss of the posterior pituitary bright spot on T1 magnetic resonance imaging (4). It is thought to be caused by specific degeneration of vasopressin-secreting magnocellular neurons in the hypothalamic paraventricular and supraoptic nuclei (1,2,5). Affected individuals respond clinically to exogenous AVP and its analogs such as desmopressin (DDAVP).

The genetic locus for FNDI is the AVP–neurophysin II (AVP–NPII) gene on chromosome 20 (6,7). The AVP–NPII gene contains 3 exons which encode a preprohormone consisting of a signal peptide followed by AVP itself, its 93 amino acid carrier protein NPII and a 39 amino acid glycopeptide of unknown function, copeptin (Fig. 1) (8). The prohormone is cleaved within neurosecretory granules and then eventually reassembled into AVP–NPII tetramers prior to secretion from the posterior pituitary.

All published mutations causing FNDI have an autosomal dominant mode of inheritance (9), have been located in either the signal peptide or the NPII carrier protein and are known or reasonably presumed to disrupt the normal cellular processing of the AVP–NPII preprohormone leading to neuronal damage (10,11). We report a kindred with an autosomal recessive form of FNDI caused by a mutation in the AVP hormone itself, resulting in the progressive loss of antidiuretic activity despite continued hypersecretion of the mutant hormone.

RESULTS

Sequence analysis of the AVP–NPII gene

All three affected children carried a homozygous missense mutation at nucleotide 301, Pro7→Leu (P7L, CCG→CTG), within the vasopressin peptide moiety of exon 1 (Fig. 1). Both unaffected parents were heterozygous carriers for the same missense mutation.

In vitro functional analysis of mutant Leu-AVP hormone

The ability of the P7L mutant hormone (Leu-AVP) to bind the human V2 receptor was investigated in LV2 murine cells stably transfected with cDNA encoding this receptor. The Ki was greater for Leu-AVP (70 nM; Fig. 2) than for normal AVP (4 nM; 13). Similarly, the EC50 for adenylate cyclase activity (the concentration of agonist required to achieve 50% of the maximum stimulated cAMP level) was >10-fold higher (0.9 versus 0.05 nM) for Leu-AVP than for normal AVP (Fig. 2). These results demonstrate that Leu-AVP is a weak agonist with decreased binding to the V2 receptor.

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Clinical investigations

Both parents tolerated 18 h of water deprivation without difficulty. Serum osmolarity remained in the upper end of the normal range while urine became maximally concentrated, and serum sodium levels remained within normal limits (Table 1). Water deprivation testing on all three children yielded results consistent with central DI although patient 3 was less severely affected than patients 1 and 2, her elder brothers. On arrival at the hospital 18 h after their last DDAVP dose, serum sodium and osmolarity levels for the two brothers were already elevated and the initial urine samples were very dilute. Their tests had to be terminated after an additional 4 h water deprivation due to further elevation of serum sodium and osmolarity. In contrast, baseline serum sodium and osmolarity of patient 3 were within normal limits and she tolerated 10 h of water deprivation.

Plasma AVP levels of both parents remained within normal limits during water deprivation (Table 1). The corrected baseline plasma Leu-AVP levels were elevated in all three children and were further increased several-fold by the end of water deprivation.

Magnetic resonance imaging (MRI) studies demonstrated that only patient 3 still had a normal hyperintense T1 signal over the posterior pituitary (data not shown). MRI studies were not performed on the parents.

DISCUSSION

This kindred differs in important ways from others affected with FNDI. First, the disease is inherited in an autosomal recessive manner and no abnormality can be detected in the parents’ responses to water deprivation. Second, the P7L missense mutation alters AVP hormone action by virtue of its location within the hormone itself. Although this study did not examine whether preprohormone processing or oligomerization per se is affected by this mutation, the autosomal recessive mode of inheritance suggests that abnormal oligomers are not formed to a significant extent in heterozygous carriers.

