Radioimmunoassay of adrenocorticotropic hormone (ACTH)— with special reference to paradoxical binding phenomenon

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There is a phenomenon that the binding of ^{125}I-ACTH with its antibody is increased with an increase of unlabeled ACTH, when very small amount of ^{125}I-ACTH is added to relatively high concentration of antisera. We have studied further on this paradoxical phenomenon in order to clarify the reason for occurrence of the phenomenon.

Both macroglobulin and globulin fractions of antisera obtained by gel filtration gave similar paradoxical curves. Prolongation of incubation period or incubation with constant shaking did not affect the paradoxical binding phenomenon.

However, the paradoxical phenomenon disappeared and usual standard curve was observed when radioimmunoassay was performed with the first piece (Fab fraction) of papain-treated IgG fraction of antisera. This paradoxical phenomenon also disappeared with an increase in antibody titer caused by repeated immunization in an animal.

These findings suggest the presence of a kind of allosteric effect in antigen-antibody reaction with such specific antisera similar to that in enzyme-substrate interaction.

This phenomenon can be utilized to develop a sensitive and reliable radioimmunoassay for ACTH.

A sensitive radioimmunoassay for glucagon using talc method

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A sensitive radioimmunoassay for glucagon has been developed. Anti-glucagon antisera were prepared by immunizing rabbits and guinea-pigs with either pork glucagon-albumin conjugate or polymerized glucagon, the latter of which produced more sensitive antisera.

Glucagon was labeled with ^{125}I by the method of Hunter and Greenwood. The spesific activity of the labeled hormone ranged from 300 to 800 mc/mg. Bound and free hormones were separated by the talc method, which enable to check incubation damage easily. This method can mesure as little as 40 pg/ml.

The antiserum used in this experiment cross-reacted with gut glucagon-like immunoreactivity (GLI). Cross-reactivity of porcine insulin, secretin,

synthetic gastrin and CCK-PZ was not observed, except for purified CCK-PZ (Kalolinska Institute) which exhibited little cross-reactivity at such a high level as 1 Ivy dog U/ml. Serial dilution of a pancreaticoduodenal vein plasma from a dog gave parallel curve to that of standard pancreatic glucagon.

Arginine was infused through a polyethylene catheter into the pancreatic artery of anesthetized dogs over a period of 10 min. Pancreatico-duodenal vein blood was collected through an other catheter inserted into the mesenteric vein or a small branch of the portal vein. Immediately after the start of intrapancreatic infusion of arginine (23.7 mM/Kg/min) in five dogs, immunoreactive glucagon (IRG) in pancreatico-duodenal vein plasma increased to the mean peak level 2.4 times the preinfusion level, and then subsided rapidly returning to the basal level 5 min after the cessation of the infusion. The infusion of 5% glucose into the same artery rapidly lowered the pancreatico-duodenal vein plasma IRG.

These results demonstrate that this radioimmunoassay is enough to use for the study of secretory mechanism of pancreatic glucagon under certain conditions.

Radioimmunoassay of Gastrin

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A double antibody radioimmunoassay technique for measuring serum gastrin using 1: 5,000 titer antigastrin serum of Wilson aboratories was studied.

Synthetic human gastrin I was labeled with ^{125}I by a modification of the Method of Hunter and Greenwood (specific activity 320–350 μCi/μg). (1) Each 0.1 ml of I-SHG I (4,000 cpm), serum sample or standard gastrin, guinea pig antiserum to porcine gastrin and buffer were mixed and incubated for 24 hours at 4°C. (2) 0.2 ml of rabbit antiserum to guinea pig γ-globulin and 0.1 ml of normal guinea pig serum were added and incubated for 24 hours at 4°C. After radioactivity counting, Bound % was calculated. Standard diluent was 0.15 M NaCl-0.01 M phosphate buffer (pH 7.4) containing 0.3% BSA and 0.01 M EDTA 2Na.

A radioimmunoassay calibration diagram using SHG I from 0.1 to 1,000 pg was sigmoidal in shape, presenting straight line from 20 to 500 pg. Best results were obtained at a dilution from 1: 50 to 1: 100 of antigastrin serum and at a dilution of 1: 5 of second antibody.

Within assay variation was 8.6% (above 100 pg/ml) and 12.9% (below 100 pg/ml) of relative standard deviation. Relative potencies normalized to SHG I were as follows: SHG I 1, Caerulein 0.01, Pentagastrin 0.0004, Benzyloxycarbonyl tetragastrin 0.00002.

Fasting serum gastrin levels measured were as follows: control group 202.4 $\pm$ 101.1, gastric ulcer 137.7 $\pm$ 100.6, duodenal ulcer 163.0 $\pm$ 98.2, renal insufficiency 763.9 $\pm$ 507.3, liver cirrhosis 197.4 $\pm$ 124.2, diabetes mellitus 177.5 $\pm$ 79.8. (pg/ml: mean $\pm$ standard deviation).