Small changes in left atrial pressure and plasma antidiuretic hormone titers in dogs

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Acute studies were conducted on 16 pentobarbital-anesthetized dogs to determine the effect of small increases in left atrial pressure (LAP) on plasma ADH levels. The LAP was increased by inflating a balloon inserted into the left atrial appendage. Peripheral arterial plasma ADH concentrations were determined by bioassay in the ethanol anesthetized rat after extraction and concentration of the hormone. No significant changes in Psem, Csem, Csem, mean arterial blood pressure, or pulse pressure were observed following balloon inflation. The plasma ADH level decreased linearly with increasing LAP up to 7 cm of water. As the plasma ADH levels decreased, a significant increase in the urine volume and a decrease in Usem/Psem and TNO were seen. These findings lend strong support to the concept that the left atrial "volume-sensitive" stretch receptors play a functional role in the regulation of ADH secretion.

Numerous investigators have demonstrated that the release of antidiuretic hormone (ADH), in addition to being controlled by the plasma osmolality (1, 25), is also influenced by the blood volume. In the dog, hemorrhage produces a decrease in urine flow (10, 18) which is associated with an increase in blood ADH concentration (26). Conversely, expansion of the blood volume of the dog by means of whole blood (28), isotonic dextran solution (5, 20), or isotonic albumin solution (3) produces an increase in urine flow, with little or no change in creatinine clearance.

The importance of left atrial stretch receptors in this mechanism has been demonstrated by Henry et al. (11) who showed that inflation of a balloon placed in the left atrium of the dog would increase the urine flow, whereas tightening of sutures placed around the pulmonary veins would not. Shu’ayb et al. (24) reported an increase in urine volume and a decrease in the blood ADH concentration in the dog following left atrial balloon inflation. Both could be prevented by vagotomy. Both hormonal and hemodynamic changes were found by Arndt et al. (2) to occur following left atrial balloon inflation in the anesthetized dog. They observed increases in osmolar and free water clearances, and in the clearances of inulin and PAH, despite decreases in cardiac output and aortic blood pressure. Lydtin and Hamilton (14) found that increasing the left atrial pressure in unanesthetized dogs by tightening a purse string around the mitral valve would increase the urine flow, urinary sodium excretion, arterial blood pressure, and renal blood flow. Baisset and Montastruc (4) demonstrated that left atrial balloon inflation produces a decrease in the antidiuretic activity of the plasma, and Shu’ayb et al. (24) showed that acute mitral stenosis induces a decrease in blood ADH concentration.

In each of these studies, however, large increases in left atrial pressures were used. We reasoned that if the left atrial stretch receptor mechanism is normally of functional significance to the animal, then it should respond to much smaller increases in left atrial pressure. With this in mind, an experimental series was begun to determine whether small increases in left atrial pressure would suffice to produce a decrease in plasma ADH concentration, with a resulting increase in urine flow and decrease in solute-free water reabsorption (TNO).

Materials and Methods

The experimental data were obtained from a total of 16 mongrel dogs (5 male and 11 female), ranging in weight from 14 to 18 kg. The animals were maintained by the laboratory animal care facilities for at least 1 week prior to use. During this time they were fed a routine dog diet, and water was available at all times. Approximately 18 hr before experimental use, 10 of the dogs were offered 1,800 ml of milk, which they rapidly consumed. All food was removed at this time. At the start of the experiment each dog was anesthetized by the intravenous injection of a solution of sodium pentobarbital, 30 mg/kg. The dog was placed on its right side on an animal table, and this position was maintained throughout the surgical and experimental procedures. The trachea was intubated with a cuffed endotracheal tube, and a positive-pressure respiration pump was used to insure adequate ventilation.

