The Role of Blood Osmolality and Volume in Regulating Vasopressin Secretion in the Rat

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Abstract A sensitive and specific radioimmunoassay for plasma arginine vasopressin (AVP) has been used to study the effects of blood osmolality and volume in regulating AVP secretion in unanesthetized rats. Under basal conditions, plasma AVP and osmolality were relatively constant, averaging 2.3±0.9 (SD) pg/ml and 294±1.4 mosmol/kg, respectively. Fluid restriction, which increased osmolality and decreased volume, resulted in a progressive rise in plasma AVP to about 10 times basal levels after 96 h. A 2–3-fold increase in plasma AVP occurred as early as 12 h, when osmolality and volume had each changed by less than 2%. Intraperitoneal injections of hypertonic saline, which had no effect on blood volume, also produced a rise in plasma AVP that was linearly correlated with the rise in osmolality (r > 0.9) and quantitatively similar to that found during fluid restriction (plasma AVP increased 2–4-fold with each 1% increase in osmolality). Intraperitoneal injection of polyethylene glycol, which decreased blood volume without altering osmolality, also increased plasma AVP but this response followed an exponential pattern and did not become significant until volume had decreased by 8% or more. At these levels of hypovolemia, the osmoregulatory system continued to function but showed a lower threshold and increase sensitivity to osmotic stimulation. We conclude that AVP secretion is regulated principally by blood osmolality but that the responsiveness of this mechanism may be significantly altered by modest changes in blood volume.

Introduction Arginine vasopressin (AVP) is a nonapeptide hormone of the posterior pituitary that causes antidiuresis in man and most other mammals. Although secretion of the hormone is known to be affected by changes in both blood osmolality and volume (1), the relative importance of these two variables in physiologic control of AVP has not been well established. This has been due largely to the lack of a suitable method for accurately measuring blood AVP at the low concentrations normally present under basal conditions. The development in our laboratory of a sensitive and specific radioimmunoassay for plasma AVP prompted us to reinvestigate these control mechanisms. By using techniques that permitted small changes in either blood osmolality or volume to be made independently, we have been able to quantitate the relative effectiveness of these two variables in altering AVP secretion in unanesthetized rats. Our findings indicate that, under normal conditions, small changes in osmolality are more potent than small changes in blood volume in affecting AVP secretion. However, the responsiveness of the osmoreceptor mechanism appears to be significantly altered by modest changes in blood volume, indicating a close interrela-

1 Abbreviations used in this paper: AVP, arginine vasopressin; BV, blood volume; Hct, hematocrit; PEG, polyethylene glycol; P\textsubscript{AVP}, plasma AVP; P\textsubscript{osm}, plasma osmolality.
tionship between the two variables in the control of AVP secretion.

METHODS

Male Sprague-Dawley rats, 150–250 g body wt, were maintained on ad lib food and water intake at constant temperature and humidity for at least 72 h before use. Three techniques were used to produce changes in blood osmolality and/or volume: a) complete fluid restriction for periods of from 12 to 96 h; b) i.p. injection of distilled water, isotonic or hypertonic saline; and c) i.p. injection of a non-absorbable polymer, polyethylene glycol (Carbowax 4000, Union Carbide Corp., Los Angeles, Calif.) dissolved at varying concentrations in hypotonic, isotonic, or hypertonic saline.

At appropriate intervals after these procedures, the rats were guillotined without anesthesia and the blood issuing from the vessels of the trunk collected in chilled heparinized tubes. Small aliquots were drawn immediately into capillary tubes for microhematocrit determinations, and the remainder centrifuged at 4°C. The plasma was carefully aspirated and an aliquot taken for determination of the concentrations in saline. Polyethylene glycol (Carbowax 4000, Union Carbide Corp., Los Angeles, Calif.) dissolved at 20°C in distilled water, isotonic, or hypertonic saline, was injected i.p. with 2 ml/100 g body wt of either distilled water, isotonic, (290 mosmol/kg) or hypertonic (1,000 mosmol/kg) saline, sacrificed at 15, 30, or 60 min, and the plasma osmolality, AVP, and hematocrit determined. Uninjected animals served as zero time controls. Mean blood hematocrit was 36.8% in control rats and 36.1% in rats sacrificed 30 min after hypertonic saline injection. The values shown for plasma osmolality and AVP at each time interval represent the mean and range of individual determinations on three animals. Asterisks indicate those values which differ significantly from zero time controls (P < 0.05 by unpaired t-test).

The effect of fluid deprivation on plasma osmolality, hematocrit, and plasma AVP. Rats were deprived of water for periods of 0, 12, 24, 48, 72, or 96 h, then sacrificed and the hematocrit, plasma osmolality, and AVP determined. The values at each time interval represent the mean and range of individual determinations on five animals. Asterisks indicate those values which differ significantly from the controls (P < 0.05 by unpaired t-test).

