The posterior pituitary and diabetes insipidus

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The syndrome of diabetes insipidus is characterised by the passage of large volumes of dilute urine and results from failure of the normal urine concentrating mechanism. Such failure may occur because of inadequate production of vasopressin or because of failure of vasopressin to exert its normal action on the collecting duct of the kidney.

Vasopressin

SYNTHESIS

Vasopressin is synthesised in hypothalamic neurones, predominantly concentrated in the supraoptic and paraventricular nuclei, which appear to be separate from those concerned in oxytocin synthesis. After synthesis, which may begin with the formation of a high molecular weight, biologically-inactive precursor molecule (Sachs and Takabatake, 1964), the vasopressin is packaged together with one or more neurophysins into neurosecretory granules (Sachs *et al.*, 1969). These then pass down the axons of the neurons to reach the secretory terminal where vasopressin is released after appropriate stimulation. Secretory terminals are found not only in the posterior pituitary but also in the region of the median eminence.

Because of the relatively wide anatomical extent of the vasopressin-producing system diabetes insipidus rarely results from disease of the pituitary itself. It is much more commonly seen arising either in the absence of any clinically or radiologically obvious cause or as a result of primary or secondary neoplasms (especially from breast) involving the hypothalamus. It may also be associated with granulomatous lesions of the hypothalamus—for example, histiocytosis X or sarcoidosis. Craniopharyngiomas or large suprasellar extensions of pituitary tumours may interfere with vasopressin release or synthesis, but more commonly diabetes insipidus results from the trauma associated with the treatment of such lesions.

RELEASE

The main physiological factors which cause or inhibit vasopressin release are shown in Table 1. Under normal circumstances changes in serum

Vasopressin release

Stimulated by:	Inhibited by:
Serum hypertonicity	Serum hypotonicity
Decrease in ECF volume	Increase in ECF volume
Hypotension	
Stress	
Exercise	

Table 1 Major physiological factors affecting vasopressin release

osmolality are probably mainly responsible for variation in vasopressin release (Robertson and Athar, 1976). If serum osmolality is kept permanently low by excessive fluid intake vasopressin release is of course, physiologically inhibited and the renal effect is identical with that seen in pathological states of vasopressin deficiency. This situation occurs most commonly in psychologically maladjusted people and needs to be differentiated from true diabetes insipidus.

RENAL EFFECT

Vasopressin acts mainly on the collecting duct of the kidney and increases its permeability to water. It also affects transport of sodium and urea, possibly through different pathways (Hays, 1976). The effect on water permeability can be inhibited by hypercalcaemia and hypokalaemia and also by a number of drugs, including demeclocycline. Drugs such as chlorpropamide or carbamazepine seem to potentiate the effect. The mechanism of the renal insensitivity to vasopressin seen in congenital nephrogenic diabetes insipidus is unknown.

Diagnosis of diabetes insipidus

Once other causes of polyuria such as diabetes mellitus have been excluded two basic questions must be answered before a definitive cause can be assigned to the syndrome: (1) Does the condition result from vasopressin deficiency or renal insensitivity? (2) If from vasopressin deficiency is this due to physiological inhibition of release or to pathological causes?

Factors interfering with the renal effect of vasopressin

pressin—for example, hypercalcaemia, hypokalaemia, or drug treatment—may as a rule be easily eliminated. Once this has been done it is usually simplest to approach the problems in the reverse order to that in which they have been stated.

MEASUREMENT OF SERUM OSMOLALITY

As pointed out by Barlow and de Wardener (1959), measurement of basal serum osmolality will often distinguish patients with compulsive water drinking, in whom it tends to be low, from those with true diabetes insipidus, where osmolality is commonly high. There is, of course, some overlap between the two groups but this simple test often gives a useful diagnostic clue.

WATER DEPRIVATION TESTS AND RENAL RESPONSE TO EXOGENOUS VASOPRESSIN

In many cases a diagnosis of significant vasopressin deficiency may be established or refuted simply by considering the clinical picture in conjunction with measurements of basal serum osmolality and of that of an overnight urine specimen. When vasopressin deficiency seems obvious the response to a therapeutic dose of vasopressin will often serve to clinch the diagnosis.

The most usual situation in which formal testing is required is when a patient complains of thirst and polyuria in the absence of any detectable organic lesion. In many cases a simple eight-hour fluid deprivation test, as described by Dashe *et al.* (1963), confirms normal hypothalamic-pituitary-renal function and no further test is needed. When, however, the response is abnormal it must be remembered that prolonged, excessive water intake may impair the renal response both to dehydration and to exogenous vasopressin. The findings of a high basal serum osmolality, a failure to concentrate the urine to more than 600 mOsm/kg after eight hours of fluid deprivation with an increase of serum osmolality to greater than 300 mOsm/kg, and an increase in urine osmolality to greater than 800 mOsm/kg after exogenous vasopressin will confirm a diagnosis of significant vasopressin deficiency. When the response to both parts of the test is abnormal the diagnosis lies between nephrogenic diabetes insipidus and compulsive water drinking.

In difficult cases further help may be obtained by more prolonged fluid deprivation tests (though these can be dangerous) and from the observations of Barlow and de Wardener (1959) that when the hypothalamic-pituitary system is basically normal urine osmolality after prolonged fluid deprivation is always higher than after exogenous vasopressin, whatever the final osmolality, while the reverse is true in states of vasopressin deficiency.

