Abstract. The osmoregulation of arginine-8-vasopressin (AVP) was investigated in 14 patients with primary hypothyroidism and in 6 with Addison's disease. Plasma AVP was measured by radioimmunoassay. Patients with primary hypothyroidism were classified into subgroups with elevated (6.81 ± 1.12 pmol/l) or normal (3.92 ± 0.96 pmol/l) basal levels of plasma AVP. Following the infusion of 2.5% saline, a positive correlation was established between plasma AVP and plasma osmolality. A decreased osmotic threshold was found in hypothyroid patients with augmented basal AVP levels (pAVP = 0.37 (POs-265), r = 0.71, P < 0.01) as compared with that in hypothyroid patients with a normal AVP level (pAVP = 0.42 (POs-280), r = 0.93, P < 0.001). A relationship was demonstrated between the alteration in the AVP osmoregulation and the severity of the thyroid insufficiency. Patients with Addison's disease exhibited an increased basal level of plasma AVP (9.59 ± 1.25 pmol/l) and a decreased osmotic threshold (pAVP = 0.42 (POs-261), r = 0.63, P < 0.01) contrasted to that of healthy volunteers (pAVP = 0.41 (POs-280), r = 0.83, P < 0.001).

The osmoregulation disturbance of the AVP secretion may play a major role in the impaired water metabolism in primary hypothyroidism and in Addison's disease.

Primary hypothyroidism and Addison's disease are associated with an impaired water metabolism. Mechanisms proposed to account for the water retention include an altered osmoregulation of arginine-8-vasopressin (AVP). However, somewhat contradictory data have been published concerning the AVP osmoregulation in these diseases. Thus, both normal (Hochberg & Benderly 1983) and abnormal (Skowsky & Kikuchi 1978) osmoregulation have been reported in primary hypothyroidism, whereas inappropriate AVP secretion (Salomez-Granier et al. 1983), normal osmoregulation (Kleeman et al. 1964) and 'reset osmostat' (Robertson 1983) have been found in Addison's disease.

The present study was undertaken to obtain more detailed data on the osmoregulation of AVP secretion in primary hypothyroidism and primary adrenocortical insufficiency.

Materials and Methods

The osmoregulation of AVP secretion was investigated in 14 patients with primary hypothyroidism (12 females and 2 males; age 28–63 years, x̄: 33.8 years; body weight 66 ± 3 kg), in 6 patients with Addison's disease (4 females and 2 males) age 34–57 years, x̄: 46.8 years; body weight 58 ± 4 kg), and in 10 healthy controls (8 females and 2 males; age 17–68 years, x̄: 35.6 years; body weight 63 ± 5 kg). Thyroid hormone substitution (triiodothyronine) in the hypothyroid patients and glucocorticoid replacement therapy (cortisone acetate) in those with Addison's disease had been withdrawn 14–16 and 3 days, respectively, before the commencement of the present investigation. In patients with adrenal insufficiency, the sodium intake and the dose of mineralocorticoid substitution were increased temporarily, and then suspended totally 12 h before study.

Thyroid evaluation studies involved measurements by radioimmunoassay (RIA) of serum thyroxine (T₄, normal range: 52–155 nmol/l), serum triiodothyronine (T₃, normal range: 1.2–3.1 nmol/l), and serum thyrotropin (TSH, normal range: 0.6–3.8 mIU/l). Serum sodium levels were determined with flame photometry,
and cortisol with fluorimetry (08.00 h specimen; normal cortisol range: 221–550 nmol/l).

For measurement of water retention, all subjects underwent a standard oral water loading (Skowsky & Kikuchi 1978). Blood pressure and laboratory parameters of renal function were checked just before the commencement of the study of AVP regulation.

The osmoregulation of AVP secretion was investigated with the aid of hyperosmotic. 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 test they 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 osmolality and AVP were obtained from the other arm before and at 30-min intervals during the infusion.

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).

