A NOVEL EXTRACTION METHOD FOR PLASMA VASOPRESSIN AND ITS APPLICATION IN A RADIOIMMUNOOASSAY

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Only a few of the radioimmunoassays for vasopressin (AVP) published previously (Dunn, Brennan, Nelson & Robertson, 1973; Möhring & Möhring, 1975) have been sensitive enough for use in the rat. This communication describes a novel method for extraction of AVP from plasma involving the use of a solid-phase antibody and its application in a radioimmunoassay sufficiently sensitive to detect this hormone in rat plasma.

Antiserum to AVP (102/5, 250 μl; Morton, Padfield & Forsling, 1975) was coupled to CNBr-Sepharose 4B (2 g) by mixing for 18 h at room temperature in 0·1 m-NaHCO₃-0·5 m-NaCl (15–20 ml). The solid antiserum was mixed with 0·2 m-Tris–HCl, pH 8·0, for 18 h and then washed with 1 m-acetic acid (200 ml). The solid antibody was finally equilibrated with 42·5 ml 0·05 m-Tris–HCl, pH 7·5, to give an antiserum dilution of 1:170.

Solid AVP antiserum (0·5 ml at a dilution of 1:170) was added to single or duplicate plasma samples (1 ml or less) in 2 ml polystyrene tubes to give a final antiserum dilution of 1:510 and a final volume of 1·5 ml. Plasma samples of less than 1 ml were made up to 1 ml with 0·9% saline. The samples were extracted by mixing end-over-end for 18 h at 4 °C. The tubes were then centrifuged and the plasma discarded. The solid antiserum containing the extracted AVP was washed twice with 2 ml water and centrifuged after each wash. The extracted AVP was eluted from the solid antiserum by mixing end-over-end for 30 min at room temperature with 1 ml 0·05 m-acetic acid, pH 3·1. The elution was repeated and the combined acetic acid extracts dried under a stream of air. The dried extracts were dissolved in 0·05 m-Tris–HCl, pH 7·5, and 50 μl samples were removed for assay. Each assay also included duplicate standards of between 0·125 and 8·0 fmol synthetic AVP in 50 μl Tris–HCl, pH 7·5. To these standards or to unknown plasma extracts were added AVP antiserum at a dilution of 1:3500 (kindly supplied by Dr J. Möhring, Centre de Recherche, Merrell International, Strasbourg, France) and 1 fmol ¹²⁵I-labelled AVP (50 μl). Tubes were incubated for 2 days at +5 °C. The separation of bound and free labelled hormone was achieved using plasma-coated charcoal (0·5 ml). The smallest amount of AVP detectable was 0·2 fmol/tube.

The recoveries of 100, 500 and 1000 fmol ¹²⁵I-labelled AVP from human plasma were 65, 66 and 67% respectively and recoveries of 1, 2·5, 5 and 10 fmol unlabelled AVP in ten separate experiments were 58 ± 10 (s.d.), 64 ± 11, 63 ± 10 and 62 ± 8% respectively. The mean concentration of AVP in the plasma of 15 normal subjects after overnight fasting and 30 min recumbency was 1·6 pmol/l (range 0·9–2·3 pmol/l). After an oral water-load of 20 ml/kg in five subjects, the level of AVP in the plasma fell from 1·9 ± 0·38 to 0·7 ± 0·3 pmol/l (P < 0·01); after fluid deprivation for 24 h, the level rose from 2·1 to 2·9 pmol/l. The concentration of AVP in the plasma correlated with both plasma and urine osmolality (r = 0·66; P < 0·001, n = 32; r = 0·65, P < 0·001, n = 32, respectively).

The recoveries of 5 and 10 fmol AVP from rat plasma were 53 and 59% respectively (n = 3). The mean concentration of AVP in the plasma of five conscious, unrestrained rats who had been bled (1 ml) via a catheter in the carotid artery was 3·9 ± 1·2 pmol/l. Sequential removal of 1 ml blood from two conscious unrestrained rats gave rise to increases in the concentrations of AVP: after the removal of only 2 ml blood, the concentrations rose from 5·4 and 2·4 to
6·2 and 2·6 pmol/l respectively; after the removal of 6 ml blood, the concentrations of AVP rose exponentially to 4008 and 701 pmol/l respectively (Fig. 1).

Fig. 1. Increases in the concentration of vasopressin (AVP) in the plasma of two conscious unrestrained rats after sequential removal of 1 ml blood samples via a catheter in the carotid artery. Values on the ordinate are plotted logarithmically.

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REFERENCES