Abstract. The arginine-8-vasopressin (AVP) responses to osmotic and histamine stimuli were evaluated in 21 patients with central diabetes insipidus (CDI) and compared to those of 10 healthy controls. Plasma AVP was measured by radioimmunoassay. Following the infusion of 2.5% saline, the AVP responses of CDI patients fell into two distinct groups: CDI I gave no response at all, while CDI II responded subnormally. Histamine increased the plasma AVP level significantly in healthy volunteers. Patients with CDI II gave subnormal AVP responses to histamine. The AVP reactions of the patients with CDI I fell into two subgroups: CDI I/A had undetectable plasma AVP, while histamine evoked AVP release in CDI I/B. Histamine trial did not lead to any change in plasma osmolality. The authors conclude that patients with CDI II suffer from a partial CDI, while those with CDI I/A represent a complete form of the disease. The remainder (CDI I/B) presumably have an osmoreceptor failure. Osmotic and non-osmotic stimulation may provide a useful tool in the differential diagnosis of CDI.

Due to the complexity of the osmoregulation of arginine-8-vasopressin (AVP) release, a variety of functional abnormalities can be assumed under the broad concept of diabetes insipidus (DI). Defects may exist in the osmoreceptors, the AVP-synthesizing neurons or their axons, in which the hormone traverses the hypothalamo-neurohypophysial tract, the storage site of AVP in the posterior pituitary, or the site of AVP action in the renal tubule (Moses et al. 1976). The differential diagnosis of the various forms of the disorder was earlier based on standardized indirect tests (Carter & Robbins 1947; Moses & Streeter 1967; Miller et al. 1970). However, sensitive and specific radioimmunoassays (RIA) for AVP determination have been developed in the past few years, and several new diagnostic procedures have now been introduced into the clinical investigation of DI, using the direct measurement of plasma AVP after osmotic (Baylis & Robertson 1980; Zerbe & Robertson 1981; Millers et al. 1983) or non-osmotic (Baylis et al. 1981; Blackett et al. 1983; Toth et al. 1984) stimulation.

Histamine has been demonstrated to exert a strong stimulatory effect on AVP release in animals (Dogterom et al. 1976; Tuomisto et al. 1980; Mens et al. 1980).

In this paper, our main objective was to evaluate the AVP responses to osmotic and histamine stimuli in patients with central diabetes insipidus (CDI), in order to determine the value of these tests in the differential diagnosis of the disorder.

Materials and Methods

Ten healthy individuals (aged 17–68 years, 2 males) and 23 patients with CDI (aged 19–51 years, 10 males) were studied after they had given informed consent. The urine volume for the patients with CDI was 7.8 ± 0.7 l/day (mean ± SEM). The diagnosis of CDI was based on generally accepted indirect tests, including water and salt challenges (László et al. 1969; Skowsky & Kikuchi 1978), the Carter-Robbins test (1947), and the administration of lysine-8-vasopressin (LVP) or 1-de-
Determination and those curves a Two Budapest, in infection amino-8-D-arginine-vasopressin (dDAVP) (Moses 1977; Zerbe & Robertson 1981) to exclude nephrogenic DI. Eleven patients had idiopathic CDI, and one patient suffered from Hand-Schüller-Christian disease. The cause of CDI was head injury in 6 cases, and virus infection in 3. Three patients with inborn CDI came from two families that had included polyuric members during many generations. One patient had suffered from CDI since birth, but without a family history of the disease. Two patients with posterior and anterior pituitary deficiencies were receiving full replacement of adrenocortical and thyroid hormones during the study. Plasma glucose and urea levels of all the subjects studied were within the normal range. Antidiuretic therapy had been discontinued at least 48 h before the commencement of the present investigation.

Each subject fasted, abstained from smoking, and drank only water for 12 h before the study, which started at 08.00 h. For 2 h before the tests, the subjects were not allowed to drink at all. After a 2-h rest in the supine position, hypertonic (2.5%) saline was infused into an antecubital vein for 2 h at a rate of 0.11 ml/kg body weight/min. Venous blood samples for the determination of plasma AVP and osmolality were obtained from the other arm before and at 30-min intervals during the infusion. The onset of thirst during the infusion was recorded. Urine samples of the patients were collected in 15 min intervals before and during hyperosmotic load via indwelling catheter.