For the determination of plasma AVP, 10-ml blood samples were collected in chilled polystyrene tubes containing 14 mg of Na2EDTA in 300 µl of isotonic NaCl, and centrifuged at 4°C without delay. Two-ml aliquots of plasma were transferred into polypropylene tubes containing 400 µl of 1 mol/l HCl, and were kept at −20°C until assayed. RIA was performed within a week after sampling. For the purpose of standard curves, AVP-free human plasma was obtained from healthy individuals 30 min after completion of an oral water load with 20 ml/kg body weight.

**Measurement of plasma AVP (RIA)**

A RIA was set up 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 employed as reference preparation for antibody production and radiolabelling. AVP antibody was generated against AVP-(ε-aminocaproic acid)-thryoglobulin conjugate in sheep. On each immunization, the animals were given about 1 mg (1 ml) immunogen emulsified in 1 ml of Freund’s adjuvant. The emulsion was injected intradermally into as many sites as possible on the back.

The immunization regimen consisted of injections every 2 weeks for a 12-week period, followed by further boosters monthly. The antisera were properly titrated so as to bind about 50% of iodinated AVP. The final antibody dilution used in the assay tube was 1:550 000. The cross-reaction was 23.3% with lys-8-vasopressin (Organon, Oss, The Netherlands), 0.12% with desglycinamide-9-arg-vasopressin (Organon, Oss, The Netherlands), 0.03% with arg-8-vasotocin (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 1-deamino-8-d-arg-vasopressin (donated by Dr L. Balásipri, Szeged, Hungary).

125I-labelling of AVP was performed by the chloramine T method of Hunter & Greenwood (1962). Reverse phase chromatography was used for purification of the labelled hormone (Janáky et al. 1982). The specific activity of the 125I-AVP was established as 49.9–611 TBq/mmol.

AVP was extracted from the plasma samples with thermally activated Vycor glass powder (140 mesh; Corning Glass Works, Corning, New York, USA) (Dogterom et al. 1978). The standard curves ranged between 0.5 and 32 pg/assay tube. Each dilution of the reference preparation was extracted from 2 ml of AVP-free human plasma containing 200 µl of 1 mol/l HCl and 1.4 mg of Na2EDTA per ml. Extraction was carried out in duplicate. The extraction recovery of 2, 8, 32 pg of AVP added to 2 ml of AVP free human plasma was 89.0 ± 3.0, 76.6 ± 7.1, 60.4 ± 5.3% ± SEM, respectively (N = 14); however, the extraction of the reference preparation automatically corrected the results for the extraction loss. Serial dilutions (1:2) of a plasma sample with an AVP concentration of 14.2 pmol/l gave results with RIA after extraction as follows: 7.0–3.7–1.8 pmol/l. The linearity of the decrease showed the specificity of the RIA. The dry residue was redissolved in 125 µl of assay buffer (Dogterom et al. 1978) and 50-µl aliquots were transferred to the RIA system in duplicates. The RIA procedure was the same as that of Dogterom et al. (1978) and sensitive to 1.21 pmol/l. The intra-assay and interassay coefficients of variation proved to be 13.3% and 16.3%, respectively. B₀ samples (antibody and tracer without unlabelled hormone) were always radioimmunoassayed simultaneously both with and without previous extraction procedure. 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, and were evaluated by Student’s t-test and linear regression analysis. Regression lines were compared with the aid of a t-statistic (Brownlee 1965).

**Results**

Table 1 lists the basal values of plasma AVP, plasma osmolality, and serum sodium measured simultaneously. Patients with Addison’s disease (serum cortisol: 144 ± 12 nmol/l, not stimulable with ACTH; BUN: 6.3 ± 0.5 nmol/l, serum creatinine: 83 ± 8 µmol/l, serum uric acid: 229 ±
Table 1.
Basal values of plasma AVP, plasma osmolality and serum sodium in primary hypothyroidism and in Addison's disease.

