Plasma vasopressin response to hypertonic saline infusion to assess posterior pituitary function[1]

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Summary: Hypertonic saline was infused into 11 volunteers to osmotically stimulate vasopressin secretion. A strong positive correlation between plasma arginine vasopressin (PAVP) and plasma osmolality (Pos) was obtained, defined by the function PAVP = 0.63 (Pos − 284), $r = +0.80$, $P < 0.001$. The sensitivity of vasopressin secretion to osmotic stimulation was represented by the slope of the expression and the theoretical threshold of vasopressin release by the abscissal intercept. Plasma osmolality at the onset of thirst was 298.5 ± 1.1 mmol/kg. Application of hypertonic saline infusion to 10 polyuric patients clearly separated those with normal osmoregulation of vasopressin secretion from those with cranial diabetes insipidus.

Introduction

Most of the major regulatory systems that control the secretion of arginine vasopressin (AVP), the antidiuretic hormone of most mammals including man, were described following a series of elegant experiments performed on conscious dogs by E B Verney and his colleagues (Rydin & Verney 1937, Verney 1947). Using indirect methods to assess the action of vasopressin, these investigators formulated the concept that the dominant physiological determinant of vasopressin secretion was body fluid tonicity. They went on to identify other regulatory factors that influenced vasopressin secretion. Large changes in blood pressure or volume were recognized as potent stimuli to release vasopressin, but uncertainty existed as to the importance of haemodynamic changes in relation to osmotic stimuli (Gauer 1968, Johnson *et al.* 1970). Progress in defining precisely the regulatory factors controlling vasopressin secretion was delayed until the development of sensitive and reliable methods of measuring the hormone in body fluids. With the development of radioimmunoassays for plasma vasopressin, a new tool was available to investigate vasopressin function. Although many of the traditional concepts including the central role of osmoregulation were confirmed (Robertson *et al.* 1976), other potentially important stimuli to vasopressin release were discovered (Shelton *et al.* 1976, Baylis & Heath 1977) and the interrelationship between osmoregulation and baroregulation of vasopressin secretion was defined (Dunn *et al.* 1973).

Now that precise measurements of plasma vasopressin and osmolality can be made and the fundamental importance of osmoregulation of vasopressin secretion has been established (Robertson 1977), more direct tests of vasopressin function in patients with deranged water metabolism, particularly those suffering from polyuria, may be contemplated. Until recently the method of investigating posterior pituitary function of polyuric patients was some form of dehydration test (Dashe *et al.* 1963, Miller *et al.* 1970). However, dehydration does not provide a sole osmotic stimulus but also an hypovolaemic influence most noticeable in the polyuric patient. It has now been recognized that even in the absence of vasopressin, as in the Brattleboro rat that is congenitally deficient in the hormone, considerable urinary concentration may be attained by dehydration alone (Gellai *et al.* 1979). Thus, certain advantages may be gained by giving a direct osmotic challenge to the posterior pituitary, since more information

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can be obtained about the function of the neurohypophysis and a more precise diagnosis can then be achieved. Infusion of hypertonic saline with measurements of plasma vasopressin and osmolality will attain this objective. We describe here the assessment of osmoregulation of vasopressin following hypertonic saline infusion in normal volunteers and assess the application of stimulus to polyuric patients.

Methods

Eleven healthy volunteers (5 male, age range 20–30 years) and 10 polyuric patients (5 male, age range 25–50 years, urine output > 2.5 litres/24 hours) gave informed consent prior to study. Each fasted, abstained from smoking and drank only water for 12 hours before the study which started at 09:00. After voiding urine and being weighed, a supine subject had indwelling cannulae sited in both antecubital veins, and a blood pressure cuff was applied to the thigh and connected to an automatic blood pressure recording device (Arteriosonde, Model 1216). After 30 minutes rest, 2 basal 10 ml blood samples, separated by an interval of 15 minutes, were drawn from one indwelling needle into chilled heparinized vacutainers. Thereafter, infusion of 5% saline was started into the other antecubital vein at the rate of 0.06 ml/kg/min for 2 hours using a rotary pump. Blood samples were taken at intervals of 20 minutes during the infusion and 2 further samples were drawn after the infusion had finished. The time at which the subject experienced thirst was noted. Blood pressure was recorded at intervals of 2 minutes throughout the whole study.