On a separate occasion, AVP release was assessed by the administration of histamine (1 ng. Peremin, Chinoim, Budapest, Hungary). After arrangements similar to those mentioned above, 0.5 mg histamine was given sc. Venous blood samples for the measurement of AVP and osmolality were drawn before and 3, 10 and 20 min after the injection of histamine.

In all studies, for the determination of plasma AVP, 10 ml blood samples were collected in chilled polyethylene tubes containing 14 mg Na2EDTA in 300 µl isotonic NaCl, and centrifuged at 4°C within 10 min. Two ml aliquots of plasma were transferred into polypropylene tubes containing 400 µl 1 M HCl, and were kept at −20°C until assayed. RIA was performed within a week after sampling.

AVP-free human plasma for the purpose of standard curves was obtained from healthy individuals 30 min after the completion of oral water load with 20 ml/kg body weight.

Blood samples were taken in heparinized vials for the measurement of osmolality. Plasma osmolality was determined via the freezing point depression method (Advanced Digi Matic Osmometer, USA).

**Determination of plasma AVP**

A RIA was developed for plasma AVP determination. The method was based on a technique described by Dogterom et al. (1978) with some modifications.

Synthetic AVP (Organon, Oss, The Netherlands; antidiuretic activity 408 IU/mg) was used as reference preparation, for antibody production and radiolabeling. AVP antibody was generated against AVP-(ε-aminocaproic acid)-thryoglobin in sheep. The antisera were properly titred so as to bind approximately 50% of iodinated AVP. The final antibody dilution used in the assay tube was 1:500 000. The cross-reaction was 23.3% with LVP (Organon, Oss, The Netherlands), 0.12% with desglycaminide-9-arg-8-vasopressin (dGAVP, Organon, Oss, The Netherlands), 0.03% with arg-8-vasopressin (Organon, Oss, The Netherlands), less than 0.01% with oxytocin (Gedeon Richter, Budapest, Hungary), 0.03% with 1–24 ACTH (Organon, Oss, The Netherlands), and 10.7% with dDAVP (donated by Dr. L. Baláspiri, Szeged, Hungary). 125I-labelling of AVP was performed by the chloramine T method of Hunter & Greenwood (1962). Reverse phase chromatography was employed for purification of the labelled hormone (Janáky et al. 1982). The specific activity of the 125IAVP was established by the method of Morris (1976) as 1348 ± 1652 Ci/mmol (49.9 ± 61.1 TBq/mmol).

AVP was extracted from 2 ml plasma samples with thermally activated Vycor glass powder (Corning Glass Works, Corning, New York, USA; 140 mesh) (Dogterom et al. 1978). The standard curves covered the range 0.5–32 pg/assay tube. Each dilution of the reference preparation was extracted with glass powder from 2 ml AVP-free human plasma containing 200 µl 1 M HCl and 1.4 mg Na2EDTA per ml. Extraction was carried out in duplicate. The extraction recovery was 77%; however, the extraction of the reference preparation automatically corrected the results for the extraction loss. The dry residue was re-dissolved in 125 µl assay buffer (Dogterom et al. 1978), and 50 µl aliquots were transferred to the RIA system in duplicate. The RIA procedure was the same as that of Dogterom et al. (1978) and sensitive to 1.21 fmol/ml. The coefficients of variation within and between assays proved to be 13.3% and 16.3%, respectively. B0 samples (antibody and tracer without unlabelled hormone) were always radioimmunoassayed both with and without previous extraction procedure simultaneously. Their identical values witnessed for the absence of the hormone in the 'AVP-free plasma' used as diluent. B0 corrected the results for any non-specific reaction.

**Statistical analyses**

Results were expressed as means ± SEM unless stated otherwise. Since all data met the requirements of non-parametric tests, biometric analyses of non-parametric types were employed. Group effects were evaluated by Kruskal-Wallis one-way analysis of variance, treatment effects by Wilcoxon's signed rank test and Friedman's two-way analysis of variance. Kruskal-Wallis and Friedman's tests were followed by multiple comparisons to the controls (Siegel 1956; Hollander & Wolfe 1973).
Table 1.
Effect of hypertonic saline infusion on plasma AVP, osmolality and thirst in central diabetes insipidus (CDI).