Similar to other children with FNDI, each child in our kindred was asymptomatic for at least the first year of life, as evidenced by normal growth and lack of polyuria or polydipsia. Moreover, water deprivation tests on patients 1 and 3 at 2 years of age yielded equivocal results, suggesting that some degree of antidiuretic function persisted until that age. Unlike other patients with FNDI, no normal AVP could have been synthesized in our patients even at birth. Thus, Leu-AVP apparently had sufficient in vivo activity to delay development of signs and symptoms of DI in our patients for at least 1 year of life. Presumably, increased secretion of the mutant hormone was able to compensate for its decreased activity.

There are insufficient data to determine why our patients developed DI despite continued secretion of mutant hormone.
Although longitudinal data are unavailable, secretory capacity may have decreased somewhat over time, as evidenced by comparing Leu-AVP levels among the three children at similar serum osmolality (patient 3, 13.5 pg/ml at 310 mOsm; patient 2, 8.4 pg/ml at 317 mOsm; patient 1, 6.8 pg/ml at 312 mOsm; Table 1) and by the loss of the hyperintense signal over the neurohypophysis in patients 1 and 2 but not patient 3. Such levels may not be sufficient to maintain antidiuresis if, as suggested by the in vitro data, the mutant hormone is 10- to 20-fold less active than normal. Alternatively, continued exposure to high levels of mutant hormone might desensitize renal AVP receptors or downstream signaling pathways in some manner.

Although we are not aware of systematic studies addressing this issue, it is also possible that renal sensitivity to AVP may be generally higher in infancy than later in childhood.

### MATERIALS AND METHODS

#### Case reports

A Palestinian Arab family presented with three children who all developed symptoms of DI by 2 years of age. The parents were first cousins and had never had symptoms of DI. Indeed, they were practicing Muslims who routinely tolerated abstaining from food and drink from sunrise to sunset during the Muslim month of Ramadan.

Patient 1 was born weighing 10 lb following an uncomplicated term pregnancy and delivery. He developed polydipsia and polyuria at 18–24 months. At 2 years of age he was referred to two consecutive 8 h water deprivation tests at another hospital. The initial test resulted in hypernatremia but the second test was considered normal. Over the next 2 years his polyuria and polydipsia worsened and he was referred to an endocrinologist at age 4 years 3 months. His height was 105.4 cm (50th percentile for age) and his weight was 23.3 kg (>95th percentile). His examination was remarkable only for moderate obesity and bilateral cryptorchidism. When allowed free access to water his daily fluid intake was 3–3.5 l and his serum Na increased to 149 with a urine specific gravity of 1.005. DI was diagnosed and he was given a trial of 5 µg of intranasal DDAVP every morning for 3 days. Afterwards his weight increased by 0.5 kg and his serum Na decreased to 140. A brain MRI scan showed an absence of the normal hyperintense T1 signal over the neurohypophysis but no evidence of any abnormal mass. By age 5 years he required twice daily doses of DDAVP. He was first seen at this institution at 7 years of age. Because of continued cryptorchidism, he was given an hCG stimulation test and had a good testosterone response (3.1 to 108 ng/dl). Testicular descent was achieved after 3 weeks of 1500 U hCG injections twice a week. At 8 years of age, when this investigation began, his DI was well controlled by 10 µg of intranasal DDAVP twice daily.

Patient 2 weighed 8 lb at birth following a normal term pregnancy. Between 9 and 12 months of age he began to grow poorly and his height fell to the 25th percentile, his head circumference to the 5–10th percentile and his weight to below the 5th percentile for his age. He had normal head computed tomography at 21 months of age. By 2 years of age he began to drink water excessively and at 28 months of age he was referred to an endocrinologist. At that time, his serum Na was 146 meq/l and serum osmolality was 300 mOsm/kg, with a urine osmolality of 79 mOsm/kg and specific gravity of 1.002. He was placed on intranasal DDAVP (5 µg at bedtime), with an improvement in laboratory values. A brain MRI showed lack of a hyperintense T1 signal over the neurohypophysis and a diminutive infundibulum. While on DDAVP his appetite for solid foods improved and his height and weight increased to the 75th percentile by 4 years of age, by which time he required 5 µg of DDAVP twice a day.

