UDC 577.175.32:57.088.6
ISSN 1330-9862

scientific note

A Fast Immunoradiometric Assay of Human Growth Hormone

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Received: February 27, 1997
Accepted: March 21, 1997

Summary

Using the components of »Pharmacia« two-site immunoradiometric assay (IRMA) it is possible to determine human growth hormone (hGH) in serum in the time interval of approximately 2 h if the reaction is performed at 37 °C (modified IRMA). The standard IRMA, that is the assay performed at room temperature as recommended by the manufacturer last at least 4 h. Comparison of the results obtained by the standard »Pharmacia« protocol, i.e. modified IRMA vs. standard IRMA, yielded a correlation coefficient of r = 0.9996. The results of hGH determinations obtained by two IRMA assays were also compared with the results obtained by standard competitive radioimmunoassay (RIA) of hGH. The following correlation coefficients were obtained: RIA vs. standard IRMA r = 0.9997; RIA vs. modified IRMA r = 0.9989. The modified IRMA procedure has found its value in determination of hGH in serum during and immediately after the selective removal of the pituitary (somatotroph) adenoma.

Keywords: human growth hormone, acromegaly, pituitary adenoma, RIA, IRMA

Introduction

Acromegalic patients, who were either not treated or with whom the conservative therapy has failed, are often candidates for the selective removal of the adenoma of the pituitary somatotrophic cells (*1*). One of the requirements during the selective removal of the pituitary adenoma is to monitor the serum hGH concentration during and immediately after the selective adenoma removal (*2,3*). The exsisting hGH assays last at least 4–24 h, which is too long to be of any practical help to the surgeon. Since selective transnasal hypophysoectomies are performed at the hospital »Sestre milosrdnice«, there was an urgent need to develop an accurate and fast hGH assay method.

Materials and Methods

Materials

Chemicals

Iodination grade hGH (isolated from human pituitaries) and radioimmunoassay grade bovine serum albumin (BSA) were purchased from Calbiochem; polyethyleneglycol (PEG) 6000 was obtained from Merck; Sephadex G-25, Sephadex G-100 and »Phamacia hGH RIA 100« kit for estimating hGH concentrations were obtained from Pharmacia; hGH standard sera were obtained from Serono. Goat anti-rabbit IgG serum was purchased from Zavod za transfuziju, Zagreb and anti-hGH serum was prepared as described (*4*). All other chemicals were analytical grade and were obtained from Kemika, Zagreb.

Sera

Acromegalic patient sera were obtained during the transnasal hypophysoectomy performed at the Department of Neuroscience, University Hospital »Sestre milosrdnice«, Zagreb. After the sera were brought into the Laboratory of Endocrinology, University hospital »Sestre milosrdnice«, they were centrifuged and immediately used for hGH estimation or stored in aliquotes at –20 °C.

Methods

RIA method

Standard competitive RIA procedure was performed as described (*4,5*). Briefly, the mixture of sera (or hGH standards), anti-hGH antibodies and ^{125}I-hGH, in total

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volume of 0.3 mL were incubated overnight at room temperature. The bound antigen was precipitated by addition of goat-anti-rabbit IgG antiserum – PEG 6000 mixture, and centrifuged. The immunoprecipitated radioactivity in obtained pellets was counted in an LKB-Wallac Clinigamma gammacounter.

IRMA method

When hGH concentrations were determined by utilizing »Pharmacia hGH RIA 100« kit, manufacturers instructions were followed. Briefly, the mixture consisting of immobilized anti-hGH antibodies (50 μL), hGH standards or human sera samples (50 μL) and ^{125}I-anti-hGH (50 μL), was incubated for 3 h at room temperature (20–25 °C). The reaction was stopped by addition of 2.0 mL of the »Pharmacia precipitating solution«, the suspension was centrifuged (10 min, 2000xg) and the radioactivity which remained in the pellet (after decanting the supernatant) was counted in a Clinigamma counter. The modified IRMA assay was performed exactly as described, except that the incubation of the assay mixture was at 37 °C for 1 h. For both IRMA assays the results were expressed as B/T ratio, where B represents the measured radioactivity in the pellet, and T corresponds to the total amount of the radioactivity added into an assay tube (50 μL of ^{125}I-anti-hGH).