<table>
<thead>
<tr>
<th>Plasma AVP (fmol/ml)</th>
<th>Plasma osmolality (mosm/kg)</th>
<th>Thirst onset at plasma osm. (mosm/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>Peak</td>
<td>ΔPeak</td>
</tr>
</tbody>
</table>

Controls (n = 10)

<table>
<thead>
<tr>
<th>Plasma AVP</th>
<th>Plasma osmolality</th>
<th>Thirst onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>Peak</td>
<td>ΔPeak</td>
</tr>
<tr>
<td>3.92 ± 0.60</td>
<td>10.46 ± 0.59f</td>
<td>6.54 ± 0.61</td>
</tr>
</tbody>
</table>

CDI I. (n = 13)

<table>
<thead>
<tr>
<th>Plasma AVP</th>
<th>Plasma osmolality</th>
<th>Thirst onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>Peak</td>
<td>ΔPeak</td>
</tr>
<tr>
<td>&lt;1.21&lt;1.21</td>
<td>&lt;1.21&lt;1.21</td>
<td>0</td>
</tr>
</tbody>
</table>

CDI II. (n = 8)

<table>
<thead>
<tr>
<th>Plasma AVP</th>
<th>Plasma osmolality</th>
<th>Thirst onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>Peak</td>
<td>ΔPeak</td>
</tr>
<tr>
<td>&lt;1.21&lt;1.21</td>
<td>&lt;1.21&lt;1.21</td>
<td>0</td>
</tr>
</tbody>
</table>

Kruskal-Wallis ANOVA

<table>
<thead>
<tr>
<th>P</th>
<th>P</th>
<th>P</th>
<th>P</th>
<th>N.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>–</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Linear regression analysis of the plasma AVP vs corresponding plasma osmolality was applied to the blood samples taken from control subjects during hypertonic saline infusions. Similarly, regression line was calculated from plasma AVP and plasma osmolality data on a group of CDI patients (CDI II). The two regression lines were compared with the aid of a t-statistic (Brownlee 1965).

Results

Hypertonic saline infusions were tolerated well by all subjects. However, 2–3 min after histamine injection they complained of a strong, but quickly disappearing flush. Five patients experienced a headache too.

During the infusion of 2.5% saline, the plasma osmolality rose from a basal value of 289.3 ± 0.6 to a peak of 304.0 ± 1.0 mosm/kg in the control subjects (Table 1). The onset of thirst was recorded at a mean plasma osmolality of 297.2 ± 1.2 mosm/kg. The plasma AVP rose by 6.54 ± 0.61 fmol/ml from a basal value of 3.92 ± 0.60 fmol/ml during a 2-h infusion.

Two patients, classified earlier as having idiopathic CDI, were found to have mild dilutional

![Graph](https://via.placeholder.com/150)

Fig. 1.
Relationships between plasma osmolality and plasma AVP during hypertonic saline infusion in control subjects and in patients with CDI II (for abbreviations, see the text).
Table 2.
Effect of histamine on plasma AVP and osmolality in central diabetes insipidus (CDI).

<table>
<thead>
<tr>
<th></th>
<th>Time after histamine (0.5 mg sc) administration</th>
<th>Friedman’s ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>3 min</td>
</tr>
<tr>
<td>Plasma AVP (fmol/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n = 10)</td>
<td>3.80 ± 0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.28 ± 0.85&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>CDI I/A. (n = 6)</td>
<td>&lt;1.21&lt;sup&gt;d&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt; (&lt;1.21–&lt;1.21)</td>
<td>&lt;1.21&lt;sup&gt;d&lt;/sup&gt; (&lt;1.21–&lt;1.21)</td>
</tr>
<tr>
<td>CDI I/B. (n = 7)</td>
<td>&lt;1.21&lt;sup&gt;d&lt;/sup&gt; (&lt;1.21–&lt;1.21)</td>
<td>6.29 ± 0.79&lt;sup&gt;c,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>CDI II. (n = 8)</td>
<td>&lt;1.21&lt;sup&gt;c&lt;/sup&gt; (&lt;1.21–3.81)</td>
<td>4.71 ± 0.74&lt;sup&gt;d,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kruskal-Wallis</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>ANOVA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Plasma osmolality (mosm/kg) |       |       |        |        |                   |
| Controls (n = 10)           | 291.0 ± 1.6 | 291.0 ± 1.4 | 291.2 ± 1.6 | 292.3 ± 1.5 | N.S. |
| CDI I/A. (n = 6)            | 299.0 ± 1.4<sup>g</sup> | 298.4 ± 1.6 | 300.1 ± 1.7 | 301.2 ± 2.2 | N.S. |
| CDI I/B. (n = 7)            | 298.7 ± 1.3<sup>g</sup> | 298.8 ± 1.4 | 299.3 ± 0.8 | 299.8 ± 0.9 | N.S. |
| CDI II. (n = 8)             | 298.4 ± 1.9<sup>g</sup> | 298.3 ± 1.9 | 300.0 ± 2.1 | 299.0 ± 2.2 | N.S. |
| Kruskal-Wallis             | P < 0.02 | P < 0.01 | P < 0.01 | P < 0.01 |                   |
| ANOVA                     |       |       |        |        |                   |

