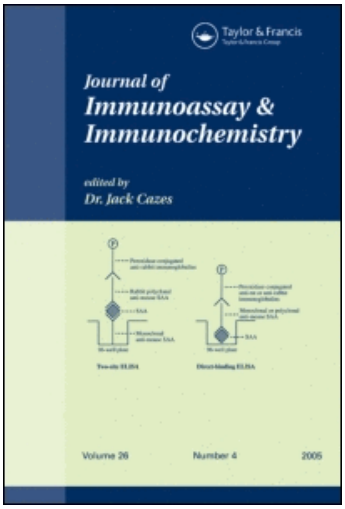

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EFFECT OF PLASMA INTERFERENCE ON DIFFERENT VASOPRESSIN ANTISERA

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ABSTRACT

The effect of plasma interference upon 2 AVP antisera both of high affinity and specificity, was investigated. Chromatography of plasma from a normal subject on Sephadex G-25 gave 3 peaks of AVP-like material with antibody A eluting in the void volume, salt volume and in the AVP volume; the quantity of AVP-like material detected in the void volume was 95% less with antibody B. Similar elution profiles were observed in a patient with cranial diabetes insipidus but the third peak was absent. AVP-like material in the first 2 peaks was probably due to interference in the radioimmunoassay. Extraction of plasma by Florisil reduced the first 2 peaks (antibody A) and eliminated the void volume peak (antibody B). Plasma interference affected the absolute values of plasma AVP concentrations of osmotically-stimulated normal and diabetic subjects. AVP antisera displaying least plasma interference are recommended for AVP radioimmunoassay.

INTRODUCTION

During the past decade numerous radioimmunoassays for the measurement of plasma arginine vasopressin (AVP) have been described. Although some have been able to readily detect low physiological concentrations of the hormone (e.g. 1, 2, 3) others have needed to concentrate large volumes of samples to enable measurement of physiological levels (e.g. 4, 5, 6). One major problem encountered by the latter groups has been the inability

to achieve very low or undetectable plasma AVP values after adequate suppression of AVP secretion. These assays appeared to suffer from non-specific interference which became particularly noticeable when measuring low AVP concentrations, and consequently the absolute values given for plasma AVP may have been spuriously high.

The purpose of this study was the investigation of the nature and magnitude of the non-specific interference, the efficacy of extraction methods to remove it and to assess its effect on AVP immunoassay measurements using 2 different antisera to AVP.

MATERIALS AND METHODS

Two antisera which have been well characterised were investigated; one (antibody A) was described by Baylis & Heath (5) and the other (antibody B) by Rooke & Baylis (7). All reagents were Analar Grade.

Chilled fresh heparinised plasma was obtained from a normal volunteer and a patient with severe familial cranial diabetes insipidus (24 h urine volume 13 l) after osmotic stimulation with hypertonic saline. One aliquot (2 ml) was applied to a column (45 cm x 0.9 cm) containing Sephadex G-25 fine (Pharmacia Fine Chemicals) and was eluted with 0.25% acetic acid containing 1 g/l BSA and 0.3 mmol/l sodium azide at 4°C. The eluate was collected in 2 ml fractions over a period of 3 h and were then stored at -30°C.

AVP was extracted from the other aliquot (2 ml) by a modification of the absorption technique described by Beardwell (4).

The extraction involved adding 20 mg of cleaned and heat-activated Florisil to the plasma which was rotated for 10 min. The plasma was removed and the Florisil washed twice with distilled water and then acidified with 0.2 mol/l HCl. The hormone was eluted by the addition of 2 ml aqueous-acetone (1:10) to the Florisil. Eluates from 2 aqueous-acetone treatments were pooled and added to an equal volume of diethyl ether. The solutions were mixed vigorously and, after allowing to stand for 5 min, the top phase was aspirated. The lower phase was dried under a stream of nitrogen, at 40°C. The residue was reconstituted with 2 ml 50 mmol/l Tris-HCl buffer containing 3.5 g/l BSA and 0.3 mmol/l sodium azide before application to the Sephadex column and elution with the acetic acid eluant. Studies were repeated using a column (90 x 2 cm) of Sephadex G-150.

Before immunoassay, the pH of each fraction was adjusted to 7.5. Immunoreactive AVP or AVP-like material was measured using antibody A and B in chromatographed neat and extracted plasmas from the normal subject and the patient with cranial diabetes insipidus.

The influence of the 2 different antisera in the immunoassay, on the absolute values of plasma AVP concentrations was investigated by observing the progressive rise in plasma AVP in response to increasing plasma tonicity. The same 2 volunteers were infused with 5% saline (0.06 ml/kg/min) for 2 h after overnight fasting with only water to drink. Venous blood samples were taken before infusion (x 2), at intervals of 30 min during the infusion, and 15 min after

cessation of saline infusion, into chilled heparinised plastic tubes. After plasma was separated from the cells at 4°C within 15 min of venepuncture, one 2 ml aliquot was deep frozen immediately at -30°C to be assayed for AVP within 2 weeks; plasma osmolality was measured in the other aliquot within 1 h (Advanced Osmometers Incl, Model 3R).

