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

J. J. MORTON AND A. J. G. RIEGGER

MRC Blood Pressure Unit, Western Infirmary, Glasgow, G11 6NT

(Received 19 December 1977)

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.

A. J. G. R. received support from the Deutsche Forschungsgemeinschaft.

REFERENCES