In both assay methods (RIA and IRMA), the hGH concentrations in unknown samples were calculated from the standard curves using the LKB-Wallac RIA-CALC program. Except where noted otherwise, all assays were performed as duplicates.

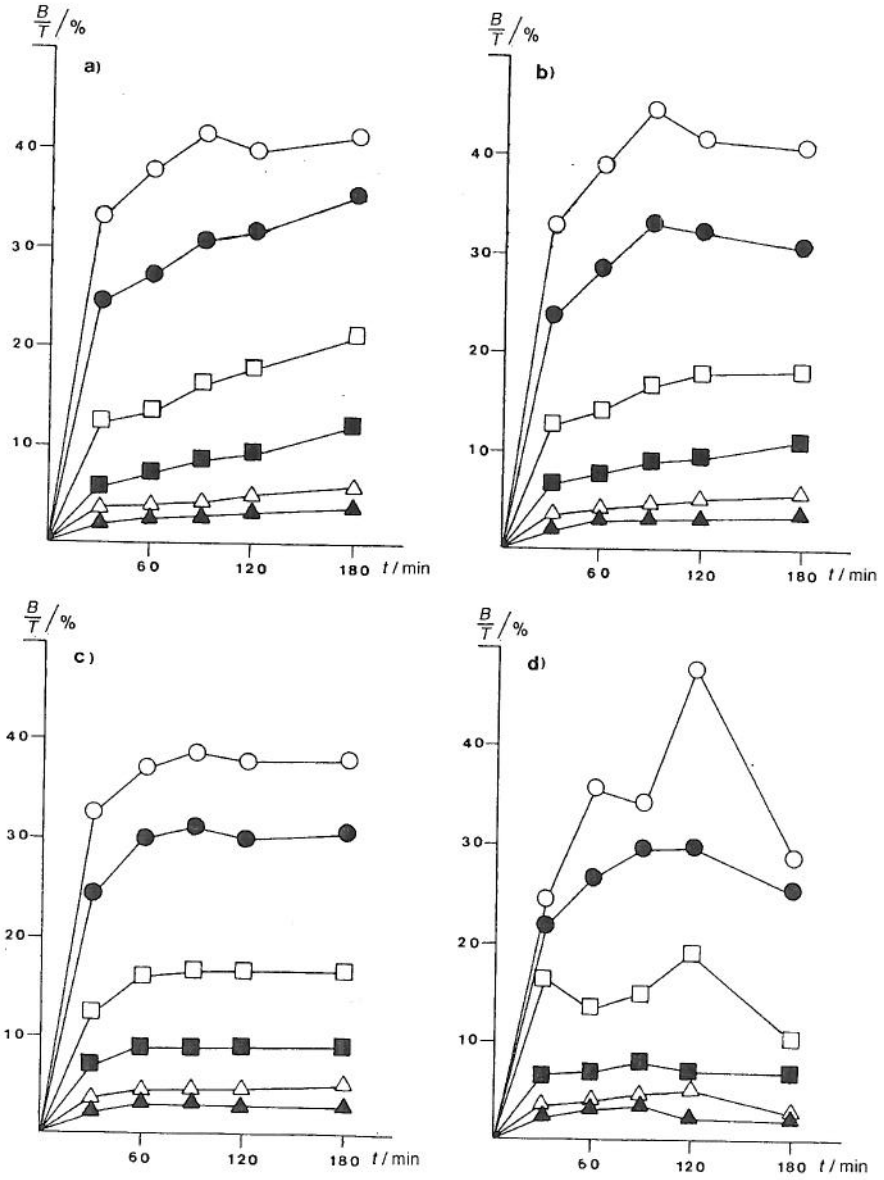

Fig. 1. The percentage of ^{125}I-antibody binding in different time intervals and temperature. Presented results are the mean of three independent experiments run in duplicate.
All mixtures were prepared identically, i.e. by incubating the suspension of 50 μL immobilized antibody, 50 μL of hGH standard and 50 μL of ^{125}I-anti-hGH. After the particular time of incubation the reaction was stopped as described in methods. In a) the incubation was performed at 21 °C, in b) at 30 °C, in c) at 37 °C and in d) at 48 °C. The concentrations of hGH standars are shown as: 0.25 μg/L ▲; 0.75 μg/L Δ; 2.5 μg/L ■; 7.5 μg/L □; 25 μg/L ●; 75 μg/L ○.