Aliquots of whole blood were drawn into heparinized capillary tubes to measure haematocrit (Hawksley microhaematocrit centrifuge). The remaining blood was centrifuged at 4°C within 30 minutes of sampling and plasma was separated, 2 ml aliquots being removed for osmolality measurement (Advanced Instruments osmometer, Model AD 3R) and the rest being deep-frozen at −20°C to measure plasma AVP by a sensitive and specific radioimmunoassay (Robertson *et al.* 1973). Vasopressin remains immunologically stable in plasma when stored under these conditions. Osmolality of the basal urine was also determined.

Results were analysed by paired and unpaired *t* tests, and linear regression analysis (Documenta Geigy Scientific Tables). Mean arterial blood pressure (MAP) was calculated by adding two-thirds pulse pressure to the diastolic pressure, and blood volume was estimated from changes in haematocrit using standard formulae (Documenta Geigy Scientific Tables). The study was approved by the Human Research Ethical Committee of Indiana University Medical Center.

Results

All subjects and patients tolerated the study well. Osmolality of the pre-infusion urine in the normal subjects was 788 ± 85 (mean $\pm$ SEM) mmol/kg which was significantly higher than the osmolality of the polyuric patients (253 ± 56 mmol/kg), $P < 0.001$.

Following infusion of 5% saline into normal volunteers, plasma osmolality rose by 17.6 ± 0.7 mmol/kg from a basal value of 287.2 ± 0.8 mmol/kg (Figure 1), and the plasma osmolality at which thirst was experienced ranged from 293 to 305 mmol/kg, mean 298.5 ± 1.1 mmol/kg (Table 1). Plasma vasopressin rose smoothly from 2.0 ± 0.3 to a peak of 14.8 ± 1.8 pg/ml after 2 hours infusion (Figure 1). Mean arterial pressure rose significantly by 14.6 ± 3.6 mmHg towards the end of the infusion, while haematocrit fell indicating an increase of blood volume of 6.7%.

Simple linear regression analysis of plasma vasopressin and plasma osmolality was applied to each individual infusion of hypertonic saline and to the pooled data from the normal subjects (Figure 2). The regression line had the equation $PAVP = 0.63\ (Pos - 284)$, where PAVP represents plasma arginine vasopressin and Pos, plasma osmolality. There was a highly significant positive correlation between the 2 indices: $r = +0.80$, $P < 0.001$. Table 1 shows the results of regression analysis applied to each normal individual and it displays the slope, abscissal intercept, and correlation coefficient of each regression function which was calculated on a minimum of 10 value pairs.

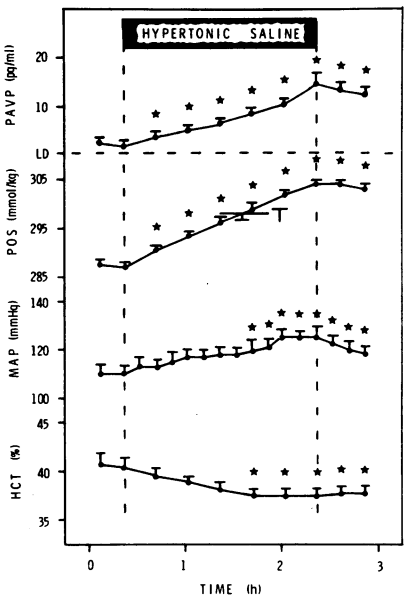

Figure 1. Effect of infusion of 5% saline at a rate of 0.06 ml/kg/min on plasma vasopressin, osmolality, mean arterial pressure and haematocrit in normal subjects. 'T' represents thirst onset. The asterisks indicate values which differ significantly from basal controls ($P<0.05$)

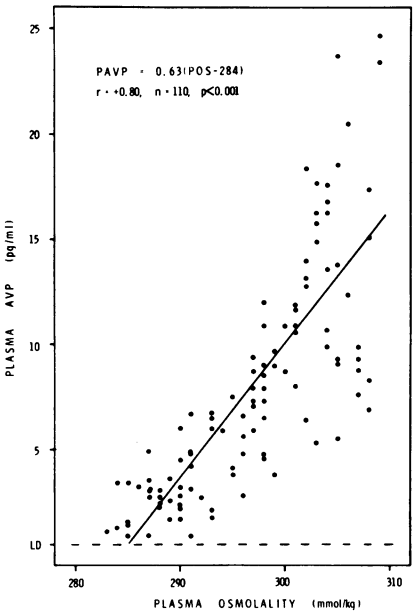

Figure 2. Relationship of plasma vasopressin to plasma osmolality in normal subjects. 'LD' represents limit of detection of immunoassay