MEASUREMENT OF VASOPRESSIN IN PLASMA

Rather than using indirect tests such as those described above it might seem simpler to measure plasma vasopressin concentrations under standard conditions and, by matching these with simultaneously determined measurements of plasma and urine osmolality, obtain an accurate picture of the state of the hypothalamic-pituitary-renal system. Although such measurements may shortly be possible measurement of plasma vasopressin has presented formidable difficulties. Bioassay techniques, though now reasonably sensitive and accurate, are quite unsuited to routine clinical use. Radioimmunoassay techniques have been developed over the last few years but still present considerable problems, of which the following are some.

PRODUCTION OF SPECIFIC ANTIBODIES

Although vasopressin has a relatively low molecular weight (1084) it has not proved difficult to raise antibodies to it in a variety of animals. The vasopressin has been coupled to a large molecule—for example, albumin or thyroglobulin—before immunisation, but antibodies can be produced using vasopressin alone as antigen (Robertson *et al.*, 1973b). Recently, however, it has become apparent that antibodies differ sharply in their specificity as shown by their ability to react with closely related peptides and with degradation products of vasopressin (Chard, 1973; Czernichow *et al.*, 1974; Thomas and Lee, 1976). These observations probably account for many of the difficulties in developing clinically useful assays for vasopressin and raise hope that more specific assays will give a much better separation between plasma vasopressin concentrations in different physiological and pathological states than most assays have done so far (see below).

Non-specific interference

Attempts to apply radioimmunoassay to the measurement of arginine vasopressin (AVP)—the human form—in unextracted serum were soon abandoned when it was found that the results suggested immunoreactive-vasopressin concentrations many times higher than those expected from bioassay. Furthermore, there seemed to be little or no change with varying states of hydration. The gel filtration studies of Robertson *et al.* (1970) showed that with certain antisera nearly all the total immunoreactive material detected in plasma eluted from a Sephadex G25 column well ahead of AVP, most being associated with the plasma proteins but much also eluting with the salt peak. Although the amount of non-specific interference varies considerably with different anti-

Water-loaded	Normal hydration	Prolonged fluid deprivation	Hypothalamic diabetes insipidus	Extraction procedure	Reference
1.4 ± 0.8*	2.7 ± 1.4*	5.4 ± 3.4*	0.8 ± 0.3*	Acetone	Robertson <i>et al.</i> (1973)
<1.2	—	2.5 ± 8.5	—	„	Husain <i>et al.</i> (1973)
—	3.5	18	<1.25	Bentonite	Skowsky <i>et al.</i> (1974)*
3.5 ± 1.6*	—	4.9 ± 2.3*	—	Florasil	Beardwell <i>et al.</i> (1975)
3.7 ± 0.9†	4.9(3.6 ± 7.4)	9.5 ± 0.6†	—	„	Morton <i>et al.</i> (1975)†
0.16 ± 0.1*	0.57 ± 0.2*	3.2 ± 2.52*	—	„	Thomas and Lee (1976)

Table 2 Plasma vasopressin concentrations in various states measured by various radioimmunoassay methods (results in ng/l except where indicated)

*Results converted from $\mu\text{U/ml}$ to ng/l, assuming $1\mu\text{U} = 2.5\text{ pg}$.

†Results expressed as pmol/l⁻¹, 1 pmol AVP = 1.08 ng.

sera none has yet been found which reliably detects vasopressin using unextracted serum.

Choice of extraction procedure

Extraction procedures form an integral part of all currently available immunoassay techniques. These have been found necessary not only to overcome the problems of non-specific interference outlined above but also to increase assay sensitivity by allowing the concentration of vasopressin from several millilitres of plasma into a smaller volume. The physiological range of immunoreactive vasopressin concentrations in plasma seems to be low, varying from less than 1 to about 10 pmol/l⁻¹ in most assay systems and the concentrations are near the limit of detection of most assays. The choice of extraction procedure depends to some extent on the specificity of the antiserum. Some groups, using antisera which are relatively little subject to non-specific interference, have been able to use simple acetone extraction with a petroleum ether wash to remove lipid (Robertson *et al.*, 1973a; Husain *et al.*, 1973) while others have used non-specific adsorbents such as Florasil (Beardwell, 1971; Morton *et al.*, 1975; Thomas and Lee, 1976), Bentonite (Skowsky *et al.*, 1974), or ion exchange resins. With these techniques recoveries of AVP from plasma greater than 90% have been reported recently.

Assay sensitivity and replicability

Available data suggest that small changes in plasma vasopressin concentration can cause substantial changes in urinary concentration, and most published results show considerable overlap between plasma AVP levels in water-loaded subjects, normally hydrated subjects, and those who are dehydrated. Furthermore, most patients with hypothalamic diabetes insipidus who have been studied have had some detectable vasopressin in the serum, though whether it has biological activity is uncertain. Some data from recently published papers are shown in Table 2. Possibly much of the overlap between patients in various states of hydration may be

accounted for by lack of assay precision and precision can be improved it may be less of a problem.

Clearly, even if overlap between plasma AVP levels in various states of hydration cannot be entirely eliminated separation can be much improved by considering the AVP concentration and the simultaneously measured serum osmolality together. However, it would also be useful, particularly in hormone deficiency states, if some dynamic test of vasopressin secretion were available analogous to those used in the assessment of anterior pituitary function. Although a number of pharmacological and physiological stimuli have been shown to cause vasopressin release, as determined by radioimmunoassay, none has yet been tested which is both consistent in its effect and reasonably free from side effects.

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