Testing Water Regulation

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Introduction

A complex regulatory system is responsible for the stability of plasma osmolality despite large variations in water consumption or water loss. This osmoregulatory system is controlled by the hypothalamic neurohypophyseal axis, which includes the organs responsible for sensing variations in plasma osmolality or volume, as well as for the synthesis, storage, and secretion of arginine vasopressin (AVP), the antidiuretic hormone. The osmoregulatory system also includes osmoreceptors of thirst, which control drinking behavior. The osmoreceptors are also stimulated by osmotic changes in plasma.

The second component of the osmoregulatory system involves the kidney. The renal collecting duct is sensitive to the action of AVP. In response to AVP, the functional units of the kidney allow dramatic variations in urinary flow in order to maintain water balance.

In order to test water regulation, it is therefore necessary to evaluate not only vasopressin secretion and sensitivity of the kidney to vasopressin, but also thirst.

Normal Physiology

Release of Vasopressin

AVP is synthesized in neurons located in two bilateral clusters of cells in the hypothalamus, the paraventricular nuclei (PVN) and the supraoptic nucleus (SON). The tracts proceeding from the two nuclei on either side of the
hypotheses converge into a single supraopticohypophyseal tract which runs through the pituitary stalk to the posterior pituitary.

**Osmoreceptor**

In recent years it has been recognized that nausea, glucopenia and angiotensin may stimulate the release of vasopressin. However, the physiology and pathophysiology of AVP can be adequately understood in terms of the traditional regulatory system consisting of the osmoreceptor and the volume receptor. The osmoreceptor is exquisitely sensitive to changes in osmolality, and an increase of as little as 1% in plasma osmolality is sufficient to bring about an increase in plasma vasopressin [Robertson et al., 1982]. In normal subjects, approximately 280 mosm/kg H$_2$O is considered the ‘osmotic threshold’ for the release of AVP. Given an osmolality ranging from 280 to more than 300 mosm/kg H$_2$O, the level of AVP increases linearly with increased osmolality (fig. 1).

**Volume**

The major input to the hypothalamus for the recognition of volume originates in the high-pressure baroreceptors of the carotid sinus and the aortic arch and reaches the SON and PVN via the ninth and tenth cranial nerves. The baroreceptor system is much less sensitive than the osmoreceptor, since a change in blood volume of 5–10% is necessary before AVP is released. However, once the blood volume threshold is exceeded, release of AVP may

![Fig. 1. Relationship between plasma osmolality and plasma AVP for normal subjects [Robertson et al., 1982]. The threshold for AVP secretion is lower than that for sensation of thirst.](image-url)
increase exponentially and reach levels ten times higher than those reached after osmotic stimulation.

**Thirst**

Thirst has not been studied in detail in humans. However, it has been suggested that thirst in humans is not sensed until a plasma osmolality of 295 mosm/kg H₂O is reached [Robertson, 1983]. At this level, AVP is maximally stimulated, and urine osmolality is very high (fig. 2). Because most adults drink sufficient water by habit and social custom, intense thirst is rarely experienced.

**Role of the Kidney in Water Regulation**

Variations in urinary concentration and volume are a function of the spatial arrangement of the renal tubules and the action of AVP on the collecting tubules.

In the renal cortex, the interstitial fluid is isosmolar with respect to plasma. In the medulla, it becomes progressively hyperosmolar toward the papillary tips. The corticomedullary gradient is maintained by the countercurrent multiplication system of the loops of Henle. In the collecting ducts, which extend across the hyperosmolar medullary interstitium, the water permeability of the luminal membrane is regulated by AVP. In its absence, permeability is low, and urine remains dilute. With rising concentrations of AVP, the permeability increases steeply. The resultant extraction of water brings about a more efficient equilibration of tubular urine with the interstitium. This results in a sharp rise in urinary osmolality (fig. 2).

The action of vasopressin on the collecting duct is mediated by the movement of a vasopressin-regulated water-channel protein to the apical membrane of the cells of the duct. The aquaporins are a family of membrane proteins present
in many water-transporting tissues. The aquaporin-2 is specific of the collecting duct and responsible for the transfer of water to the interstitial fluid. Aquaporin-2 is present in urine and its concentration is increased during antidiuresis [Kanno et al., 1995].

**Functional Physiologic Relationships**

The exquisite physiologic regulation of water balance is due to the sensitivity of the kidney to minute changes in the levels of AVP. This relationship is illustrated in figure 2. As shown in this figure, the lower limit of urine osmolality occurs in the absence of AVP and corresponds to the maximal capacity of the kidney to excrete free water. The higher limit of urine osmolality corresponds to the maximal ability of the kidney to concentrate urine in response to increased levels of AVP.

**Diabetes insipidus**

The term diabetes insipidus describes different disorders of water regulation due to vasopressin deficiency or lack of action (see diagnostic algorithm, Appendix B, Chart 6). As a rule, they are manifested by polyuria and polydipsia with varying degrees of plasma hypertonicity. When caused by insufficient AVP, the disorder is sometimes called central diabetes insipidus. A lack of response to AVP is referred to as nephrogenic diabetes insipidus. Usually, polydipsia is secondary to polyuria. Primary polydipsia due to a specific disorder of thirst regulation is rarely seen in pediatric practice.