Table 1. Results of linear regression analysis of plasma vasopressin and osmolality (Pos) in individual normal volunteers

Subject	Slope	Abcissal intercept	Correlation coefficient	Thirst Pos
1	0.83	286	0.96	298
2	0.49	282	0.92	301
3	0.60	283	0.98	294
4	1.07	287	0.95	299
5	0.44	280	0.97	293
6	1.09	288	0.95	301
7	0.44	284	0.94	298
8	0.63	280	0.97	295
9	0.33	286	0.93	305
10	0.81	284	0.95	298
11	0.46	287	0.93	302
Mean ±SD	0.65 ±0.26	284 ±2.80	0.95 ±0.02	299 ±3.6

Subjects 1 to 5 were male

Basal plasma osmolality of the polyuric patients was not significantly different from the normals (286.4 ± 1.3 and 287.2 ± 0.8 mmol/kg respectively; $P>0.05$), but a greater rise in plasma osmolality of 22.5 ± 2.0 mmol/kg was achieved after infusion. Vasopressin response to osmotic stimulation fell into two distinct groups: patients A to D had a normal response (left panel, Figure 3) while patients E to J had either a subnormal response (G,H,I) or, as in the

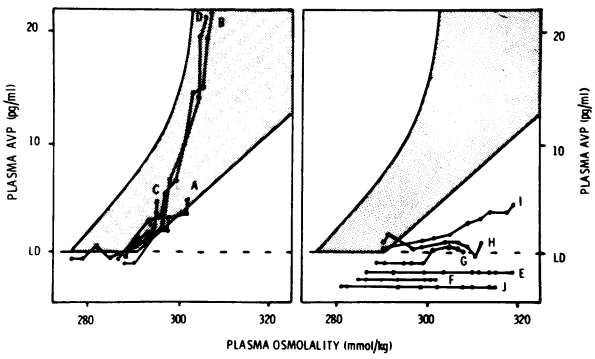

Figure 3. Result of hypertonic saline infusion in 10 polyuric patients. Left panel shows normal response (shaded area represents normal range) and right panel shows subnormal response

case of E,F,J, no response at all (right panel, Figure 3). The only patient who had abnormal osmoregulation and yet retained an osmotically active vasopressin response, albeit subnormal, was patient I. The regression equation of this patient was PAVP = 0.1 (Pos − 287), $r = +0.98$, $P < 0.001$. Thirst was experienced by every patient except one (patient H) at plasma osmolalities similar to the normal volunteers. The latter patient remained completely adipsic despite achieving a plasma osmolality of 313 mmol/kg at the end of saline infusion. All patients demonstrating polyuria with normal osmoregulation (A, B, C, D) had low basal urine osmolalities (130, 135, 109 and 119 mmol/kg, respectively) associated with undetectable plasma vasopressin (PAVP < 0.5 pg/ml). Table 2 summarizes the functional diagnoses of the patients with possible causes for their abnormalities.

Table 2. Functional vasopressin abnormalities and their possible causes in polyuric patients

Patient	Functional diagnosis	Aetiology
A	Primary polydipsia	Psychogenic
B	Primary polydipsia	Idiopathic
C	Primary polydipsia	Idiopathic
D	Primary polydipsia	Idiopathic
E	Complete CDI	Post-hypophysectomy
F	Complete CDI	Idiopathic
G	Partial CDI	Post-hypophysectomy
H	Partial CDI and adipsia	Surgery to anterior communicating artery
I	Partial CDI	Road traffic accident
J	Complete CDI	Familial

CDI = cranial diabetes insipidus

Discussion

Infusion of hypertonic saline to assess neurohypophysial function was suggested over 30 years ago (Hickey & Hare 1944, Carter & Robbins 1947), but because it produced a considerable osmotic diuresis and because methods to determine vasopressin action relied on indirect urinary methods, the test fell into disrepute. Consequently, dehydration remained the mainstay of stimulating the posterior pituitary. However, the ability to directly measure physiological concentrations of vasopressin in plasma has led to a reappraisal of hypertonic saline infusion in the assessment of neurohypophysial function (Robertson *et al.* 1976).

The results of our studies in healthy volunteers clearly show that at a constant rate of saline infusion a smooth increase in plasma osmolality can be achieved which is associated with a comparable smooth rise in plasma vasopressin. The infusion does not cause a fall in either blood volume or pressure, two factors known to affect the sensitivity of the osmoregulatory system (Robertson & Athar 1976). On the contrary, blood pressure rose by 13% and blood volume by 7%. Whether such increases in haemodynamic factors affect osmoregulation is unknown, but theoretical considerations would suggest that vasopressin secretion may be slightly diminished under those circumstances.