Simple linear regression analysis by the method of least squares (8) was performed on the plasma AVP and osmolality data.

RESULTS

Immunoreactive AVP or AVP-like material was detected in the void volume, the salt volume, and in the position at which synthetic AVP elutes, after chromatography of normal unextracted plasma using antibody A in the immunoassay (Figure 1). Assay with antibody B showed a similar pattern but the immunoreactivity in the void volume was reduced by 95%, and there was no immunoreactivity detected in the salt volume (Figure 1). Chromatography of unextracted plasma from the patient with cranial diabetes insipidus showed that, with antibody A, there were 145 fmol of AVP-like material in the void volume, 16 fmol in the salt volume, but AVP was not detected at the elution position of AVP. With antibody B, immunoreactive AVP-like material (5 fmol) was measured only in the void volume.

Elution profiles of unextracted plasma from these 2 volunteers after chromatography on Sephadex G-150 showed that the first peak of immunoreactive AVP-like material (void volume on Sephadex G-25,

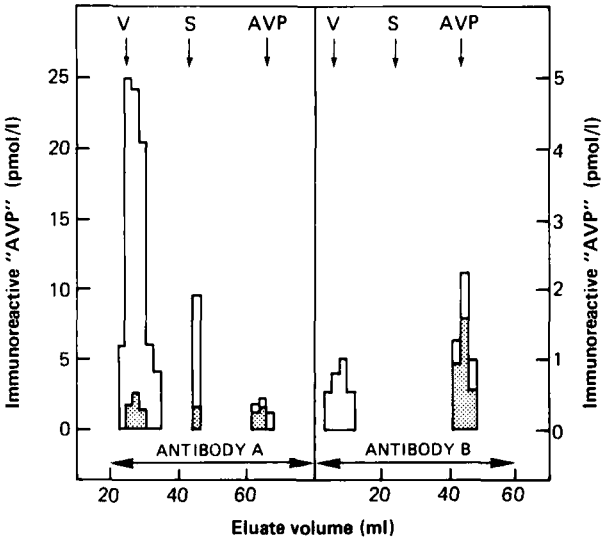

Figure 1

Elution profile of plasma from an osmotically-stimulated normal adult after chromatography on Sephadex G-25 (fine). Eluant was 0.25% acetic acid containing 1 g/l BSA and 0.3 mmol/l sodium azide. Open and stippled bars represent immunoreactivity detected by antibodies A and B, respectively.

antibody A) appeared to elute at a position which corresponded to a molecular size of approximately 150,000 daltons. The rest of the pattern of the elution profile on Sephadex G-150 was similar to Sephadex G-25.

The effect of extraction of plasma by the Florisil technique on the elution profiles on Sephadex G-25 is given in Figure 2. With antibody A, there was 90% reduction in immunoreactive AVP-like material in the void volume, 80% reduction in the second peak and 30% reduction in the third peak after extraction (left panel). Extraction removed all immunoreactivity in the first peak, and AVP was reduced by 20% in the second peak, with antibody B.

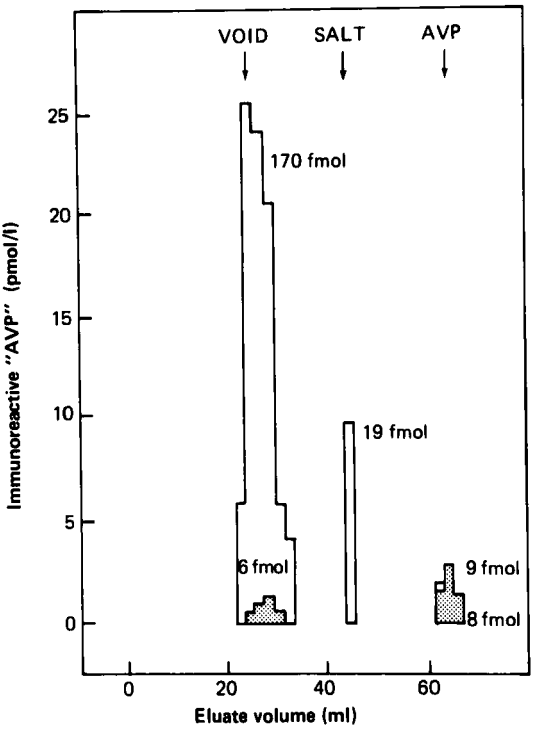

Figure 2

Chromatography on Sephadex G-25 (fine) of neat and extracted plasma (open and stippled bars, respectively) from an osmotically-stimulated normal adult. Results obtained with antibody A are in the left panel, and those with antibody B in the right panel.

