Radioimmunoassay for Arginine-Vasopressin in Cold Ethanol Extracts of Plasma

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A radioimmunoassay for arginine-vasopressin in human plasma with use of a commercially available antibody was developed and evaluated. The hormone was extracted from plasma with cold 98% ethanol, which showed a significantly higher (p < .001) and more precise recovery than with the acetone–ether procedure (65.3 ± 3.7% vs 50.8 ± 6.0%, respectively). The sensitivity was 0.31 pg per tube. Results for normal subjects in different physiological conditions and in patients with diabetes insipidus and inappropriate antidiuretic hormone secretion showed the good reliability of the method.

Additional Keyphrases: hormones · normal reference interval · diabetes insipidus

There are several reports on the radioimmunoassay of 8-arginine-vasopressin (AVP) (1–3), but application of this technique in human plasma is fraught with difficulties related to raising antibodies with high affinity and good specificity and the need to extract the hormone from plasma. The appearance of commercially available, highly specific antisera for AVP makes measurement of the hormone easier for clinical laboratories. However, AVP extraction from plasma is still a complex procedure with, generally, low and variable recoveries, and its application is still controversial (2, 4, 5).

Here we describe a radioimmunoassay for AVP and a method for extracting AVP from plasma with cold ethanol. The effectiveness and reliability of this technique are compared with those of a classical extraction procedure (acetone–ether). The reference interval for normal subjects and results in some characteristic AVP disorders are also reported.

Materials and Methods

Sample preparation and extraction. Blood was collected into chilled lithium heparin-containing plastic tubes, centrifuged (2000 g, 10 min, 4 °C), and the plasma was stored at −20 °C for one or two weeks before it was extracted and assayed. A previous study from our laboratory (J. Camps, unpublished observations) showed no measurable losses of AVP during such storage. We tested two techniques for the extraction: (a) acetone–ether, following the method of Robertson et al. (4) and (b) ethanol extraction. In the latter, 4 mL of cold 98% ethanol was added to 1 mL of plasma. The mixture was centrifuged (2000 g, 20 min, 4 °C). The ethanol phase was decanted, evaporated under a stream of nitrogen, and the residue was reconstituted with 100 μL of RIA buffer (see below).

Looking for nonspecific interference in such plasma extracts, we compared binding of standards diluted in AVP-free plasma extract and in buffer. We studied also the parallelism between a standard AVP curve and a dilution curve of an extract of AVP-high plasma and compared the nonspecific binding with the nonspecific binding for plasma extracts.

Radioimmunoassay. Synthetic AVP (400 kio. units/ Calbiochem–Behring Corp., La Jolla, CA) was labeled with 125I by the Chloramine T method (6), modified as follows: 30 μL of 0.2 mol/L phosphate buffer (pH 7.5), 20 μL of the solution of Chloramine T (1.7 g/L, in distilled water), and 0.5 mCi of 125I were mixed in a vial. The mixture was shaken gently for 45 s and 35 μL (7.5 μg) of AVP was added. The vial was shaken again for 30 s and its contents were transferred to a tube containing 1 mL of distilled water and 150 mg of the anion-exchange resin Dowex AG 1-X10 (100–200 mesh) to remove free iodine and to stop the labeling reaction (3). After centrifugation, the supernate was applied to a column of Sephadex G-25 (350 × 10 mm) and eluted with 5 mmol/L acetic acid, to separate labeled from unlabeled AVP (7). One-milliliter fractions were collected, and aliquots measured for radioactivity. Undamaged labeled AVP was identified by its ability to bind the antibody.

The buffer used for radioimmunoassay was barbital (20 mmol/L, pH 8.6) containing, per liter, 0.14 mol of NaCl, 0.01 mol of EDTA, and 10 mL of normal rabbit serum. The radioimmunoassay was performed by mixing 100 μL of either standard or sample with 100 μL of anti-AVP serum (Calbiochem–Behring). After incubation for 24 h at 4 °C, 5000 dpm of 125I (100 μL) was added and the tubes were incubated again for 24 h at 4 °C. These incubation times were selected after kinetic studies. Free and bound hormones were separated by the charcoaldextran method (5).

