Symposium III. Radioimmunoassay

Peptide hormones

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Radioimmunoassay, which was first introduced by Berson and Yalow for the assay of plasma insulin, has been extensively used to measure various peptide hormones in the past decade. During this period, however, certain problems have been recognized in techniques and validation of radioimmunoassay. We would like to discuss about these problems as well as advantages of this procedure.

1. Production of antibody against peptide hormones of small molecular weight.

It is usually difficult to produce antisera of high titer against peptides of small molecule. We have immunized animals with ACTH and glucagon using various procedures, such as conjugation with albumin, polymerization and absorption to particles. Some antisera were obtained which were sensitive enough to use for radioimmunoassay. Although it is difficult to conclude which is the best procedure for raising antibody, repeated blood sampling and determination of antibody titer appear important. Because considerable fluctuations were noted in antibody titer during the immunization periods.

2. A paradoxical phenomenon in radioimmunoassay.

There is a phenomenon that binding of ^{125}I-ACTH is increased in proportion to an increase of unlabeled ACTH, when very small amount of ^{125}I-ACTH is added to relatively high concentration of antisera. We studied further on this paradoxical phenomenon. Both macroglobulin and globulin fractions of antisera obtained by gel filtration gave similar paradoxical curves. Occasional shakings of incubation mixture or prolongation of incubation period did not significantly affect the standard curves. This paradoxical phenomenon was also observed in radioimmunoassay of glucagon. Although an explanation for this phenomenon is still uncertain, it is useful for radioimmunoassay of peptide hormones of small molecule, against which production of antisera with high association constant is extremely difficult.

3. Heterogeneity of antisera and antigens.

We studied reactions of ACTH and its fragments with 5 different antisera. Some antisera reacted more markedly with the N-terminal fragment, whereas others reacted with the C-terminal fragments. These results indicate heterogenity of anti-ACTH antisera. We also observed size heterogenity of plasma and tissue ACTH, by using gel filtration. These heterogeneities of antisera or antigens may influence radioimmunoassay under certain conditions.

4. Relationships between biologic and immunologic activities.

When plasma ACTH was measured both by bioassay and radioimmunoassay, discrepancy was noted under certain conditions. Disappearance of endogenous and exogenous ACTH from the blood was more rapid when measured by bioassay than when measured by radioimmunoassay. It is possible that metabolites of ACTH are still measurable by radioimmunoassay.

5. Advantages of radioimmunoassay.

Since radioimmunoassay is more sensitive and simple than bioassay, it is useful for studies on the state of hormones in blood and on dynamics of hormone secretion.