The chest was opened at the 4th intercostal space with an electrosurgical unit, and the pericardium was slit to expose the heart. The left atrial appendage was incised and a small balloon, made from the distal 2 cm of a latex finger cot and attached to the end of a length of PE-205 tubing, was inserted through the appendage and into the atrium. A cannula of Vivosil tubing (2 mm id) for recording mean left atrial pressure was also placed in the atrium rostral to the balloon. The appendage was closed by placing a ligature around it about 1 cm from its free edge. The balloon tube was retracted until the base of the balloon rested against the ligature. The pressure-recording cannula was retracted until
were recorded on a Beckman/Offner type R inkwriting (17). The pressures and arterial pressure was recorded by use of a Statham gauge pressure transducers (PR23-2, 5D-350, or P23De), intrapleural pressure were connected to Statham strain-gauge pressure transducer (P23AA). The pressures were recorded on a Beckman/Offner type R inkwriting recorder. All of the pressure transducers were placed so that the transducer diaphragms were on the same horizontal plane as the left atrium. Urine conductance was measured continuously by use of the system described by Rothe et al. (17).

Approximately 1 hr following the completion of all surgery, each dog was given an intravenous priming dose of creatinine and PAH, consisting of 1 ml/kg of a 7% creatinine and a 2.5% PAH solution in isotonic saline, and an intravenous infusion of a solution of 1.3% creatinine and 0.17% PAH in isotonic saline was begun at a rate of 1 ml/min. Because pentobarbital will increase ADH release (8), this anesthetic was infused continuously with the creatinine and PAH at a rate of 0.5–1.0 mg/min to maintain the level of anesthesia as constant as possible. The experimental procedures were not begun for at least 3 hr after the completion of all surgery because surgical stress stimulates ADH release (15). The urine conductance was stable at the time of onset of the experimental protocol.

Figure 1 shows the protocol of all experiments. After a 15-min preexperimental control period, the atrial balloon was inflated with a volume of saline adequate to increase the mean left atrial pressure by approximately 2, 4, or 8 cm of water. The sequence of inflation levels was chosen at random. In a few instances the balloon was inflated to increase the mean left atrial pressure by about 12 cm of water, but it was soon apparent that this level of inflation was unsatisfactory because a marked fall in mean arterial blood pressure occurred. Therefore, this level of left atrial pressure increase was abandoned. A 15-min period was allowed after the balloon inflation before the experimental clearance period of 15 min duration was begun; 15 min were allowed to transpire between the balloon inflation and the start of the experimental clearance period in order to allow the blood ADH concentration to decay down to its new level. This timing was chosen because the half-life of ADH in the dog has been shown to be about 6 min (12, 19).

At the end of the experimental clearance period the balloon was deflated, and after a 15-min interval a postexperimental control clearance of 15 min duration was begun. The sequence was repeated at a different balloon-inflation level. The number of inflations per animal ranged from 2 to 6. At the midpoint of each clearance period, 17 ml of arterial blood were drawn for the determination of blood ADH concentration, hematocrit ratio, plasma osmolality, plasma creatinine and PAH concentrations, and serum sodium concentration. Prior to obtaining each blood sample, the dead space in the arterial cannula was cleared by withdrawing 10 ml of blood and returning it by way of the venous cannula. All blood which was removed was replaced with citrated blood from a cross-matched donor animal. To reduce the ADH concentration, the donor blood was collected early in the day and allowed to stand for several hours before use.

Urine and plasma samples were analyzed for creatinine and PAH on the Technicon AutoAnalyzer. Urine and plasma osmolalities were determined by use of the Advanced Osmometer (model 31-LAS or model 31 CM). Urine and serum sodium and potassium concentrations were measured by flame photometry (Baird KY-2), and urine chloride concentrations were determined by use of a Buchler-Cotlove (model 4-20510) chloridometer. Hematocrit ratios were obtained in Wintrobe tubes centrifuged at a high speed until stable readings were obtained.

The blood ADH concentrations were determined by bioassay. The ethanol-anesthetized rat was used as the assay animal. Prior to assay, the blood ADI was extracted and concentrated according to Yoshida's modification (27) of the method reported by Weinstein et al. (26).