**Diagnostic Procedures**

**Basic Parameters**

**Urine Volume**

At first appearance, it might seem that determination of urine volume is the simplest and easiest diagnostic procedure to be carried out. In fact, the precise and reliable calculation of urine volume requires either hospitalization or, in a home setting, a certain amount of skill. In addition, the urinary flow is in some cases not grossly elevated, and differences between normal and abnormal urinary volume are relatively difficult to distinguish. Differential diagnosis should exclude other causes of polyuria, such as osmotic diuresis of glucose or urea and intrinsic renal disease. Routine biochemical analysis can eliminate polyuria resulting from osmotic diuresis or chronic renal disease.
Osmolality

Plasma and urine osmolality are the most important parameters, which are determined by osmometer using freshly collected, unfrozen specimens. The apparatus should be well calibrated. For plasma, simultaneous measurement of sodium is a good practice in order to establish a control for the plasma osmolality level. Measurement of urine density is no longer frequently performed, although it has been shown that the correlation between urinary osmolality and density is good. Urinary density is easy and inexpensive to calculate, and the procedure can be performed at the patient’s bedside during a dehydration test in order to obtain information quickly.

AVP Measurement

AVP is measured by radioimmunoassay (RIA) of 1 ml plasma. A quantity of 2 ml blood is collected in chilled, heparinized tubes and centrifuged immediately in a refrigerated centrifuge. Platelets contain a certain amount of AVP. Therefore, the blood should be centrifuged for 20 min at 2,000 rpm, and care should be taken when collecting the supernatant to leave the pellet intact. The assay sensitivity is 0.5 pg/ml plasma. Normal values vary according to the technique used. In our laboratory, the average AVP value for normal children is \(1.9 \pm 0.2\) (pg/ml \(\pm\) SD) for a plasma osmolality of \(283 \pm 1\) (mosm/kg H\(_2\)O \(\pm\) SD). Since the AVP level varies according to osmotic stimulation, it should be compared with plasma osmolality measured at the same time [Czernichow et al., 1979].

Test Procedure

Dehydration Test

Purpose. The dehydration test evaluates plasma AVP secretion and action by introducing a certain degree of hypertonicity and volume contraction. These two physiologic inputs work additively to stimulate the release of AVP. Measurement of plasma and urine osmolality at the end of the test is usually sufficient to establish a diagnosis of diabetes insipidus [Edelman et al., 1967; Frazier et al., 1967; Czernichow et al., 1984].

Protocol. The test is started postprandially at 6 p.m. and finished at 8 a.m. for patients without frank polyuria. In very young children or in patients with massive polyuria (urine volume >41/24h, it is started at midnight or in the early morning. No food or drink is allowed during the test. The patient must be kept under close surveillance throughout the test, for two reasons: (1) some children drink water surreptitiously and (2) blood pressure and weight should be measured every 1–4 h and signs of dehydration observed.

The test should be terminated if 5% of body weight is lost. At the end of the test, spot urine and blood specimens are collected to determine urine and
Table 1. Plasma AVP and plasma and urine osmolality after dehydration test [from Czernichow et al., 1979]

<table>
<thead>
<tr>
<th></th>
<th>Plasma AVP pg/ml ± SD</th>
<th>Osmolality, mosm/kg H₂O ± SD</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>plasma</td>
</tr>
<tr>
<td>Normal</td>
<td>1.9 ± 0.2</td>
<td>283 ± 1</td>
</tr>
<tr>
<td>Partial deficiency</td>
<td>2.0 ± 1.8</td>
<td>298 ± 9</td>
</tr>
<tr>
<td>Complete deficiency</td>
<td>1.3 ± 0.8</td>
<td>312 ± 15</td>
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Fig. 3. Urinary osmolality (U Osm) in relation to plasma osmolality (P Osm) in normal children and in patients with partial and complete diabetes insipidus. In many patients with massive polyuria, water deprivation of a few hours’ duration is enough to demonstrate elevated P Osm and inadequate U Osm. Several patients with partial diabetes insipidus have U Osm close to normal at the end of the test. All of them had elevated P Osm at that time.

plasma osmolality and AVP concentration. Thirst sensation should be recorded throughout the test.

Results. During a test involving 14-hour water deprivation, normal children showed almost no variation in plasma osmolality (table 1), while urine osmolality became elevated. All patients with diabetes insipidus became hypernatremic and maintained hypo-osmolar urine. Patients with partial AVP deficiency were clearly distinguishable from those with total AVP deficiency in that the former showed a moderate elevation in plasma and urine osmolality (fig. 3, 4).
If upon initial observation the patient has an elevated plasma osmolality due to hypernatremia and an inappropriately low urine osmolality, the diagnosis of some form of diabetes insipidus may be established without further testing (fig. 4; see also diagnostic algorithm in Appendix B, Chart 6). If the diagnosis is not immediately obvious, it is necessary to perform a dehydration test. Patients with complete inability to secrete vasopressin have no measurable control.

**Fig. 4.** Dehydration test lasting 14 h in children. Plasma vasopressin (AVP), plasma osmolality (P Osm), and urine osmolality (U Osm) for control subjects (hatched bars), patients with severe central diabetes insipidus (dark circles), partial central diabetes insipidus (open circles), and patients with nephrogenic diabetes insipidus (triangles). Patients with severe central diabetes insipidus have elevated plasma osmolality with markedly dilute urine, while patients with partial diabetes insipidus have moderate elevation of plasma osmolality and moderate inability to maximally concentrate urine [Czernichow et al., 1984]. Note that patients with polyuria of renal origin show either partial or complete insensitivity to AVP. In this situation, AVP measurement is clearly diagnostic.

**Comments.** If upon initial observation the patient has an elevated plasma osmolality due to hypernatremia and an inappropriately low urine osmolality, the diagnosis of some form of diabetes insipidus may be established without further testing (fig. 4; see also diagnostic algorithm in Appendix B, Chart 6). If the diagnosis is not immediately obvious, it is necessary to perform a dehydration test. Patients with complete inability to secrete vasopressin have no measurable