

Measurement of Plasma 18-Hydroxy-11-Deoxycorticosterone (18-OH-DOC) Levels by Radioimmunoassay

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A radioimmunoassay method for the measurement of plasma 18-OH-DOC has been developed. The antiserum against 18-OH-DOC was produced in 1,2-³H-18-OH-DOC and standard 18-OH-DOC were stored in ethanol at -20°C. 1,2-³H-18-OH-DOC was added to all samples to correct for recovery. Plasma was extracted with dichloromethane and chromatographed on LH-20 column (1 × 55 cm). The purified extracts were incubated with antiserum at a 1/1000 dilution for 30 minutes at 25°C, and then for 16 hours at 4°C. Saturated ammonium sulfate was used to separate free from bound 18-OH-DOC.

Recovery after extraction was 4.6 ± 7.5 (SD) %. The accuracy and precision of the method were acceptable, and a sensitivity of 5 pg per sample enabled the measurement of very low levels of plasma 18-OH-DOC. High specificity of the method was obtained by high-specificity of the

antiserum and a good separating ability of Sephadex LH-20 column chromatography.

The mean plasma 18-OH-DOC levels at 8 a.m. were 16.8 ± 5.6 ng/dl in 22 normal subjects, and 15.5 ± 8.7 ng/dl in essential hypertension. In low renin hypertension, the levels were lower than the other hypertensive groups. Plasma 18-OH-DOC was normal or high levels in primary aldosteronism, and high in DOC producing tumor and in 17- α -hydroxylase deficiency.

Plasma 18-OH-DOC was increased markedly by ACTH and suppressed by dexamethasone. Upright position after furosemide administration, dietary sodium restriction or angiotensin II infusion increased moderately plasma 18-OH-DOC.

These results confirm that 18-OH-DOC secretion is regulated primarily by anterior pituitary, and renin-angiotensin system plays a minor role in 18-OH-DOC secretion.

Clinical Problems of Commercial Kits for Human LH Radioimmunoassay

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Basic studies of human LH radioimmunoassay were performed using two kinds of commercial kits provided by Carbiochem Co. U.S.A. (Kit A) and CEA-IRE-SORIN (Kit B).

Kit A: Sensitivity was 1.95 mIU/ml and LH concentration up to 500 mIU/ml was able to be determined. The dilution curve of plasma was parallel to the standard curve. Coefficient of variation (C.V.) and recovery of added LH ranged from 7.8 to 250 mIU/ml were 5.23–13.95% and 85.7–119.3%, respectively. Intra-assay variability and inter-assay variability were 4.78–9.21% and 4.4–15.8%, respectively. In this assay system, the remarkable cross-reactions of TSH and FSH with antiserum for LH were observed, that is, TSH

crossreacted 104% and FSH 114.7%. High concentration of prolactin and GH also crossreacted, but they were able to be neglected clinically. ACTH did not crossreact. Plasma LH concentration in normal male subjects was 8.72 ± 2.0 mIU/ml (Mean \pm SD).

Kit B: Sensitivity was 0.5 ng/ml and LH concentration up to 50 ng/ml was able to be determined. The dilution curve of plasma was parallel to the standard curve. C.V. and recovery of added LH ranged from 1 to 25 ng/ml were 2.95–25.39% and 63.2–115.8%, respectively. Especially in low concentration of added LH, recovery was not satisfactory. Intra-assay variability was 8.42–10.89%. Regression equation between two different

assays was $Y=1.06X+0.18$ and coefficient of correlation was 0.99. TSH crossreacted 15% with antiserum for LH, FSH also crossreacted less than 7% and prolactin 0.4%. GH and ACTH did not crossreact.

Correlationship between Kit A and Kit B: Regression equation was $Y(\text{ng/ml})=0.08X (\text{mIU/ml})+0.06$ ($X=0-300 \text{ mIU/ml}$) and Coefficient of correlation was 0.97. Plasma LH level in normal males calculated using this expression was $0.7 \pm$

0.17 ng/ml .

In these findings two problems were indicated. (1) Kit B was inadequate in observing the small LH changes near the resting level. (2) In both kits, especially Kit A, not only HCG but TSH and FSH remarkably crossreacted with antiserum for LH. When plasma samples containing high concentration of TSH or FSH are assayed, LH values may be modified by these hormones.

Basic Studies and Clinical Applications of Radioimmunoassay Kit for Human Prolactin

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Recently, a specific and sensitive radioimmunoassay (RIA) kit for human prolactin (PRL) has been developed by CEA-IRE-SORIN(CIS). By using this kit, the following basic and clinical experiments were performed.

The standard curve of this kit was almost completely paralleled with the standard curve by MRC 71/222 added in this kit. Moreover, the dilution curve of the plasma from a patient with hyperprolactinemia was also paralleled with this standard curve. The assay sensitivity of this kit was from 2.5 ng/ml to 200 ng/ml of serum or plasma. The cross-reactivity of with LH, FSH, TSH, GH, HCG, HPL, TRH, LH-RH and Somatostatin were not seen in this assay. Plasma samples with human PRL concentrations of 3 ng/

ml, 15 ng/ml, 120 ng/ml and 250 ng/ml had intra-assay coefficients of variation of 23.4%, 7.6%, 15.2% and 10.5% respectively. Inter-assay coefficient of variation of human PRL 20 ng/ml was 16.9%.

In clinical use, the fasting PRL level of normal male subjects ($n=20$) was $11.3 \pm 6.0 \text{ ng/ml}$. This value was almost coincided with the datum obtained from our NIH kit. The patients with hyperthyroidism, breast cancer and anorexia nervosa showed normal PRL values. However, patients with acromegaly and Cushing's syndrome showed a slightly high PRL values.

These data suggest that this kit is clinically a very useful tool for measuring human PRL.

Studies of Gastrointestinal Hormones by Radioimmunoassay

Report II. Radioimmunoassay of Secretin

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In our previous papers we reported on the radioimmunoassay of gastrin and secretin. The present study was undertaken to investigate further fundamental aspects of secretin assay. The results obtained are reported in this paper.

For the study were employed an antiserum preparation with an antibody titer of 1: 6000 (supplied by Eisai K.K.), Schwarz-Mann 6-tyrosyl

secretin as labelled secretin and purified secretin from raw extracts as reference standard.

Under various conditions (involving dilution of antibody, one- or two-step method, concentration of protein added, addition or non-addition of trasyllol, and pH value of buffer) calibration curves were prepared and compared.

For assay samples containing secretin at a con-