Male Holtzman rats of approximately 150 g body wt were used as the assay animals. Each rat was given orally a volume of 12% ethanol equal to 5% of its body weight, and 30 min later was given an equal volume of tap water. When adequately anesthetized, a tracheotomy was performed and the urinary bladder was exposed and intubated with PE-205 tubing. The femoral vein was cannulated with PE-10 tubing. The rats were kept in an incubator at 35 C during the assay procedures. Intravenous infusion of a solution containing 0.3% sodium chloride, 1.6% glucose, and 2% ethanol with a Harvard infusion pump at a rate (either

**Figure 1.** Protocol of experiments.
0.128 or 0.256 ml/min) adjusted to approximate that of urine flow insured constant levels of hydration and anesthesia. A conductance electrode, consisting of a pair of 10 mm lengths of 16-gauge stainless steel tubing placed 10 mm apart, was placed in the bladder catheter, and the urine conductance was continuously recorded on a Sanborn (model 150) recorder with a bridge system as described by Rothe et al. (17). A drop counter placed in the system recorded drops of urine and served to give a rough estimate of urine flow rate.

The area under the change in the urine-conductance curve was used as the response parameter. Each rat was standardized by the intravenous injection of two dose levels of synthetic arginine vasopressin (Sandoz Pharmaceuticals). Usually 5- and 10-micronit standards were used. The unknown samples were assayed by the intravenous injection of two dose levels which would overlap the levels of the standards. The standards were in a solution of 0.03% acetic acid in saline and were kept frozen until used. No loss of potency was seen with this procedure. As many samples were assayed in each rat it was necessary to restandardize the rat periodically. All of the ADH values reported are in terms of microunits of synthetic arginine vasopressin (AVP). The sensitivity of the assay is at least 1 punit of synthetic AVP. Further details of the assay method have been reported previously (16). When possible, the blood samples taken during the two control periods and during the experimental period of a given balloon inflation were assayed in sequence in the same rat.

Because the blood ADH is confined to the plasma compartment (6), the plasma ADH concentration was determined by dividing the blood ADH concentration by the value of 1 minus the hematocrit ratio.

RESULTS

Table 1 gives the means ±SE for the control and experimental periods for each factor studied. The preexperimental control and the postexperimental control values for each factor were averaged, and the difference between the average of the controls and experimental value indicates the change induced. The test statistic used to determine whether or not a significant change occurred was the Student t-test for paired observations (13).

Changes in left atrial stretch-receptor activity should correlate more closely with changes in the pressure gradient across the atrial wall than with changes in mean left atrial pressure alone. By subtracting the intrapleural pressure (IPP) from the mean left atrial pressure (LAP), the left atrial transmural pressure (LAP-IPP) was obtained. The mean increase in left atrial transmural pressure for all experiments was 4.4 cm of water (+ 0.3 se).

The increase in left atrial transmural pressure was associated with a significant (P < 0.005) decrease in plasma ADH concentration (P_{ADH}). Other changes associated during LAP elevation and the decrease in P_{ADH} include a significant (P < 0.005) increase in urine flow, and significant (P < 0.005) decreases in urine osmolality (U_{osmol}), U/P osmolar ratio, and T_{i,o}. Creatinine clearance (C_{cr}) did not change significantly. No consistent changes occurred in the plasma osmolality (P_{osmol}), serum sodium concentration (S_{Na}), or the urinary excretion rates for sodium, potassium, or chloride. Left atrial balloon inflations of 2, 4, and 8 cm of water produced no change in either mean arterial blood pressure or pulse pressure. Figure 2 shows a plot of the increases in left atrial transmural pressure (LAP-IPP) of 7 cm of water or less versus the changes in plasma ADH concentrations. A significant (P < 0.005) negative linear relationship is seen, and the regression line has a slope of -0.45 (± 0.09 se) microunits of ADH per milliliter of plasma per centimeter of water increase in left atrial transmural pressure. This plot does not include experiments in which the left atrial transmural pressure increased more than 7.0 cm of water as this linear relationship does not hold above this level. An experiment in which an increase in plasma ADH concentration was seen is also excluded from this plot by the use of an outlying observations test (P < 0.01) (13).