Absolute values of plasma AVP concentrations during osmotic stimulation are given in Figure 3. In the patient with cranial diabetes, plasma AVP remained undetectable (<0.3 pmol/l), except for the final estimation, with antibody B. Immunoassay with antibody A of the same samples showed no rise in plasma AVP which fluctuated about 1 pmol/l. Regression analysis of the data points obtained from the normal subject defined the equation pAVP = 0.21

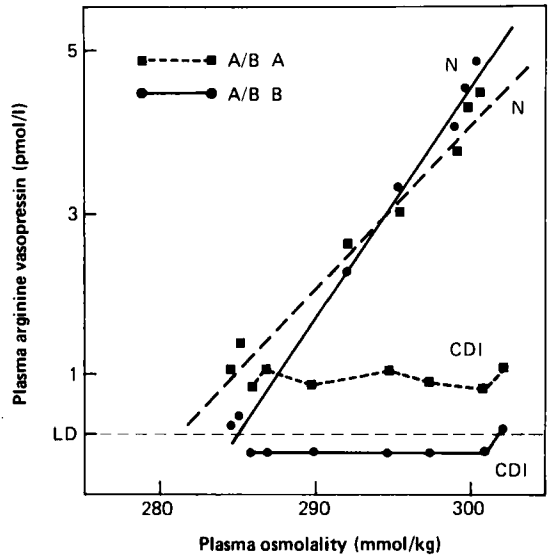

Figure 3

Relationship between plasma AVP, measured by antibodies A and B, and plasma osmolality during 5% saline infusion in a normal adult (N) and a patient with severe familial cranial diabetes insipidus (CDI).

(pOS - 280), $r = +0.97$, $p < 0.01$ for antibody A, and pAVP = 0.27 (pOS - 283), $r = +0.99$, $p < 0.01$ for antibody B, where pAVP represents plasma AVP and pOS represents plasma osmolality.

DISCUSSION

The results of these studies indicate that the two AVP antisera appear to detect different patterns of AVP or AVP-like material in normal fractionated plasma. Three peaks of apparent AVP immunoreactivity were observed using antibody A with the greatest quantity of immunoreactivity present in the void volume frac-

tions. A similar elution profile (9) was observed in a patient with the syndrome of inappropriate antidiuretic hormone secretion using one antibody raised by Wu & Rockey (10). In contrast, the Glick-I antibody gave an elution profile of this patient's plasma that was similar to antibody B described in the study (9). The previous work with plasma from the patient with the syndrome of antidiuresis can be criticised because the AVP may have been in unusual molecular forms, as part of precursor molecules, or bound to plasma constituents. However, there is considerable evidence that the majority of physiological AVP circulates in an unbound form (11, 12).

The nature of the immunoreactivity in the first two peaks defined by antibody A is not clear. Theoretically it is possible that in the first peak, antibody A bound to AVP that was attached to AVP-specific neurophysin, but the molecular weight of the neurophysins is of the order of 10,000 daltons. Our studies suggest that the immunoreactivity was detected in constituents approximately 150,000 daltons. Furthermore, the elution pattern of the patient with familial cranial diabetes insipidus showed that the first 2 peaks detected with antibody A were similar to the normal's profile. It is possible, but most unlikely, that this patient was secreting protein-bound AVP that was physiologically inactive. The more probable conclusion is that the first 2 peaks do not reflect true immunoreactive AVP, but that the depression of binding in the immunoassays is due to non-specific interference. In the antibody A radioimmunoassay, depression of binding can be readily achieved by high concentrations of BSA or increasing the salt

concentration (5), confirming that this antibody is subject to non-specific interference. These conclusions are in keeping with previous observations (9).

Antibody B measured consideraby less AVP-like material in the first peak than antibody A, and none was detected in the salt volume. Following extraction with Florisil, the chromatographic profile of plasma showed substantial reductions in "AVP immunoreactivity" in the first 2 peaks with antibody A, absence of the first peak with antibody B, and slight reduction in the third peaks with both antibodies. It appears, therefore, that this extraction method will not remove all non-specific interference with antibody A and consequently the absolute values of plasma AVP concentration will be spuriously elevated. This is not so with antibody B. The difference can be readily appreciated in Figure 3. The patient with familial cranial diabetes insipidus appears to have detectable plasma AVP, measured by antibody A. As anticipated, the basal plasma AVP concentrations in the normal subject are also raised with antibody A, but the values after osmotic stimulation are similar to those obtained with antibody B. The latter discrepancy may be accounted for by slight variations in the efficacy of the extraction procedure removing non-specific interference.

We conclude that, in the selection of antisera for AVP radioimmunoassays, one important consideration must be the effect of non-specific interference. We recommend that only those subject to low interference should be selected for radioimmunoassays. Failure to do so will result in high basal values, as reported

by some workers (3, 6, 13, 14), and distort data on AVP secretion.

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