Table 1. The precision of hGH determination between the IRMA assays

Modified IRMA (incubation 60 min, 37 °C)				
Sample	n	γ / μg L^{-1}	SD	CV/%
A	5	1.32	0.20	15.44
B	5	6.32	0.44	6.89
C	5	38.30	2.23	4.82
Standard IRMA (incubation 180 min, room temperature (20–25 °C)				
Sample	n	γ / μg L^{-1}	SD	CV/%
A	10	1.27	0.10	7.83
B	10	6.87	0.40	5.87
C	10	36.31	1.63	4.50

Standard serum hGH concentrations (Serono A, B, C standards) were included in $n = 5$ (modified IRMA) or in $n = 10$ (standard IRMA) assays. In all instances the individual concentration values were obtained from duplicate samples.

Results and Discussion

Determination of hGH in sera by competitive RIA method(s), that is, methods based on competitive binding of ^{125}I labeled antigen which competes with the unlabeled antigen for a limited and constant number of antibody binding sites, is an accurate assay, but it lasts more than 18 hours *(4–6)*. Immunoradiometric assays (IRMA) which are based on binding of the antigen's epitope »1« onto the immobilized antibodies, and on the binding of antigen's epitope »2« onto the ^{125}I-labelled antibodies *(7)*, are more widely used since the specific monoclonal antibodies (specific for different antigen's epitopes) are available. IRMA assays (often called *sandwich assay*) provide also some other advantages over the »classical« competitive RIA assays. So, for example, the antigen can be determined over the wider range (broader range of the standard curve), the assays are usually performed in shorter period of time, and techni-