Human studies. These were carried out in normal volunteers. Blood was sampled 15 min after placing a “butterfly” in an antecubital vein, with the subjects recumbent. Baseline values for AVP (after an overnight fast) were measured in 35 normal subjects, two untreated patients suffering from a partial neurogenic diabetes insipidus, and one patient with oat-cell carcinoma associated with a syndrome of inappropriate antidiuretic hormone secretion. In 11 of these normal subjects and in the three patients the effect of increasing plasma osmolality on AVP was also studied by infusing, intravenously, hypertonic saline solution (50 g/L) at the rate of 50 μL per kilogram of body weight per minute during 2 h. Blood was sampled at 30-min intervals from the beginning of the infusion. Plasma osmolality was determined from osmometric depression of the freezing point (Advanced Instruments, Needham Hts., MA 02194).

Results

Sample extraction. Recovery of 125I-labeled AVP added to plasma averaged 50.8 ± 6.0% (n = 30) when acetone–ether was used as a method of extraction and 65.3 ± 3.7% when the ethanol method was used (p < .001). Thus we chose the ethanol method for the extraction of AVP from plasma. We corrected results for samples for extraction losses, using 65% as the mean percentage of recovery.
The curve obtained after diluting standard AVP in buffer was identical to that obtained after diluting the hormone with AVP-free plasma extract (Figure 1). Both curves paralleled that obtained by diluting a plasma extract high in AVP. Nonspecific binding was 4.9 ± 2.4%. Nonspecific binding for plasma extracts was similar, 4.4 ± 2.3%. Therefore it was not calculated routinely.

Radioimmunoassay. The specific activity of the iodinated AVP was greater than 1500 kCi/mol, which is comparable to the specific activity reported for moniodinated AVP (8). The antibody showed high affinity for AVP (Kd = 1.2 × 10^{-11} L/mol) as calculated from a Scatchard plot (9). The specificity of the antibody was tested. No cross reaction was obtained with oxytocin. Cross reaction was 2% with lysine-vasopressin.

The smallest amount that could be detected was 0.31 pg of AVP per tube; the more sensitive portion of the standard curve was between 1.2 and 5.0 pg. The intra-assay CV was 11% (mean ± SD of the controls: 3.9 ± 0.4 ng/L). The interassay CV was 14% (mean ± SD of the controls: 4.2 ± 0.6 ng/L). Analytical recovery was as follows: at 10 pg per tube, mean 88% (n = 4); at 5 pg, 85% (n = 4); at 2.5 pg, 83% (n = 4); at 1.25 pg, 81% (n = 4); at 0.62 pg, 82% (n = 4); and at 0.31 pg, 90% (n = 4).

Human studies. The mean baseline AVP concentration for the 35 normal subjects was 2.4 ± 0.9 ng/L (osmolality, 285 ± 7 mOsm/kg). Figure 2 shows results obtained after the intravenous infusion of hypertonic saline solution. For normal subjects, we observed a highly significant direct linear correlation between osmolality and AVP concentration in plasma (r = 0.80; p < .001). In the two patients with neurogenic diabetes insipidus, AVP was markedly de-

![Figure 2](image-url)  
**Fig. 2.** Relation of plasma AVP to osmolality during infusion of 50 g/L saline in 11 normal subjects (○), two patients with neurogenic diabetes insipidus (DI), and one patient with a syndrome of inappropriate antidiuretic hormone secretion (SIADH) (△). LD represents the lowest AVP concentration that can be reliably detected in the assay. The shaded area represents the 99% confidence limits for the regression of y on x.

creased in relation to plasma osmolality, both in the basal state and during the hypertonic saline infusion. In the case with the inappropriate antidiuretic hormone secretion, AVP concentrations were markedly increased.