Patient 3 also had an uncomplicated birth and normal infancy. She developed polyuria and polydipsia at 15–20 months of age. A random laboratory test at 20 months revealed a serum Na of 144 and a serum osmolality of 298 with a urine osmolality of 137. She was empirically started on 5 µg of intranasal DDAVP at bedtime, which relieved her symptoms. Her height increased along the 50–75th percentile and her weight increased along the 50–75th percentile. At 30 months she was placed on twice daily DDAVP, near the time of this investigation.

### Table 1. Water deprivation test results

<table>
<thead>
<tr>
<th>Subject</th>
<th>Start* (h w/o H2O)</th>
<th>End (h w/o H2O)</th>
<th>UOP* (ml/kg/h)</th>
<th>Weight loss (%)</th>
<th>AVP* (pg/ml)</th>
<th>Serum Na+ (mmol/l)</th>
<th>Serum osmolality (mOsm)</th>
<th>Urine osmolality (mOsm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>S</td>
<td>E</td>
<td>S</td>
<td>E</td>
<td>S</td>
</tr>
<tr>
<td>1. 8-year-old male</td>
<td>2</td>
<td>6</td>
<td>8.0</td>
<td>4.6</td>
<td>6.8</td>
<td>17.3</td>
<td>149</td>
<td>155</td>
</tr>
<tr>
<td>2. 4-year-old male</td>
<td>2</td>
<td>6</td>
<td>7.8</td>
<td>4.7</td>
<td>8.4</td>
<td>69.6</td>
<td>147</td>
<td>152</td>
</tr>
<tr>
<td>3. 2-year-old female</td>
<td>2</td>
<td>12</td>
<td>3.1</td>
<td>3.0</td>
<td>3.8</td>
<td>13.5</td>
<td>140</td>
<td>148</td>
</tr>
<tr>
<td>Mother</td>
<td>14</td>
<td>18</td>
<td>0.5</td>
<td>1.6</td>
<td>1.4</td>
<td>139</td>
<td>139</td>
<td>139</td>
</tr>
<tr>
<td>Father</td>
<td>14</td>
<td>18</td>
<td>0.5</td>
<td>1.2</td>
<td>3.0</td>
<td>140</td>
<td>148</td>
<td>136</td>
</tr>
</tbody>
</table>

S, values obtained at start of test; E, values at end of test; T, values after administration of a single dose of DDAVP.

*Subjects had not had anything to drink for the indicated number of hours by the start of the test. The children had received their last DDAVP doses 18 h before the start of the test.

Urinary output. The 2-year-old girl did not urinate spontaneously and a sample was obtained by catheterization at the end of the test.

Serum vasopressin levels measured by radioimmunoassay. Levels are not adjusted for the 92% cross-reactivity between mutant Leu-AVP and wild-type AVP on this immunoassay.
PCR amplification and sequence analysis of the AVP gene

All human studies were performed under Institutional Review Board-approved protocols. Blood samples were collected from both parents and all three children and genomic DNA was isolated. Specific DNA primers were used for PCR and DNA sequencing of the AVP–NPII gene (Fig. 1a). Nucleotide numbers corresponded to those previously reported (8). Primers A (nt 34–53) and D (nt 2473–2454) were used to generate full-length AVP–NPII gene products. Primer pairs E+B, C+G and H+D were used to generate exons 1–3, respectively, as described (12). An additional primer, J (nt 142–162), was used solely for DNA sequencing (14). Direct DNA sequencing of AVP–NPII gene fragments was performed using the Thermo Sequenase α-33P-radiolabeled terminator cycle sequencing kit (Amersham Life Science, Cleveland, OH).