![Figure 2](http://ajplegacy.physiology.org/Downloaded from 10.1202.63.1 on April 13, 2017)

**Fig. 2. Change in left atrial transmural pressure vs. change in plasma ADH levels.**

**Table 1. Effect of increasing left atrial transmural pressure on plasma ADH concentration, mean arterial blood pressure, serum solute and sodium concentration, and renal function.**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Control</th>
<th>Exp. Period</th>
<th>Δ</th>
<th>N</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>P_{ADH} μU/ml</td>
<td>6.3 ± 4</td>
<td>4.4 ± 3</td>
<td>-1.9 ± 3</td>
<td>45</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>P_{osmol} mOsm/liter</td>
<td>209±2</td>
<td>209±2</td>
<td>0±5</td>
<td>42</td>
<td>NS</td>
</tr>
<tr>
<td>MABP, mm Hg</td>
<td>119±2</td>
<td>117±2</td>
<td>-2±1</td>
<td>49</td>
<td>NS</td>
</tr>
<tr>
<td>V_{u}, ml/min</td>
<td>0.82±0.06</td>
<td>0.92±0.07</td>
<td>+1.1±0.03</td>
<td>49</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>U_{osmol}, mOsm/liter</td>
<td>777±14</td>
<td>716±12</td>
<td>61±9</td>
<td>49</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>U_{s}/P_{osmol}</td>
<td>2.60±14</td>
<td>2.39±14</td>
<td>-0.22±14</td>
<td>40</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>G_{osmol}, ml/min</td>
<td>1.83±11</td>
<td>1.85±11</td>
<td>+0.03±04</td>
<td>49</td>
<td>NS</td>
</tr>
<tr>
<td>T_{i,o}, ml/min</td>
<td>0.10±0.07</td>
<td>0.93±0.06</td>
<td>-0.80±0.02</td>
<td>49</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>S_{Na}, meq/liter</td>
<td>130±8</td>
<td>130±1.1</td>
<td>+0.4±0.33</td>
<td>29</td>
<td>NS</td>
</tr>
<tr>
<td>C_{cr}, ml/min</td>
<td>30±3</td>
<td>30±3</td>
<td>-0.6±1.7</td>
<td>49</td>
<td>NS</td>
</tr>
<tr>
<td>C_{osmol}, ml/min</td>
<td>191±8</td>
<td>192±9</td>
<td>+1.4</td>
<td>48</td>
<td>NS</td>
</tr>
<tr>
<td>Na_{excr}, meq/min</td>
<td>81±9</td>
<td>80±10</td>
<td>-0.0±1</td>
<td>41</td>
<td>NS</td>
</tr>
<tr>
<td>K_{excr}, meq/min</td>
<td>66±5</td>
<td>64±6</td>
<td>-0.5±2</td>
<td>43</td>
<td>NS</td>
</tr>
<tr>
<td>Cl_{excr}, meq/min</td>
<td>55±5</td>
<td>57±8</td>
<td>+2.1±3.6</td>
<td>36</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ±SE. The mean increase in left atrial transmural pressure was 4.4 cm of water (±.31 se). N indicates the number of observations. Test statistic used was the Student t test for paired observations. NS indicates P > 0.05.
DISCUSSION

These experiments were designed to study the effect of small increases in left atrial transmural pressure, produced by inflating a small balloon placed in the left atrial appendage, on plasma ADH concentration and on the renal handling of water. This method of increasing left atrial pressure has been used by many investigators in the study of the role of the left atrial stretch receptors in ADH release. However, it should be recognized that this procedure may also produce other circulatory alterations. The changes in plasma ADH concentration and renal function which result from this procedure may be due to a combination of several factors, and not be the sole result of pressure or volume changes in the left atrium. This is especially so if very large balloon inflations are used. For this reason, small increases in left atrial pressure, produced by minor degrees of balloon inflation, are not only more physiological, but also involve less chance that factors other than left atrial pressure influence the responses being studied.

Recent experiments have shown that changes in arterial blood pressure can influence ADH release. Share and Levy (22) have reported that carotid occlusion produces an increase in blood ADH concentration in the vagotomized dog. This effect was blocked by carotid sinus denervation. If the systemic arterial blood pressure is maintained at a constant level, an increase in plasma ADH concentration results from carotid occlusion in dogs with intact vagi (20). However, the rise in plasma ADH concentration which normally results from carotid occlusion can be prevented by the simultaneous inflation of a balloon in the left atrium. Furthermore, when the carotid sinuses of an anesthetized vagotomized dog are perfused at normal constant mean and pulse pressures, no increase in plasma ADH concentration occurs following hemorrhages of up to 30% of the blood volume (21). Clark and Silva (7) have demonstrated that carotid occlusion in the vagotomized cat results in an increase in the plasma concentration of antidiuretic substances. These findings indicate that decreases in mean arterial blood pressure, acting through the carotid baroreceptor mechanisms, bring about a reflex increase in ADH release. In our experiments the mean arterial blood pressure decreased, on the average, 2 mm Hg during left atrial transmural pressure clavation. However, this change in mean arterial blood pressure is so slight that it is doubtful that it has functional significance. Furthermore, an increase in plasma ADH concentration would be predicted to result from a fall in mean arterial blood pressure, whereas in these experiments a decrease in the plasma ADH occurred. Thus, it is probable that changes in mean arterial blood pressure did not contribute to the change in plasma ADH concentration observed during left atrial balloon inflation.

