Radioimmunoassay of Human Chorionic Gonadotropin

S. Tojo and M. Mochizuki

Department of Obstetrics and Gynecology, Kobe University, School of Medicine, Kobe

The biological activity and antigenicity of HGG were comparatively studied by radioimmunoassay (sensitivity 0.005 IU/ml) using ^{131}I and purified HGG (8.000~29,000 IU/mg specific activities).

The results thus obtained are as follows.

First of all, the antigenicity of HGG became the lower as the HGG was purified to the higher biological specific activity.

On the other hand, experiments on the relationship between the antigenicity and biological activity by various inactivation methods revealed that antigenicity of HGG is more resistent than biological activity to the treatment with heat, 6M-urea, 0.1 M KOCN, sialidase, α-chimotrypsin and streptokinase, indicating that the biologically active fragment of HCG was dissociated from the site of antigenicity.

Comparisons among biological activity, antigenicity and optical density (280 mμ) of each fraction of gel filtration on Sephadex G-100 or of DEAE-column chromatography also revealed that htere was a constant parallelism between the patterns of immunological activity and optical density, but the biological activity did not fluctuate in parallelism with the pattern of optical density.

Therefore, it is clear that in some conditions only a part of biologically active fractions can be detected by radioimmunoassay.

However, radioimmunoassay is useful in determination of small amount of HCG in fetal organs and blood, of LH in blood of nonpregnant women and in the diagnosis or follow-up of chorioma, as it detects a very little amount of HCG.

Radioimmunoassays of FSH and LH

H. Demura

Department of Internal Medicine, Tohoku University, School of Medicine, Sendai

We have developed radioimmunoassays of FSH and LH in plasma sensitive enough to measure the normal levels in 10 μl of plasma respectively. HCG was used in place of LH for radioimmunoassay of LH.

In order to establish a specificity of assays, highly purified FSH and HCG were used for standards and iodination; antisera to FSH were absorbed with HCG and antisera to HCG were absorbed with FSH.

A Sephadex G-100 column (1×25 cm) was used for purification of ^{131}I-FSH or ^{131}I-HCG. The fractinos with minimum radiation damage at the tailing part of the FSH-^{131}I or HCG-^{131}I peak were used for assays.

The bound and free labelled hormones were separated by paperchromatoelectrophoresis or by dextran coated charcoal.

Good correlations between the bioassays and radioimmunoassays of FSH and LH were found in the purified pituitary and urinary fractions.

Our mean plasma FSH and LH levels in normal young men were 16.9 and 14.0 mIU 2nd-IRP-HMG respectively comparable with values reported previously. Values in prepubertal children were more than half of those in adults. Low plasma gonadotropin levels were observed in patients with hypopituitarism and in patients with congenital adrenal hyperplasia. High values were found in postmenopausal women, in patients with Klinefelter's syndrome and occasionally in patients with acromegaly and Cushing's syn-

drome due to adrenal hyperplasia.

The plasma LH levels increased to two to three times higher than basal levels after 60 to 90 minuites of an intravenous injection of insulin (0.1 μ/kg) in normal subjects. However, no responses of plasma LH levels to insulin induced hypoglycemia were observed in patients with panhypopituitarism. The plasma LH levels decreased slightly after 30 minuites and increased moderately between 120 and 180 minuites by an intravenous injection of Premarin (20 to 40 mg) in nromal subjects.

Both maximum FSH and LH levels during normal menstrual cycles occurred near the thermal nadir.

Glucagon Immunoassay

K. SHIMA

The Second Department of Internal Medicine, Osaka University Medical School, Osaka

A two-antibody immunoassay for glucagon has been established. The outline of the procedure is as follows, 0.1 ml of guinea pig antiglucagon serum, suitably diluted in buffer, were incubated at 4°C with 1 ml of glucagon standard or of an unknown specimen and 0.1 ml of labeled glucagon solution containing 1,000 μ of Trasylol, proteolytic enzyme inhibitor. After 24 hours, 0.1 ml of precipitating antiserum and 0.1 ml of normal guinea pig serum (diluted 1:100) were added to each tube. The incubation at 4°C was continued for an additional 48 hour period, after which radioactivity of the specimen was measured and then the tubes were centrifuged at 3,000 r.p.m. for 30 minutes.

The supernatant fluid was decanted and radioactivity of the precipitate was counted.

A standard curve was obtained by plotting radioactivity recovered in the precipitate, expressed as fraction of total radioactivity as a function of the amount of unlabeled glucagon.

The method was sensitive to as little as 0.1 mμg of glucagon, permitting the measurement of glucagon in 1 ml of human serum, One of the problems encountered in the immunoassay for glucagon was due to fragilty of the hormone.

The addition of a proteolytic enzyme inhibitor (Trasylol) to this system protected glucagon from destruction promoted by proteolytic enzyme and made satisfactory recoveries of added glucagon possible. Glucagon added to serum has been destructed during a storage in deep freeze state for 3 weeks.

4,000 μ of Trasylol per ml was necessary for the satisfactory preservation of glucagon, under these condition.

Thirty-minutes after the oral administration of 100 g of glucose the average serum immunoreactive glucagon of 5 healthy subjects rose from 0.44 mμg/ml to 0.52 mμg/ml ($P<0.05$). On the other hand, the intravenous injection of glucose was followed by a prompt decline in serum glucagon to a minimum of 0.27 mμg/ml in 15 minutes ($P<0.05$).

The method described above can not distinguish pancreatic glucagon from the one originated from the gut, which might contribute to the rise of serum glucagon level after the oral administration of glucose. These facts remained the immunoassay for glucagon to be improved.