Synthesis of mutant Leu-AVP

Mutant AVP, with the Pro7 of normal AVP replaced by Leu (Leu-AVP), was synthesized by BioSynthesis (Lewisville, TX). Except for this substitution, Leu-AVP was made identical to normal AVP, including preservation of the disulfide bond between Cys1 and Cys6, as well as amidation of the C-terminus. Leu-AVP was purified to >95% purity by HPLC and the peptide structure was confirmed by both laser desorption mass spectrometry and by amino acid analysis. The concentration of peptide in the stock solution used for all further experiments was quantitated by HPLC and amino acid analysis (C. Slaughter, personal communication).

In vitro analysis of Leu-AVP-specific receptor binding and activity

A murine cell line expressing the human V2 vasopressin receptor, LV2-p11, at ~200 000 sites/cell was provided by Dr M. Birnbaumer (13,15). Specific binding of [3H]AVP (59 Ci/mmol; New England Nuclear, Boston, MA) to LV2-p11 cells in six-well plates was assayed as described (13). Fixed concentrations of [3H]AVP (0.1, 1.0 or 10 nM) in the absence (total binding) and presence of 10 μM unlabeled AVP (non-specific binding) were incubated for 2 h at 4°C with and without various concentrations of Leu-AVP (0.03–30 nM). The Ki for Leu-AVP was calculated as described (16).

To determine receptor-mediated cAMP activity generated by AVP and Leu-AVP, triplicate samples of 5 × 104 LV2-p11 cells plated in 12-well plates were incubated for 10 min at 37°C in Dulbecco’s minimal essential medium containing 0.1 mM 3-isobutyl 1-methyl xanthine (Sigma, St Louis, MO) with or without a stimulating agent [Forskolin (Sigma), AVP or Leu-AVP] and cell extracts were recovered as described (17). Sample extracts were then resuspended in cAMP assay buffer (50 mM sodium acetate, pH 5.8, 0.02% bovine serum albumin) and cAMP levels were measured using the non-acetylation protocol for the BIOTRAK cAMP enzyme immunoassay system (Amersham Life Science, Arlington Heights, IL).

Water deprivation testing of family members

Both unaffected parents and all three children with DI were admitted for monitoring during formal water deprivation (Table I). Parental weights were measured at the beginning (baseline) and end of the test whereas weight and urine output were recorded hourly for each child. Water deprivation testing ended after 18 h (parents) or earlier if 5% of initial weight was lost or when serum sodium increased to >150 or serum osmolality increased to >300 and urine osmolality decreased to <150. Upon termination of the test, family members were allowed to drink water ad libitum and eat. Routine intranasal DDAVP doses (5–10 μg) were administered to the children.

Radioimmunoassay of Leu-AVP and AVP

Blood samples were collected into ice-chilled tubes. Plasma was promptly separated and frozen until analysis for AVP by radioimmunoassay (Nichols Institute at Quest Diagnostics, San Juan Capistrano, CA). Standard curves for Leu-AVP and normal AVP were generated by adding known amounts of Leu-AVP or normal AVP to charcoal-stripped human plasma samples, which were extracted with acetone in a manner identical to that used for analysis of patient samples. In fact, the radioimmunoassay had nearly equivalent sensitivities for Leu-AVP (92%) and AVP (provided by Dr Raj Pandian, Nichols Institute, San Juan Capistrano, CA) (data not shown) and measured AVP levels were therefore not adjusted for sensitivity of the immunoassay for Leu-AVP.

ACKNOWLEDGEMENTS

We thank Dr Mariel Birnbaumer (UCLA) for the LV2 cell line, Dr Raj Pandian (Quest Diagnostics) for performing radioimmunoassays of Leu-AVP and Dr Clive Slaughter (UT Southwestern) for HPLC and amino acid analysis of Leu-AVP. This work was supported by grant R37 DK37867 from the National Institutes of Health.

REFERENCES