a: Mean ± SEM. b: Median with the lowest and highest values in brackets.
Significantly different from controls c: P < 0.05. d: P < 0.01, multiple comparisons after Kruskal-Wallis test.
Significantly different from 0 min value e: P < 0.05. f: P < 0.01, multiple comparisons after Friedman’s test.
g: Basal plasma osmolality significantly different from controls, P < 0.05, Kruskal-Wallis test. Histamine challenge did not alter plasma osmolality values as demonstrated by Friedman’s ANOVA testing.

Hyponatraemia (plasma osmolality 284 and 286 mosm/kg, respectively), and both showed normal AVP responses to hypertonic saline. Thus, primary polydipsia could be diagnosed, and they were excluded from further studies. In the remaining 21 CDI patients, the AVP responses to osmotic stimulation fell into two distinct groups: patients with CDI I gave no response at all, while those with CDI II responded subnormally (Table 1). The regression line (Fig. 1) of the plasma AVP vs corresponding plasma osmolality values of the control subjects was characterized by the equation pAVP = 0.41(pOs-280), r = 0.83, P < 0.001, where pAVP represents the plasma AVP and pOs the plasma osmolality. Similarly, regression line was calculated from the pAVP and pOs data on patients with CDI II. They showed a significant correlation between pAVP and pOs (pAVP = 0.16(pOs-280), r = 0.67, P < 0.02), though the slope of the regression lines was very shallow (Fig. 1). The osmotic threshold (abscissal intercept) in CDI group II did not differ from that for the controls (280 mosm/kg in both of them), however, a significant difference (P < 0.05) between the slopes of the regression lines of groups CDI II vs control could be demonstrated.

Thirst was experienced by CDI patient at a lower increase of the plasma osmolality than that for healthy volunteers (ΔpOs in CDI I: 3.7 mosm/kg, and ΔpOs in CDI II: 3.2 mosm/kg, versus ΔpOs in the controls: 7.9 mosm/kg). The basal plasma osmolality values in the two CDI groups were significantly higher than that for the controls, though patients with CDI exhibited a decreased basal level of plasma AVP (Table 1). The results of histamine loading are listed in Table 2. The controls showed a significant increase of the plasma AVP level, with a peak 3 min after histamine injection. CDI II patients gave subnormal plasma AVP responses to histamine.
The AVP reactions of the CDI I patients fell into two distinct subgroups: 6 patients (CDI I/A) had undetectable plasma AVP, while the histamine stimulus evoked AVP release in 7 (CDI I/B). Neither the controls nor the patients showed any significant change in plasma osmolality after the histamine trial.

The mean daily diuresis (l/24 h ± se) and urinary osmolality (mosm/kg ± se), the 15-min diuresis (ml/15 min ± se) prior to and during hypertonic saline infusion in the CDI subgroups were found as follows. I/A: 10.8 ± 1.3, 92.5 ± 6.8, 171.7 ± 19.7, 199.8 ± 25.8. I/B: 9.1 ± 0.3, 105.3 ± 7.5, 162.0 ± 11.4, 177.7 ± 10.2. II: 4.4 ± 0.4, 248.5 ± 13.9, 120.6 ± 12.9, 58.9 ± 7.9.

