

The Basic Studies of Radioimmunoassay for Glucagon

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We have gained some antiglucagon antisera from rabbits by use of bovine serum albumin bound glucagon mediated by carbodiimide as antigen. These antiglucagon antisera have considerably high titer for immunogenic binding to glucagon and labeled glucagon, and specificity for bovine-porcine glucagon and no crossreactivity for porcine insulin, human C-peptide and human growth hormone. We have performed radioiodination of glucagon with lactoperoxidase. In radioiodination procedure, the reaction time for 10 seconds might be tolerable to radioimmunoassay for glucagon as well as for 60 seconds, in which the specific activities were from 200 to 300 $\mu\text{Ci}/\mu\text{g}$. Repurification of the labeled glucagon were done by gel filtration through sephadex G-25 or -G50 column, eluted

with 0.01 M (PH 7.5) phosphate buffer. The labeled glucagon and antiglucagon antisera made us possible to carry out radioimmunoassay for glucagon. Our assay procedures for glucagon are based on the methods by Dr. Unger with minor modification. Bound/free ratio of glucagon free sample (Bo) were 0.3—0.4 and its displacement was good against standard solution of glucagon between 25 pg/ml and 2000 pg/ml. The standard curves in this system are relatively good and plasma glucagon levels measured by this system were relatively correlated to those in the assay system using 30K- antiglucagon antiserum (purchased from Dr. Unger) by parallel assay on the same samples.

Responses of Plasma Pancreatic Glucagon to Infusion of Arginine in the Patients with Various Liver Diseases

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Plasma pancreatic glucagon concentrations were determined in the patients with various liver diseases. After overnight fasting, the patients and normal subjects received an intravenous infusion of 30 g of L-arginine over a period of 30 min. Blood was withdrawn before and 15, 30, 60, 90,

120 min. after the start of the infusion. Plasma pancreatic immunoreactive glucagon (IRG) was determined by the radioimmunoassay with antiserum 30 K. In the patients with liver cirrhosis, plasma IRG concentration in the basal state was almost three times greater than that observed in

the control subjects. In the patients with liver cirrhosis and chronic hepatitis, plasma IRG response to arginine was significantly greater than in the normal subjects. Particularly, in the cirrhotic patients with abnormal glucose tolerance, hyper-

response of glucagon was highly significant. In the patients with liver cirrhosis, the prolonged disappearance curve of injected exogenous glucagon was observed.