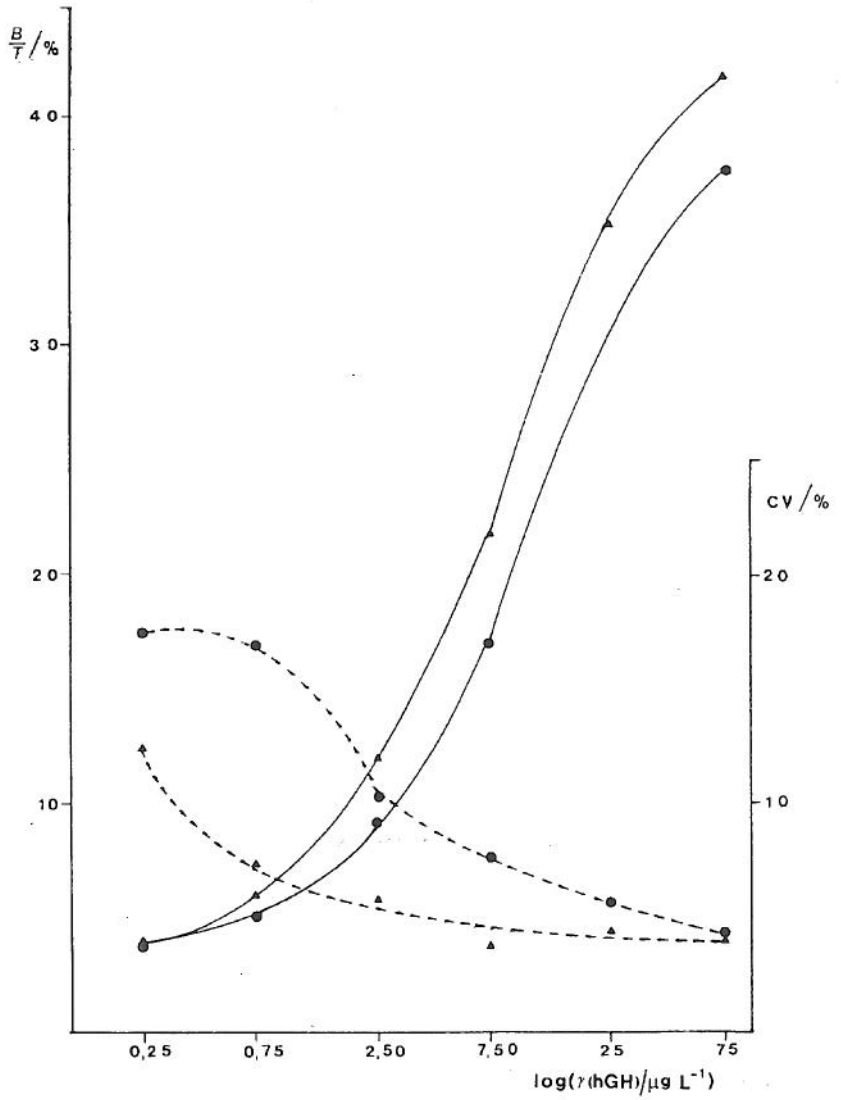

Fig. 2. The stability and the precision of the standard curves for modified (1 h) IRMA and the standard (3 h) IRMA assays. Each point is the mean of five different duplicate determinations. Standard curves: ▲ 3 h IRMA; ● 1 h IRMA; Precision profiles: ▲---▲ 3 h IRMA; ●---● 1 h IRMA

Table 2. Selective hypophysoectomy

Sera samples	Type of assay		
	1 h IRMA γ / $\mu g\ L^{-1}$	3 h IRMA γ / $\mu g\ L^{-1}$	RIA γ / $\mu g\ L^{-1}$
Anesthesia, before surgical treatment	*150.0 ± 5.9	*152.0 ± 3.0	*158.0 ± 7.0
Beginning of the surgical treatment	*162.0 ± 5.0	*165.0 ± 3.6	*175.0 ± 8.5
Tumor removal	57.6 ± 1.4	58.1 ± 2.2	*56.0 ± 2.6
0 min after tumor removal	24.4 ± 0.7	27.4 ± 0.6	26.0 ± 3.5
30 min after tumor removal	6.8 ± 7.6	7.6 ± 2.3	7.4 ± 0.4
60 min after tumor removal	4.9 ± 0.2	5.2 ± 0.1	4.6 ± 0.2
90 min after tumor removal	3.7 ± 0.2	3.6 ± 0.1	3.3 ± 0.1
120 min after tumor removal	4.0 ± 0.3	3.6 ± 0.1	3.4 ± 0.1
24 h after tumor removal	0.9 ± 0.2	1.4 ± 0.1	1.5 ± 0.0

* Samples were diluted ten-fold with the RIA assay buffer (0.015 M phosphate buffer pH = 7.5, containing 0.137 M NaCl, 0.033 M EDTA, 0.015 M NaN_3 and 33 g/L BSA).
The presented values are the mean ± SD obtained from the samples run in triplicate in a particular hGH assay.

cally they are easier to perform (possibility of automation). An additional advantage of IRMA peptide hormone kits over RIA peptide hormone kits, is that the IRMA kits are more stable (longer shelf life). The better stability of the IRMA kit is due to the increased stability of ^{125}I-anti-antigen-antibody over the ^{125}I-antigen.

In order to perform an accurate hGH detemination as fast as possible, we used the »Pharmacia hGH RIA 100« kit. In order to determine if the stable complex – immobilized antibody/antigen/^{125}I-antibody – can be formed in a shorter interval of time than recommended by the manufacturer, we started the experiments by incubating the assay mixture at different temperatures. As can be seen from Fig. 1, the constant B/T values, which are the sign of stable immunocomplexes, are obtained after 120 min (when the incubation was performed at 30 °C, Fig. 1.b) or after 60 min when the incubation of the assay mixture was performed at 37 °C (Fig. 1.c). When the incubation was performed at 21 °C (Fig. 1.a), the equilibrium was not reached until 180 min of incubation. On the other hand, when the incubation was performed at 48 °C (Fig. 1.d), equilibrium, i.e. the stable immunocomlex was not formed, which is probably the result of the instability (conformational changes) either in the hGH epitopes or in the anti-hGH paratopes.