The functional characteristics of the osmoregulatory system can be readily appreciated by examining the relationship between plasma vasopressin and plasma osmolality over a suitably wide range of values. Two major functional properties of the osmoreceptor immediately emerge: i.e. the threshold of vasopressin release and the sensitivity of the osmoregulatory mechanism. Sensitivity of the system is indicated by the slope of the regression line relating plasma vasopressin to osmolality. Regression analysis of the pooled normal data defined a slope of 0.63, which means that for every 1 unit rise in plasma osmolality, plasma vasopressin rose, on average, by 0.63 pg/ml, or, expressed in a slightly different way, 1% increase in plasma osmolality caused a 1.8 pg/ml rise in plasma vasopressin. These results are in broad agreement with the original estimates of osmoreceptor sensitivity suggested by Verney (1947).

Two other points are worth emphasizing. Although vasopressin secretion responds well to saline infusion, most other solutes administered to produce osmolar increases similar to saline fail to elevate plasma vasopressin to any great extent (Zerbe *et al.* 1977). Furthermore, vasopressin secretion depends upon the infusion rate of hypertonic saline, since infusion rates of 0.1 ml/kg/min or more cause an exaggerated exponential vasopressin response (Robertson *et al.* 1976). Thus, it is essential to maintain a constant speed of infusion for all studies.

The second characteristic property of the osmoregulatory system is the threshold of vasopressin release. This defines the theoretical plasma osmolality at which vasopressin is secreted, and may have important implications in defining the abnormalities in conditions such as inappropriate antidiuresis.

It is clear from the individual normal results that considerable variation exists in the slopes and intercepts of individual regression lines. The reason for these differences is unknown. Nevertheless, in each case, an excellent correlation between the two indices plasma vasopressin and osmolality was achieved.

A similar large variation was observed in the plasma osmolality at which thirst was first experienced. This may in part be explained by the difficulty in expressing an opinion about a subjective sensation in which there is probably a continuous increase in sensation and not an absolute threshold. However, it is interesting to note that the mean plasma osmolality of thirst onset (299 mmol/kg) occurred slightly above the plasma osmolality (295 mmol/kg) required to maximally concentrate urine (Robertson *et al.* 1976), thus implying that other factors, e.g. social habits, motivate drinking under normal circumstances.

It can, therefore, be appreciated that hypertonic saline infusion with measurements of plasma vasopressin provides substantial information about the osmoregulatory system. When the test was applied to 10 polyuric patients, two distinct groups were observed, those with normal, osmotically-stimulated vasopressin release and those with a subnormal response, i.e. cranial diabetes insipidus. Three patients had undetectable plasma vasopressin despite considerably raised plasma osmolality, suggesting that they suffered from complete diabetes insipidus. The remaining three patients with subnormal responses showed varying abnormalities. One patient (I) had an intact osmoregulatory mechanism with a subnormal 'set' of the system, another (G) secreted vasopressin only when hypertonic, and the third continued to release vasopressin independent of osmotic control. The latter patient also had adipsia, and since the thirst centre and vasopressin-synthesizing neurones are situated in the anterior hypothalamus, an isolated lesion in this area is suggested. It can be appreciated, then, that a variety of functional abnormalities exist under the broad diagnosis of cranial diabetes insipidus. Polyuria in patients with normal osmoregulation may be due to either a form of nephrogenic diabetes insipidus or primary polydipsia. The observation that all these patients

had hypotonic urine and undetectable plasma vasopressin militates against the former diagnosis. Therefore, it is probable that they suffered from an intrinsic thirst abnormality.

In medical practice, the cause of polyuria can usually be established by standard tests of dehydration (Dashe *et al.* 1963). Infusion of hypertonic saline with vasopressin measurements should be reserved for the investigation of polyuric patients of particular interest, or the differentiation between primary polydipsia in which renal responsiveness to exogenous vasopressin is often impaired (Barlow & de Wardener 1959) and partial diabetes insipidus, situations frequently causing diagnostic difficulty. However, osmotic stimulation of vasopressin release is proving an extremely useful research tool in extending our knowledge of the pathophysiology of disorders associated with abnormal water metabolism (e.g. Robertson *et al.* 1976, Boykin *et al.* 1978).

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