Share and Levy (23) have shown that variations in the arterial pulse pressure also influence ADH release. They observed an increase in plasma ADH concentration when flow through the carotid sinuses of the dog was changed from pulsatile to nonpulsatile. However, in our experiments alterations in pulse pressure were not seen. Therefore, changes in pulse pressure could not have contributed to the observed decrease in plasma ADH concentration.

It is generally accepted that changes in plasma osmolality are important in controlling ADH release (1, 25). Our data, however, indicate that the plasma osmolality and the serum sodium concentration remained constant during left atrial balloon inflation. Therefore, it is concluded that osmolar stimuli were not responsible for the resulting changes in plasma ADH levels.

From these data, it seems apparent that changes in mean arterial blood pressure, pulse pressure, and plasma osmolality cannot be significant factors which contribute to the decrease in plasma ADH concentration observed following left atrial balloon inflation. Therefore, this effect is probably produced in the main by the increased left atrial transmural pressure which stimulates the left atrial stretch receptors and inhibits ADH release. This is supported by the negative linear relationship which exists between left atrial transmural pressure and plasma ADH concentration.

The alterations in the renal handling of water are consistent with the observed changes in plasma ADH concentration. These changes include an increase in urine flow rate and decreases in the urine osmolality, U/P osmolal ratio, and TZO. These are responses which one would predict to result from a decrease in plasma ADH concentration. The renal responses were not the result of changes in glomerular filtration rate or effective renal plasma flow because the renal plasma clearances of creatinine and PAH, estimates of glomerular filtration rate, and effective renal plasma flow, respectively, showed no significant changes. Similarly, the increased urine flow was not osmotically induced because the osmolar clearance, and the urinary excretion rates of sodium, potassium, and chloride remained constant. This constancy of the osmolar clearance and the clearances of creatinine and PAH are not in agreement with the findings of Arndt et al. (2) who reported increases in solute, inulin, and PAH clearances following left atrial balloon inflation in the dog. However, they used much larger balloon inflations than those used in our studies and, thus, it is likely that they produced greater circulatory alterations.

Although the increase in urine flow rate and decreases in U/P osmolal ratio and TZA observed following left atrial balloon inflation were consistent and have high degrees of statistical significance, the magnitude of these responses is not very large. This might suggest that the change in plasma ADH concentration produced by small increases in left atrial transmural pressure are not sufficient to promote functionally important changes in urinary water loss. However, it should be recognized that the plasma ADH concentrations during the control periods were moderately elevated above the normal for the dog (9). This may be the result of a slightly dehydrated state of the animals, the anesthesia, and the surgical stress. Thus, plasma ADH levels may have been near the minimal plasma ADH concentration which will promote the maximal renal water conservation. We have found in man (unpublished observations) that maximal renal concentrating power is reached at blood ADH levels of 4–6 μU/ml. If this observation holds for the dog, then large increases in urinary water loss would not be expected, even though fairly large reductions in plasma ADH occurred. It has been reported that dogs are hydrated by the continuous infusion of a hypotonic saline solution, thus decreasing the control plasma ADH levels, a much
greater increase in urine flow is observed following left atrial pressure increase than is seen following similar left atrial pressure increases in unanesthetized dogs (14).

The data would indicate that small increases in left atrial transmural pressure (less than 8 cm of water) are sufficient to elicit decreases in plasma ADH concentration. This is presumed to be due to a decrease in ADH release. These findings lend strong support to the concept that the left atrial stretch receptor mechanisms are functionally important in the normal regulation of plasma ADH levels.

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