

Radioimmunoassay of Human Prolactin

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We have already reported on the results of human prolactin assay at the Endocrine Meeting, April, 1973, using the human prolactin and its antiserum given by Dr. H. G. Friesen. We report here on the investigation of crossreactivity of human, sheep and pig prolactin. Using anti-human prolactin, prolactin reacted in the order of human, sheep and pig. The same order was shown

for the displacement. When anti-human prolactin, ^{125}I -sheep prolactin were used, the displacement was similar with human and sheep prolactin. Using anti-sheep prolactin, and labeled human, sheep, pig prolactin, the results showed on good displacement as the assay system of human prolactin.

Radioimmunoassay of Human Prolactin (HPr)

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A sensitive radioimmunoassay for human prolactin (HPr) was developed with highly purified HPr and antibodies to HPr (kindly supplied by Dr. H. Friesen). HPr (Friesen 71-9-4) was iodinated with ^{125}I to specific activities of between 130 and 200 $\mu\text{Ci}/\mu\text{g}$ by a modified chloramin T method of Greenwood et al and also used for standards in these studies. The double antibody technique was used to separate bound and free labelled hormones. The parallelism was observed among the inhibition curves of the standard HPr and those of sera obtained from a patient with Forbes-Albright syndrome and a pregnant woman. Human GH, TSH, ACTH, LH and FSH showed no significant cross-reactivity in the assay. No cross-reactivity with rat or porcine prolactin was demonstrated. Ovine prolactin cross-reacted but given a non-parallel inhibition curve. The minimal detectable HPr value was 1–2.5 ng/ml. The

average coefficient of variation was 9.4% in different assays.

Basal levels of plasma HPr in normal subjects were less than 30 ng/ml and 90% of them were under 20 ng/ml. The mean ($\pm\text{SE}$) basal HPr levels in 32 normal subjects was 11.4 ± 1.3 ng/ml. No significant sex difference was observed. Elevated levels of plasma HPr were demonstrated in some patients with chromophobe adenoma, ectopic pinealoma, histiocytosis X, HPr producing tumor, hypothyroidism and galactorrhea due to drugs. Plasma HPr levels were normal in most of the patients with acromegaly. Plasma HPr was detectable in some patients with pituitary dwarfism (including isolated GH deficiency) and panhypopituitarism. Synthetic TRH (500 μg iv), chlorpromazine (25 mg im), insulin (0.1 U/Kg iv), arginine (0.5 g/kg iv) and 5-HTP (200 mg po) caused a significant increase in plasma

HPr in normal subjects, whereas L-DOPA (500 mg po) and CB-154 (2.5 mg po) decreased plasma HPr levels. Plasma HPr responses to TRH impaired in most of the patients with Sheehan's

syndrome. The treatment with CB-154 (2.5 mg po bid) was effective to reduce plasma HPr in a patient with Forbes-Albright syndrome.

Radioimmunoassay of Vasopressin in Human Plasma

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This report refers to a highly sensitive and specific radioimmunoassay for vasopressin.

Lysine-8-vasopressin is coupled to porcine gamma globulin by a carbodiimide reaction and the conjugate is injected into a guinea pig at three-weekly intervals. Synthetic lysine vasopressin is used for iodination. Gel filtration on Sephadex G-25 fine is used both to purify the iodinated vasopressin and to increase its specific radioactivity.

Duplicate volume of plasma, 10 ml each, is added to 200 mg of Florisil and thoroughly shaken for 10 min at room temperature. The Florisil is washed twice with 5 ml volumes of cold 0.2 N hydrochloric acid and the vasopressin is then eluted by mixing the Florisil with 10 ml of cold acid-acetone for 5 min. The eluate are then lyophilized and the residue redissolved 0.05 M phosphate buffer containing 0.1 gram of lysozyme in 100 ml (lysozyme diluent). Each tube in the standard curve contains 0.1 ml of vasopres-

sin antiserum, 0.1 ml of ^{125}I -LVP (3000–6000 cpm), and 0.6 ml of lysozyme diluent. After 1 hour of incubation at 25°C, the tubes are incubated for 72 hours at 4°C. Dextran-coated charcoal was used for the separation of bound and free vasopressin.

Ten pg of vasopressin is the smallest amount that can be detected with confidence in most assays. The antiserum does not distinguish arginine from lysine vasopressin, but oxytocin causes no displacement of ^{125}I -LVP from antiserum. The mean recovery was 50–70%. Intraassay variability was $\pm 5.0\%$. The plasma vasopressin levels are 0–3.5 pg/ml in normal subjects. In two patients smoked while hydrated a definite rise in vasopressin level was seen. Comparison of plasma AVP level with plasma osmolality shows a clear correlation.

The method should enable us to gain a more complete understanding of the part played by vasopressin in body fluid homeostasis.