I. Radioimmunoassay

Radioimmunoassay of Adrenocorticotropic Hormone (ACTH) and Its Clinical Application

Y. NAKAI and S. MATSUKURA

Second Division, Department of Medicine, Kyoto University School of Medicine, Kyoto

H. IMURA

Third Division, Department of Medicine, Kobe University School of Medicine, Kobe

We have developed a new technique for radioimmunoassay of ACTH based on the discovery of an increase in binding of ^{125}I-ACTH was added to relatively high concentration of antisera. Antisera used in the present experiment was prepared in rabbits by injecting human ACTH (Raben) or ACTH-Z (Organon). Highly purified human ACTH(Li) was used for iodination and reference standard. Bound and free ACTH were separated by the talc method of Rosselin et al. When 1 to 2pg of ^{125}I-ACTH was added to high concentration of antisera, a progressive increase in B/F ratio occured instead of the conventional decrease. This paradoxical phenomenon was observed by both macroglobulin and globulin fractions of antisera obtained by gel filtration. It was also not influenced by incubation period nor shaking of incubation mixture. Using this method, 1 to 2pg of ACTH was consistently detected. Reproducibility and specificity in this assay system were satisfactory. Serial dilution of plasma of an Addisonian patient gave parallel curve to that of standard ACTH. These results indicate the availability of this new phenomenon to the radioimmunoassay of ACTH.

Plasma ACTH levels were measured by this method in patients with various endocrine disorders. Plasma ACTH levels were elevated in patients with Addison's disease and Cushing's disease after adrenalectomy and decreased in patients with hypopituitarism and in Cushing's syndrome due to adrenal adenoma. Oral administration of metyrapone, given 30 mg/kg once at midnight, produced a marked increase in plasma ACTH after 8 to 10 hrs in normal subjects, but not in patients with pituitary insufficiency. No overlap in plasma ACTH was noted between these 2 groups. Basal morning plasma ACTH levels were moderately elevated in patients with untreated Cushing's syndrome due to adrenal hyperplasia, but normal diurnal rhythmicity was lacking. The assay of plasma ACTH may prove of value in differentiating between primary and secondary adrenocortical insufficiency and especially in helping to evaluate the etiology of Cushing's syndrome.