<table>
<thead>
<tr>
<th></th>
<th>Plasma AVP (pmol/l)</th>
<th>Plasma osmolality (mosmol/kg)</th>
<th>Serum Na⁺ (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N = 10)</td>
<td>3.92 ± 0.60¹</td>
<td>289.3 ± 0.6</td>
<td>138.4 ± 0.4</td>
</tr>
<tr>
<td>Hypothyroidism 1 (N = 9)</td>
<td>6.81 ± 1.12*</td>
<td>282.6 ± 0.5</td>
<td>134.3 ± 0.3*</td>
</tr>
<tr>
<td>Hypothyroidism 2 (N = 5)</td>
<td>3.92 ± 0.96</td>
<td>289.0 ± 0.9</td>
<td>138.0 ± 0.5</td>
</tr>
<tr>
<td>Addison's disease (N = 6)</td>
<td>9.59 ± 1.25**</td>
<td>283.0 ± 0.8</td>
<td>132.9 ± 0.5**</td>
</tr>
</tbody>
</table>

¹ Mean ± SEM, N = number of subjects.
* P < 0.05,  ** P < 0.01 (Student's two sample t-test, compared with controls).

18 µmol/l; systolic and diastolic blood pressure: 105 ± 5, 72 ± 6 mmHg, respectively) exhibited hyposmolality, hyponatraemia, and a significantly augmented basal level of plasma AVP as compared with the controls. Following administration of the oral water load, they displayed a delay in disposal rate, excreting a total of only 45.8 ± 4.9% within 4 h after the water ingestion.

Patients with primary hypothyroidism were subdivided into subgroups with elevated (hypothyroidism 1) or normal (hypothyroidism 2) basal levels of plasma AVP. Subgroup 1 consisted of patients with basal AVP values above 4.50 pmol/l (the highest basal value measured in healthy subjects). In subgroup 1, hyposmolality and hyponatraemia were also demonstrated with plasma basal osmolality less than 285 mosmol/kg (the lowest value determined in healthy subjects), whereas the plasma osmolality and serum sodium concentration in subgroup 2 did not differ from those in healthy individuals. The average age of subgroup 1 was 34.5 years and that of subgroup 2 32.6 years. No differences were found between subgroups 1 and 2 as to the parameters of renal function (BUN: 6.2 ± 0.8 vs 6.7 ± 0.4 mmol/l; serum creatinine: 72 ± 9 vs 68 ± 11 µmol/l; serum uric acid: 241 ± 32 vs 253 ± 24 µmol/l), body weight (65 ± 6 vs 68 ± 4 kg), systolic blood pressure (120 ± 6 vs 124 ± 3 mmHg), and diastolic blood pressure (83 ± 2 vs 79 ± 3 mmHg).

After oral water challenge, a more marked water retention was documented in the hypothyroid patients with a high basal AVP level (excretion within 4 h as a percentage of the total water intake: 44.0 ± 4.5%, vs that in the hypothyroid patients with a normal basal AVP: 66.0 ± 7.2%), albeit the difference was not statistically significant as a consequence of the high standard deviation. Further differences could be detected as regards the basal concentrations of serum T₄ and TSH (serum T₄ in hypothyroidism 1: 17.23 ± 7.23 nmol/l, in hypothyroidism 2: 49.15 ± 6.08 nmol/l, P < 0.05; serum TSH in hypothyroidism 1: 45.68 ± 5.74 mIU/l, in hypothyroidism 2: 19.55 ± 5.78 mIU/l, P < 0.05). The serum T₃ and cortisol values proved to be similar in the two subgroups (serum T₃ in hypothyroidism 1: 0.60 ± 0.18 nmol/l, in hypothyroidism 2: 0.58 ± 0.42 nmol/l, not significant; serum cortisol in hypothyroidism 1: 391.2 ± 22.4 nmol/l, in hypothyroidism 2: 436.8 ± 22.7 nmol/l, not significant). Although the duration of hypothyroidism varied greatly (1–10 years) in the individual patients, the two subgroups did not differ considerably in this respect. The two subpopulations of hypothyroid patients were examined separately in the further studies of AVP secretion.