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Changes in blood volume (BV) were estimated from the changes in hematocrit (Hct) by the standard formula (BV2/BV1) = (Hct1/Hct2) × 100 (3) which assumes no change in circulating erythrocyte volume.

All statistical computations were performed with the Statistics Package of the Hewlett-Packard desktop computer, model #9810A (Hewlett-Packard Co., Palo Alto, Calif.). Linear regressions were determined by the method of least squares and evaluated for independence and for identity by conventional statistical methods (4).
FIGURE 3 The relationship of plasma AVP to isovolemic changes in plasma osmolality. Rats were injected i.p. with 2 ml/100 g body wt of either distilled water, isotonic, (290 mosmol/kg) or hypertonic (600, 800, 1,000, or 1,200 mosmol/kg) saline, sacrificed 30 min later, and the blood hematocrit, plasma AVP, and osmolality determined. Each point represents the values obtained in a single animal. The regression values were calculated from only those samples with osmolalities greater than 292 mosmol/kg (n = 35).

RESULTS

Effects of fluid deprivation. In 16 rats studied during ad lib intake of food and water, the blood hematocrit averaged 36.4±0.9%, the plasma osmolality 293.6±1.4 mosmol/kg and the plasma AVP concentration 2.3±0.9 pg/ml (±SD). Complete fluid restriction for periods of 12-96 h resulted in a progressive rise in each of the three variables to maximums of 49.0±2.4%, 312.6±1.5 mosmol/kg, and 23.1±4.9 pg/ml, respectively (Fig. 1).

Effects of saline and distilled water injections. The i.p. injection of 2 ml/100 g body wt of hypertonic saline (1,000 mosmol/kg), resulted in a prompt and parallel rise in plasma osmolality and AVP, both of which peaked at 15 min and then declined at similar rates (Fig. 2). The injection of the same amount of distilled water was followed by an equally prompt fall in mean plasma osmolality to 287 mosmol/kg and in mean plasma AVP concentration to the barely detect-

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All experiments were conducted essentially as described under Fig. 3, and the regression equation relating plasma AVP and osmolality for each group of rats calculated as described in the text. The values shown represent the date each experiment was performed, the number of rats involved (n), the intercept (X-axis) and slope of the regression line, and the correlation coefficient for the relationship (r). The data from 2/15/72 were from two groups of rats from the same shipment that had been housed for one wk either in individual or group cages.

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able level of 0.5 pg/ml. The i.p. injection of isotonic saline did not significantly alter either plasma osmolality or AVP concentration.

The mean blood hematocrit in rats receiving hypertonic saline was about 1% lower than in the control rats. This change was not statistically significant for the relatively small number of samples involved in any one experiment, but analysis of pooled data from four such experiments (n = 36) revealed an overall fall in mean (±SD) blood hematocrit, from 37.7±1.0 to 36.5±1.5%, that was significant (P < 0.05). The injection of isotonic saline had no appreciable effect on hematocrit.

When rats were injected i.p. with different concentrations of saline and sacrificed 30 min later, the resultant increase in plasma AVP was found to be directly proportional to the level of plasma osmolality achieved (Fig. 3). At osmolalities above 292 mosmol/kg, a precise, linear relationship (r = 0.95, P < 0.001) between plasma AVP (PAVP) and osmolality (Posm) was observed that was described by the regression equation $P_{AVP} = 0.83P_{osm} - 242$. This equation could be rearranged to the more useful form $P_{AVP} = 0.83 (P_{osm} - 292)$, in which 0.83 represents the slope of the regression line, and 292 its intercept on the osmolality or X-axis. Representative values from six other such experiments conducted over a 12 mo period are shown in Table I. In all cases, the correlation coefficients were greater than 0.9 and the intercept on the osmolality axis very close to 292 mosmol/kg. However, considerable variation in the slope of the regression line (0.8-1.9) was observed. This variability did not appear to follow a seasonal pattern nor to correlate with slight differences in the weight, diet, or housing arrangements of the animals.

Effects of polyethylene glycol (PEG) injections. The i.p. injection of isotonic saline containing PEG produced a rapid, progressive rise in hematocrit, without altering plasma osmolality (Fig. 4). This increase in hematocrit, which reflected a fall in plasma volume, was significant by 30 min and continued to increase for at least 3 h. This was associated with a progressive rise in plasma AVP, but this rise was delayed and did not become significant until 1 h after the injection, by which time blood volume was calculated to have decreased by about 9%.