With regard to the above proposed grouping of the disease the case histories revealed that five of the CDI II patients had acquired the disease secondary to a skull trauma, and one had done so following a virus infection, while two had CDI of unknown origin. Four patients of the CDI I/A group suffered from an inborn polyuria-polydipsia syndrome, while the remainder had Hand-Schüller-Christian disease or head injuries in their histories. In 2 cases of CDI I/B, virus infections had been recorded earlier, and an idiopathic form of the disease was diagnosed in all the other members of this subgroup.

Discussion

This study indicates that the direct measurement of plasma AVP and plasma osmolality may provide a better approach to the differential diagnosis of CDI and to the pathogenesis of various forms of the disease.

The median value of the basal plasma AVP in patients with CDI was too low to be determined, though their mean basal plasma osmolality proved to be above the normal range. These data may themselves be pathognomic. However, it is clear from the individual basal AVP levels that the values found for controls and patients may overlap. Stimulation tests for the determination of AVP reserve capacity are therefore of essential diagnostic importance (Baylis & Robertson 1980; Zerbe & Robertson 1981; Milles et al. 1983).

A simultaneous follow-up of the changes in plasma AVP and osmolality during the infusion of hypertonic saline provides substantial information about the osmoregulatory system (Robertson et al. 1976).

Our data on healthy subjects (regression analyses, osmolality at thirst onset, and plasma AVP response to hypertonic saline infusion) were similar to those found in previous studies involving direct AVP measurements by RIA (Robertson et al. 1976; Hayward et al. 1976; Baylis & Robertson 1980; Milles et al. 1983). The diagnosis of idiopathic CDI in two patients, established earlier by indirect tests, was corrected through the new examination regime to primary polydipsia.

From their AVP responses to hypertonic saline, 21 patients with verified CDI were classified into two distinct groups. In group CDI I (insensitive to hyperosmosis), the plasma AVP remained unchanged during hypertonic NaCl infusion. This population represents the most severe form of the osmoregulatory disturbance of AVP secretion. In group CDI II (sensitive to hyperosmosis), the increase in plasma osmolality was able to augment the level of plasma AVP, though the AVP release was less pronounced, and the slope of the regression line was more shallow than those in healthy controls. A positive correlation between the plasma osmolality and the plasma AVP, and the normal osmotic threshold in these cases, bear witness to a normal osmoreceptor function, whereas the disturbed synthesis and/or release of AVP may play a major role in the impaired water metabolism.

Histamine has been widely used as a diagnostic challenge of gastric acid secretion in clinical gastroenterology. Animal experiments have demonstrated that it is an effective stimulus of AVP, too (Bhargava et al. 1973; Haas et al. 1975; Dogterom et al. 1976; Mens et al. 1980).

Our results show that AVP secretion can be stimulated with histamine in humans. In healthy individuals, the plasma AVP rose to a peak 3 min after sc administration of the drug; the effect expired within 20 min. The plasma osmolality was not altered by histamine loading. Accordingly, a site of action distinct from the osmoreceptors is to be suggested for AVP stimulation. As a marked cardiovascular effect has been observed both in humans and in animals after peripheral application of the preparation (Harvey & Owen 1980), involvement of the baroreceptors in the AVP-modulating effect of subcutaneously administered histamine may be proposed.
The AVP response to histamine loading was less pronounced in group CDI II (sensitive to hyperosmosis) than in the controls. The decreased effects of the osmotic and the non-osmotic stimulation suggested a partial form of CDI in these patients. Group CDI I (insensitive to hyperosmosis) might be subdivided into subgroups A and B (insensitive or sensitive to histamine, respectively). The plasma AVP of patients insensitive to histamine (CDI I/A) remained undetectable after either hypertonic NaCl infusion or histamine injection. Thus, the deficiency of AVP synthesis and/or release is total in these cases. In subgroup CDI I/B (sensitive to histamine), no AVP release was triggered by hyperosmosis, but histamine provoked an increase of the plasma AVP level. Consequently, this form of CDI may be due to the impaired function of the osmoreceptors. Since the effect of histamine was smaller in this subpopulation than in the controls, the additive role of other factors (impaired synthesis and/or release of AVP) must also be taken into consideration.

We conclude that the osmotic and the non-osmotic stimulation of AVP secretion comprises a useful tool in the differential diagnosis of CDI and extends our knowledge as regards the pathogenesis and pathophysiology of the disease.

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