Following the infusion of 2.5% saline, the plasma osmolality rose by 14.30 ± 1.40 mosmol/kg from a basal value of 289.30 ± 0.60 mosmol/kg in control subjects, by 14.60 ± 1.60 mosmol/kg from 283.00 ± 0.80 mosmol/kg in patients with Addison’s disease, by 15.00 ± 1.50 mosmol/kg from 282.60 ± 0.50 mosmol/kg in patients with hypothyroidism 1, and by 14.50 ± 1.80 mosmol/kg from 289.00 ± 0.90 mosmol/kg in patients with hypothyroidism 2. The plasma AVP rose from 3.92 ± 0.60 to a peak of 10.46 ± 0.59 pmol/l in the controls, from 9.59 ± 1.25 to 16.42 ± 2.57 pmol/l in patients with Addison’s disease, from 6.81 ± 1.12 to 12.71 ± 1.10 pmol/l in patients with hypothyroidism 1, and from 3.92 ± 0.96 to 10.62 ± 0.42 pmol/l in patients with hypothyroidism 2.

Simple linear regression analyses of the plasma vasopressin and plasma osmolality were applied to the pooled data from the control subjects and each group of patients. The regression line of the controls was characterized by the equation pAVP = 0.41 (pOs-280), r = 0.83, P < 0.001 (Figs. 1 and 2), where pAVP represents the plasma AVP and pOs the plasma osmolality. The osmotic threshold (abscissal intercept) was found at a plasma osmolality of 280 mosmol/kg. In patients with prim-
In primary hypothyroidism, the AVP responses to osmotic stimulation (Fig. 1) fell into two distinct groups: patients with a normal basal AVP level (subgroup 2) gave a normal response, whereas those with a high basal AVP level (subgroup 1) had abnormal osmoregulation. The regression equation for the latter patients was $pAVP = 0.37(pO_5-265), r = 0.71, P < 0.01$. The individual data of the patients demonstrated in Fig. 1 showed no significant differences between the regression coefficients of hypothyroidism subgroups 1 and 2 and those of the controls. The abscissal intercept of subgroup 1, however, differed significantly from that of the controls ($P < 0.01$). The AVP response to osmotic challenge was also retained in patients with Addison's disease: the regression equation for these patients was $pAVP = 0.42(pO_5-280), r = 0.93, P < 0.001$. (pO5-261), $r = 0.63, P < 0.01$ (Fig. 2). The osmotic threshold in adrenocortical insufficiency was lower than that found in the control group ($P < 0.01$).

**Discussion**

Basal plasma AVP values have been reported to be 1.1–5.8 pmol/l (Robertson et al. 1973; Uhlich et al. 1975; Itzkovich et al. 1980; Bevilacqua et al. 1985). The AVP concentrations in our controls are within this range. However, the somewhat higher level may be due to our assay technique inhibiting the loss of AVP during storage by HCl and correcting extraction loss with the extraction of the standard curve.
Water retention has been generally observed in primary hypothyroidism. In a fundamental review on the neurohypophysial function in hypothyroidism, Robinson (1985) argues on the basis of numerous literature data that AVP plays little if any role in the impaired water excretion. However, the basal plasma AVP values are elevated in about 60% of our patients with primary hypothyroidism (subgroup 1). Although the osmoregulation of their AVP secretion is maintained, its irregularity can be documented by a decreased osmotic threshold compared with that in the controls and in hypothyroid patients with a normal basal AVP level (subgroup 2).

A significant water retention is observable in both subgroups of our hypothyroid patients, though the retention is more marked in the subtype with high AVP values. Although many important factors may contribute to the impaired water metabolism in primary hypothyroidism, (decreased cardiac output and glomerular filtration rate, reduced distal delivery of fluid etc.; Robinson 1985), considering our results and those of others (Jawadi et al. 1984; Skowsky & Kikuchi 1978), an additive role of the altered AVP secretion cannot be excluded.