When isotonic saline containing various concentrations of PEG were injected and the rats sacrificed 2 h later, a dose-related rise in hematocrit and plasma AVP was observed. In those animals in whom plasma osmolality remained at or near control levels (294 mosmol/kg), the increase in plasma AVP was related to the change in hematocrit, and hence blood volume, by a curvilinear function (Fig. 5). Significant increases in plasma AVP over control levels were observed only in those animals with an hematocrit greater than 39%, which corresponded to a fall in blood volume of about 7%. Beyond this point, PAVP rose at an increasing rate relative to the degree of hypovolemia present. This relationship was best described by an exponential equation, $P_{AVP} = 1.3e^{(P_{Hem}-294)}$, in which $P_{AVP}$ represents the plasma AVP concentration and $\Delta$vol the percent change in blood volume as calculated from the change in hematocrits.

<table>
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<th>Date</th>
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<td>36.6±1.0</td>
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<td>Saline + PEG</td>
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<td>$P_{AVP} = 1.8(P_{osm}-284)$</td>
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<td>(350 mg/ml)</td>
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Experiments were conducted as described under Fig. 6. The P values refer to the difference between control and experimental values for the indicated parameters.

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**DISCUSSION**

Previous studies of the regulation of antidiuretic hormone suffer from a variety of methodologic limitations that often make them difficult to interpret in physiologic terms. For the most part these studies were based on indirect or relatively insensitive measures of hormone activity, were concerned with the effects of relatively large stresses, and often were performed under anesthetics now known to have pronounced effects on antidiuretic hormone (5, 6). The present study was designed to avoid these problems by employing a sensitive and specific radioimmunoassay for plasma AVP to study hormone secretion under conditions that better approximate the usual physiological situation. The validity of the plasma AVP assay, documented previously for man (2), is further evidenced here by the good agreement with bioassay values reported for unanesthetized rats (6) as well as by the physiologic responses obtained.

Under basal conditions, plasma AVP was relatively constant, averaging 2.3±0.9 pg/ml. This value is well above the sensitivity limit of our assay (0.5 pg/ml) and, since it did not seem to be affected by variations in laboratory environment nor by minor stresses such as the pain of injection, probably represents the true “basal” level. Nearly identical values are found in normally hydrated recumbent adult humans (2). As expected, fluid deprivation resulted in a very marked increase in plasma AVP as well as plasma osmolality and blood hematocrit (Fig. 1). This increase in AVP was readily detectable after only 12 h of fluid deprivation when plasma osmolality had increased by less than 2%, and blood volume, as estimated from the change in hematocrit, had declined by a similar amount. These results amply document the extraordinary responsiveness of AVP secretion to small changes in water balance, but do not clarify whether the hormone response was due to the associated changes in osmolality, volume, or both. To resolve this question, it was necessary to employ other experimental approaches that permitted each of the two variables to be manipulated independently.

Fortunately for these purposes, we found that i.p. injection of hypertonic saline consistently produced a dose-related rise in plasma osmolality without appreciably altering total blood volume (Fig. 2). High concentrations of hypertonic saline (>900 mosmol/kg) did cause a slight fall in mean hematocrit, but this effect was barely significant and represented a change in
volume well below that normally required to affect AVP secretion (vide infra). Thus, the changes in plasma AVP which follow the injection of hypertonic saline, can be attributed almost exclusively to the changes in plasma osmolality. The validity of this conclusion is also supported by the close linear relationship between plasma AVP concentration and plasma osmolality after hypertonic saline injection (Fig. 3, Table 1). In over a dozen such experiments conducted over a 12 mo period, the correlation coefficient for this relationship was never less than 0.9, a result which testifies to the precision of the osmoreceptor mechanisms that govern AVP secretion. The constancy of this mechanism is shown by the point of intercept of the various regression lines on the X or osmolality axis (Table 1). This value, which represents the “threshold” for the osmotically mediated release of AVP, was remarkably constant from one group of rats to the next, averaging about 292 mosmol/kg. Thus, “basal” plasma osmolality, which was also quite constant at about 294 mosmol/kg, appears to be maintained, probably by the thirst mechanism, only slightly above the osmotic threshold for AVP release.

The sensitivity of this osmoregulatory system is represented by the slope of the regression lines relating plasma AVP to osmolality (Fig. 3, Table 1). Although this parameter was less constant from experiment to experiment than the threshold values, a slope of less than 0.8 has been observed only once in normal rats. This means that an increase in plasma osmolality of only 1% (29 mosmol/kg) regularly resulted in a rise in plasma AVP of at least 2.4 pg/ml, a result that amply confirms Verney’s original conclusion concerning the sensitivity of this osmoregulatory system (7). The reason for the apparent variability in osmoreceptor sensitivity is unclear. It did not seem to follow a seasonal pattern nor to be influenced by variations in the housing arrangements for the animals (Table 1). Recent studies in our laboratory suggest that osmoreceptor sensitivity may be subject to some circadian variation but this factor alone cannot account for the differences observed here.