In further experiments, we have determined the stability of the standard curve(s) and the precision profile in *intra* and *inter* assay determination of standard hGH concentrations. As shown in Fig. 2, the coefficient of variation (CV), for the curves which were obtained by 1 hour (modified) IRMA procedure, performed at 37 °C, was higher than 10% for low hGH concentrations (0.25 and 0.75 μg/L) and below 10% for higher hGH concentrations. Also, as shown in Table 1, the precision of hGH determination, for three standard hGH concentrations, was lower when the hGH concentration was determined by 1 hour IRMA than when the concentrations were determined by the standard IRMA procedure. Although the precision (CV) of the hGH determination for 1 h IRMA peformed at 37 °C was lower than the precision of the hGH determination in the standard 3 h IRMA assay, the correlation coefficients (comparing 2 different assay procedures), for 5 hGH samples (Serono standard B) within one assay, as well as the correlation coefficient for hGH determinations within 5 different assays, were very high ($r > 0.99$).

hGH concentrations for sera samples obtained during and after the selective removal of pituitary (somatotroph) adenoma are presented in Table 2. As can be seen, almost identical results were obtained no matter which hGH assay method was performed. The calculated correlation coefficient, when the results obtained by 1 h IRMA were compared with the results obtained by 3 h IRMA, was $r = 0.9996$. Similarly, the correlation coefficients for hGH concentrations determined by RIA vs. 3 h IRMA was $r = 0.9997$ and for RIA vs. 1 h IRMA, r was 0.9989.

In conclusion, it is possible to perform an accurate determination of hGH concentration, in serum, in approximately 2 hours (30 min for serum centrifugation and pipeting, 1 h incubation, 30 min centrifugation and counting of the radioactivity). This fast determination of hGH concentration is valuable in instances when the surgeon is in doubt if he/she has completely removed the adenoma from the rest of the healty somatotrophs. In our experience *(1)*, the routine estimation of hGH concentration during the selective adenoma removal, has established the value of the described 1 h IRMA assay. The serum hGH concentration, determined 30 min after the tumor has been removed, shold be at least ten-fold lower than the hGH concentration determined during anesthesia (before surgical treatment) and in any case lower than 10 μg/L, which is a good criterion for a successful adenoma removal. The described procedure is simple to perform, and as shown, the determined hGH concentrations are almost the same as the compared results obtained by standard 3 h IRMA or the »classical« RIA procedure.

Acknowledgement

This work was supported by the Ministry of Science and Technology, Republic of Croatia, through the grant 3-01-211.

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Brza imunoradiometrijska metoda određivanja humanoga hormona rasta

Sažetak

Koristeći se pripravkom tvrtke »Pharmacia« za određivanje humanoga hormona rasta (hGH), koji se zasniva na imunoradiometrijskom postupku (IRMA), može se odrediti koncentracija hGH u serumu tijekom 2 sata ako se reakcija provodi pri temperaturi od 37 °C (modificirana IRMA). Određivanja hGH pri sobnoj temperaturi (standardna IRMA), kao što preporučuje proizvođač, traju najmanje četiri sata. Usporedbom rezultata dobivenih modificiranim postupkom s rezultatima postupka koji je propisao proizvođač (tj. modificirana IRMA/standardna IRMA), izračunan je koeficijent korelacije r = 0,9996. Ta su određivanja uspoređena s rezultatima standarnoga radioimunološkoga određivanja (RIA), pri čemu su dobiveni sljedeći koeficijenti korelacije između pojedinih vrsta određivanja: RIA/standardna IRMA r = 0,9997; RIA/ modificirana IRMA r = 0,9989. Modificirani IRMA postupak primjenjuje se pri određivanju koncentracije hGH tijekom selektivne adenohipofizoektomije (specifično za adenom somatotropnih stanica) i neposredno nakon nje.