The explanation of the existence of two separate hypothyroidism subgroups with different levels of AVP secretion is not clear. In this respect, the markedly different T₄ and TSH values in hypothyroid patients with high or normal AVP basal levels may deserve some attention. Thus, the possibility exists that the alteration in the AVP osmoregulation depends on the severity of the thyroid insufficiency. A causative role of the adrenocortical function in high or normal vasopressin secretion appears unlikely, as the plasma cortisol levels of both groups are within the normal range. The impaired osmoregulation of vasopressin in hypothyroid patients with high basal AVP values could possibly have the following explanations: 1) A decreased cardiac output and blood volume have been demonstrated in severe hypothyroidism (Graettinger et al. 1958). Since hypovolaemia may induce a reduction in the setting of the vasopressin osmoreceptor (Robertson et al. 1976; Schrier & Bichet 1981), the changed osmoregulation of the AVP secretion may represent a homeostatic mechanism correcting the volume deficit. However, no marked signs of cardiac and renal failure could be detected in any of the patients investigated in this study. 2) A thyroid hormone deficiency produces diffuse effects on most organ systems, as well as on intracellular metabolic intermediates. Thus, a thyroid insufficiency may contribute directly to the alteration of the osmoreceptor function. 3) A possible role of TRH in the observed phenomenon must also be considered, since TRH itself is able to influence the secretion of AVP (Weitzman et al. 1979; Jawadi et al. 1984). The TRH level is augmented in primary hypothyroidism (Mitsuma et al. 1976). Although the osmoregulation of AVP is maintained in primary hypothyroidism, a modulatory action of the increased TRH production on the osmoreceptor cannot be excluded.

Addison’s disease is associated with a greatly elevated basal level of plasma AVP. Our results are in accordance with earlier data in humans (Robertson 1983; Salomez-Granier et al. 1983) and in animals (Schwartz et al. 1983). Ishikawa et al. (1985) explained the increased AVP secretion in glucocorticoid and mineralocorticoid deficient rats with nonosmotic release of AVP. In our clinical study, the infusion of hypertonic saline evoked a further AVP increase, similar to that in healthy individuals. An altered osmoreceptor function is indicated, however, by the decreased osmotic threshold in adrenocortical insufficiency as compared with that in the controls. Earlier data have demonstrated that changes in blood volume and blood pressure influence the osmoregulation of AVP (Robertson et al. 1976; Schrier & Bichet 1981). On the basis of these observations, Robertson et al. (1982) explained the decrease of the osmotic threshold in isolated aldosterone deficiency by concomitant hypovolaemia and hypotension. As glucocorticoids increase the osmotic threshold of AVP secretion (Aubry et al. 1965), their deficiency in Addison’s disease may well contribute to its ‘resetting’. The role of vasopressin in blood pressure regulation under hypovolaemic conditions has been proved in animal experiments (Aisenbrey et al. 1981). Thus, it is highly possible that the altered osmoregulation of AVP in primary adrenocortical insufficiency serves as a defensive reaction to maintain the appropriate blood pressure (Schwartz et al. 1983).

It seems evident that the osmoregulation disturbance of the AVP secretion may play a major role in the impaired water metabolism in Addison’s disease. However, water retention has also been observed after adrenalectomy in Brattleboro rats with an inherited AVP deficiency (Balment et
al. 1976). Accordingly, a multifactorial causation is to be suggested for the water retention in Addison’s disease, including both an altered AVP secretion and AVP-free (possibly intrarenal) mechanisms (Schrier & Bichet 1981).

Taken together, the basal plasma AVP level is elevated in Addison’s disease, whereas patients with primary hypothyroidism can be divided into two subgroups on the basis of their AVP values. In the severe form of the latter disease, the basal AVP level was found to be augmented. The osmoregulation of the AVP release is maintained in Addison’s disease and in both subgroups of hypothyroidism. However, the osmotic challenge indicated a decreased osmotic threshold in adrenocortical insufficiency as well as in hypothyroidism with elevated basal AVP level. We conclude that among other factors, the disturbed osmoregulation of AVP secretion may play an additive role in the impaired water excretion in primary hypothyroidism and in Addison’s disease.

Acknowledgments

Thanks are due to Organon, Oss, The Netherlands, and to Dr L. Balasperi, Institute for Medical Chemistry, Szeged, Hungary, for providing all the peptides used in the AVP RIA, and in the cross-reactivity studies. Professor Tj. B. van Wimersma Greidanus and J. A. Ten Haaf are gratefully acknowledged for their valuable advice in setting up the AVP RIA and for providing us with Vycor glass powder. Statistical analyses were performed by Dr Krisztina Boda, Biometric Centre, University Medical School, Szeged, Hungary. The authors wish to thank Miss Györgyi Vorindan for excellent technical assistance.

References


Received September 30th, 1985.
Accepted October 28th, 1986.

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