To evaluate the characteristics of the volume-related control systems, it was necessary to employ a technique that would produce small, readily quantifiable reductions in blood volume without altering plasma osmolality. A further requirement was that the procedure not cause undue stress and, therefore, not require the use on anesthesia. We have found that the i.p. injection of saline solutions of PEG is well suited to this purpose. PEG is a nonabsorbable hydrophilic polymer which, when injected i.p., results in a transudation of noncolloid water from the extracellular into the peritoneal space (8). This leads to a rapid fall in plasma volume, and hence blood volume, which can be easily quantified from the resultant rise in hematocrit. The degree of hypovolemia produced can be controlled by varying either the duration of the injection (Fig. 4) or the concentration of PEG employed (Fig. 5), while the effect on plasma osmolality can be regulated by varying the concentration of saline in which the PEG is dissolved (Fig. 6).

With this approach it has been possible to show that the effects of hypovolemia on AVP secretion differ qualitatively as well as quantitatively from those of hyperosmolarity. Unlike the prompt response seen after hypertonic saline injection (Fig. 1) the rise in plasma
the rat is directly comparable to other types of volume depletion. This conclusion is also supported by preliminary studies in man where it has been found that removal of up to 6% of blood volume by phlebotomy has no effect on plasma AVP (11).

When compared on the basis of percent change from basal levels, it is readily apparent that small changes in blood osmolality are much more effective than small changes in volume in altering AVP secretion (Fig. 7). Due to the exponential nature of the hypovolemic response, however, very large increases in plasma AVP can be produced more readily by reducing volume than by raising osmolality. The present studies demonstrate that this is not necessarily synonymous with the primacy of volume in regulating AVP secretion, as previously has been suggested (12).

Our studies also confirm and extend a previous observation that hypovolemia does not abolish the influence of osmolality on AVP secretion (10). Instead, hypovolemia appears to modify osmoregulatory function in such a way as to promote increased AVP release in response to a given level of plasma osmolality (Fig. 6, Table II). The mechanisms of this effect, which manifest both a reduction in the threshold and an increase in the slope of the osmoreceptor response, are unknown. The studies of Gauer and Henry (13) and Share (14) indicate that the effects of large volume depletions on AVP secretion are mediated, at least in part, by afferent nerves from pressure-sensitive receptors in the heart and large arteries of the chest and neck. However, recent reports on the effects of angiotensin on AVP secretion (15, 16) raise the possibility that a humoral mechanism might also be involved. It is conceivable that each of the two parameters of osmoreceptor function—threshold and sensitivity—is responsive to a different volume-sensitive system, one humoral and the other nervous.

We conclude that in the usual physiologic situation, such as a short period of fluid deprivation, the major stimulus to AVP secretion must be the resultant rise in blood osmolality, since the accompanying changes in volume are usually not of sufficient magnitude to be effective. Thus, the 2% decline in blood volume observed in our rats after 12 h of fluid deprivation could not by itself have caused the observed rise in plasma AVP, whereas the 1.5% rise in osmolality could easily have had this effect. During longer periods of fluid deprivation, however, the progressive fall in blood volume probably plays a more important role in maintaining AVP secretion. Under these conditions, neurohypophyseal stores of AVP decline progressively to less than one-third normal levels (17), a circumstance that should result in a corresponding fall in the amount of AVP released in response to a given stimulus (18).

AVP after PEG injection occurred relatively late, well after the hypovolemia had progressed to substantial levels (Fig. 4). This lag in AVP release can be attributed to the exponential nature of the stimulus-response curve that characterizes the effects of isotonic volume depletion (Fig. 5). Thus, hypovolemia was not an effective stimulus to AVP release unless the fall in volume exceeded 8–10% of normal. The 2-fold rise in plasma AVP observed at this level of hypovolemia is very similar to that found in two other studies, also conducted without anesthesia, that used phlebotomy to reduce blood volume. Henry, Gupta, Meehan, Sinclair, and Share found that a 10–15% reduction in the blood volume of a dog resulted in a 2-fold rise in plasma antidiuretic activity (9), while Johnson, Zehr, and Moore observed a similar increase in plasma antidiuretic activity after removing 10% of the blood volume of a sheep (10). Although the basis for comparison is rather limited, the agreement between these two studies and our own results suggests that the PEG method for producing and quantitating blood volume changes in
The fact that a decrease in the plasma AVP-osmolality relationship was not observed after prolonged fluid restriction (the relationship was quite similar to that after hypertonic saline injection) indicates enhancement of the osmotically mediated AVP secretion by the accompanying hypovolemia. This booster effect thus serves to insure the secretion of AVP in amounts sufficient to maintain maximum antiuresis during prolonged water deprivation despite profound depletion of pituitary stores of the hormone.

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