**Discussion**

Radioimmunoassay of AVP requires a preliminary extraction, to concentrate the AVP in the plasma of healthy subjects and to eliminate nonspecific interference which probably is due to rupture of the AVP molecule by the proteolytic enzymes of plasma (4). Although it has been shown that these nonspecific interferences can be also eliminated by adding high amounts of oxytocin and protease inhibitors to the incubation medium to prevent AVP degradation, the radioimmunoassay described for unextracted samples show high nonspecific binding, which reduces the accuracy of the assay (5). The most important arguments against the use of extraction procedures in the radioimmunoassay of AVP are the complexity of these methods and the variable percentage recovery obtained with such classical extraction media as acetone–ether or Bentonite (5). The current study shows that these inconveniences may be partly obviated by using cold ethanol as the extraction medium: recovery of AVP was found to be significantly higher and the range of recovery narrower. Moreover, ethanol extraction is easier to perform.

Our results for healthy subjects indicate that the radioimmunoassay described here fulfills the requirements for an accurate, sensitive measurement of AVP in human plasma. When the extracts were 10-fold concentrated, the assay could detect up to 0.5 ng of AVP per liter, a sensitivity similar to that of the most sensitive radioimmunoassays yet described (4, 5, 10). The plasma AVP concentrations obtained in basal conditions and following a hypertonic saline infusion were comparable with those obtained by other investigators (8, 11, 12). In the two patients with partial neurogenic diabetes insipidus and in the case with a syn-
drome of inappropriate antidiuretic hormone secretion, baseline values for AVP in plasma were found to be inappropriately low and high, respectively, in relation to plasma osmolality. Furthermore, in these cases the plasma AVP concentration did not change when plasma osmolality was increased.

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References

Osmometry of CO2 in Gas Samples
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Measurement of solute concentrations in biologic fluids by an osmometric technique has been described previously. Here, we describe an osmometric technique for measuring CO2 in gas samples. A solution of NaOH is injected into a graduated syringe containing the gas sample and CO2 is trapped in the reaction: 2NaOH + CO2 → Na2CO3 + H2O. The decrease in osmolality of the NaOH solution allows estimation of pCO2 in the original gas sample.

Additional Keyphrase: blood gases

We have previously described simple and precise osmometry of total CO2 in biological fluids and of urinary ammonia (1, 2). Here we describe a technique for measurement of CO2, which utilizes the same osmometric principle.

Materials and Methods

The materials for this technique consist of two graduated syringes, 0.2 and 0.3 mol/L solutions of NaOH, and an osmometer.

Fill the first syringe with the gas sample in which the CO2 content is to be measured, and close its tip with a snugly fitting rubber cap. Using a needle to pierce the rubber cap, inject 2 mL of 0.2 or 0.3 mol/L NaOH into the syringe. A calibrated syringe can be used to inject the NaOH. We used graduated 60-mL plastic syringes for the measurement. As long as the same syringe is used, we have been able to obtain excellent reproducibility, with a CV of <1%. For 2 min, vigorously shake the syringe into which the NaOH has been injected, to trap the CO2 as shown in the following reaction:

\[
\text{CO}_2 + 2 \text{NaOH} \rightarrow \text{Na}_2\text{CO}_3 + \text{H}_2\text{O}
\]

The measurement of osmolality of NaOH solution which has taken up CO2 allows the determination of pCO2 in the original gas (1).

To determine how long it takes for all the CO2 to be reacted, we made repeated measurements on samples from a single collection of expired air, shaking the samples with NaOH for increasing intervals up to 2.5 min. The reaction was complete within 1.5 to 2 min.

Osmolality was measured with an Advanced Osmometer (Advanced Instruments, Inc., Needham Heights, MA 02194). The precision of the osmometer is ±1 mOsm/L. This would affect pCO2 measurements by ±0.42 mmHg when 60-mL syringes are used. The Corning 165/2 pH/Blood Gas Analyzer (Corning Medical, Medfield, MA. 02052) was used to measure pCO2 by another technique for comparison purposes.

Calculations. Calculate the quantity of CO2 trapped in the NaOH solution as follows:

\[
\text{CO}_2 \text{(mmol)} = \Delta \text{Osm \ (mOsm/L)} \times V / 1.323
\]

where ΔOsm is the change in osmolality of NaOH solution caused by CO2 trapping, and V the volume of NaOH.