

D. Measurement C (in vitro)

TRH Radioimmunoassay

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TRH radioimmunoassay had been developed for unextracted human serum. Anti-TRH antibody was produced immunizing with rabbits TRH-bis-diazotized-benzidine-bovine serum albumin. This antibody was little cross-reacted with L or D,L Aze3-TRH, but not other TRH analogues, amino acids or pituitary hormones.

Radioiodination of TRH was performed by Greenwood-Hunter's method. Purification of TRH-I-125 was performed on a Sephadex G-10 column and three peaks were obtained. First peak was damaged protein, second peak was TRH-I-125 and third peak was I-125. Second peak was further divided to ascending (a), top (b) and descending portion (c). Immunoreactivity of each portion was 0.23 (a), 0.52 (b) and (c). Portion b or c were used this experiment. Immunoreactivity of TRH-I-125 was decreased with several times of freeze and thaw of TRH-I-125. Immunoreactivity was stable for 20 days if TRH-I-125

was divided to small amount.

It was found that TRH immunoreactivity was inactivated with serum. This inactivation could be prevented with adding of BAL.

In this system, recovery of known amounts of TRH were approximately 100%. Dilution curve of high TRH serum was parallel to standard curve. In this system sensitivity was 0.01ng/tube.

TRH levels measuring with this system were undetectable to 2.0ng/ml in normal subjects, undetectable in hyperthyroid patients or a tertiary hypothyroid patient and high in primary or secondary patients.

Urinary excretion of TRH following synthetic TRH iv. administration in a normal subject was about 3% for 120 min.

From above data it was suggested that this system is very useful tool to study the role of hypothalmo-pituitary thyroid axis in clinical basis.

ACTH Radioimmunoassay

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The ACTH radioimmunoassay was announced to be established by R.C.C., England,

recently. This assay system is composed of two procedures, namely, (1) extraction of ACTH from plasma and (2) radioimmunoassay of ACTH. The manual of this assay kit described as follows: In extraction procedure, plasma ACTH was adsorbed to the glass particles, desorbed from them with fifty percent of acetone and then dried up under air stream. In radioimmunoassay procedure, ACTH antisera and samples/or standard solutions of ACTH were incubated for 16 to 20 hours at 4 C and then second incubation for 6 to 8 hours was performed after addition of I-125-ACTH. Bound and free forms of ACTH were separated with charcoal adsorbent.

In this study, each steps of this assay system reevaluated whether the prescribed procedure were suitable for measurement of plasma ACTH level or not.

In extraction procedure, fifty five percent of plasma ACTH was adsorbed to glass particles and approximately forty percent of

ACTH was desorbed from them. Initial plasma volume used for extraction affected on the extraction rate of ACTH. The antibody was not crossreacted with TSH, HGH, FSH or HCG. The bound percent of I-125-ACTH increased gradually according to extension of incubation time up to 6 hours, and kept in the same value after that. The bound percent was not affected by the incubation time for thirty minutes after charcoal adsorbent addition. The recovery of ACTH revealed ninety percent in this system. The intraassay variation was 120 ± 14 pg/ml (M. \pm SE).

The basal level of plasma ACTH at six o'clock varied from 20 to 100pg/ml in normal and 27 to 100 pg/ml in Cushing's syndrome (hyperplasia) In normal subjects ACTH levels in plasma were markedly increased after SU-4885 administration keeping diurnal variation.

The above data indicated that this system was useful to measure plasma ACTH and to study a role of pituitary adrenal axis.

Studies on the Regulation of Human Prolactin Secretion

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A sensitive radioimmunoassay (RIA) for human prolactin (hPRL) was developed with purified hPRL and antiserum to hPRL (kindly supplied by NIAMDD). hPRL was iodinated by the enzymatic method using lactoperoxidase. When the radioiodinated hPRL was passed through a 1.5×50 cm column of Sephadex G-100, it usually separated into three peaks. By the solid-phase RIA using antibody-coated plastic disposable microtiter trays, it was confirmed that the second peak consisted of

the immunoreactive material that was used for RIA. For the measurement of plasma hPRL levels, the double antibody technique was used to separate bound from free labeled hormones. The average coefficients of variation were 11.7% in within assays and 14.8% in between assays.

Basal plasma hPRL levels in normal subjects were less than 30ng/ml, and no statistically significant sex difference was observed. Intravenous administration of 500 μ g of TRH