Radioimmunoassay

Human Growth Hormone Secretion—Suppression and Stimulation Tests

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The rate of human growth hormone (HGH) secretion is subject to marked and rapid fluctuations in response to a variety of stimuli, e.g., physical stress, emotional stress, and food intake. These variations in the HGH levels of normal individuals decrease the diagnostic usefulness of determining HGH levels of random blood samples. Also, pituitary failure cannot be detected since normal values can be as low as zero. For these reasons, tests have been developed in which serum HGH levels are measured after pituitary stimulation and suppression. Our methodology for these tests and some case reports are presented.

Human growth hormone (somatotropin), a single-chain polypeptide containing 190 amino acid residues with two disulfide bridges, is secreted by the anterior pituitary. Serum concentration of this hormone is assayed routinely by radioimmunoassay with a normal range of 0–6.5 ng/ml in the adult. During rest, the normal concentration of circulating human growth hormone (HGH) is usually less than 3 ng/ml *(1,2)*. Since normal values can be as low as zero, pituitary failure to produce HGH cannot be detected. Random blood samples for HGH evaluation have proven useless in the differential diagnosis of pituitary disease. For example, the HGH assay cannot be used to differentiate short stature due to hypopituitarism from retarded growth due to other causes. The measurement of serum HGH levels after pituitary stimulation or suppression should be the laboratory approach to diagnosis of pituitary-related growth hormone abnormalities *(1)*. Insulin-produced hypoglycemia and L-dopa, a precursor to dopamine, are commonly used to stimulate pituitary secretion of HGH. A glucose load is used to suppress secretion of HGH.

Method

An insulin stimulation study is begun after the patient has fasted for 15 hr. The patient is informed of the frequency of blood sampling and all side effects resulting from an insulin dose, namely, hypoglycemia. The patient may experience faintness, nausea, rapid pulse, warmth, profuse perspiration, and decreased blood pressure. The time course and severity of the produced hypoglycemia will vary from patient to patient. It can last as long as 90 min. A member of the technical staff should remain with the patient during this period until the patient returns to normal, and a bottle of glucose for iv administration should be kept close at hand as there is a possibility of insulin shock. After a 10-ml blood sample is collected to determine baseline HGH levels, a stimulation dose of 0.1 units/kg insulin is injected. Ten-milliliter blood samples are then drawn at 30, 60, 90, and 120 min postinjection and serum is obtained. In addition to the HGH determinations on each sample, glucose levels should also be determined to assure that the patient develops hypoglycemia. Because of the frequent blood collection, the patient may prefer to have an iv saline drip installed with a butterfly and three-way stopcock.

Because insulin side effects can be both unpleasant and potentially dangerous, L-dopa is preferred by many as the stimulant drug. An L-dopa stimulation study is performed in the same technical manner as the insulin study. The patient fasts for 15 hr and is then informed of the side effects of L-dopa and the frequency of blood sampling. The chief complaint is nausea. This problem can be decreased by having the patient move about during the first 30 min of the study. A baseline 5-ml blood sample is obtained, after which a 0.5-gm dose of L-dopa is given orally. Five-milliliter blood specimens are drawn at 30, 60, 90, 120, and 180 min postdose. If the patient prefers, an iv saline drip may be installed.

The so-called glucose suppression test is most easily performed in conjunction with the pathology laboratory during the routine glucose tolerance test. The patient should be NPO from midnight, and the study should begin as early as possible the following morning. The procedure and the possibility of experiencing nausea should be explained to the patient before starting the study. A baseline 5-ml blood sample is drawn and a 1.75-mg/kg (100 gm maximum) dose of glucose is then given orally. Five-milliliter blood samples are drawn at 30, 60, 90, 120, and 180 min or longer postdose.

The assay of HGH is made using the Schwartz/Mann

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double antibody kit.* The kit requires two incubation periods of 24 hr each. In the first, ^{125}I-HGH is incubated with the patient serum and a primary antibody for HGH (raised in guinea pigs). The HGH in the patient's serum and the radioactive HGH compete for binding sites on the antibody. The more HGH present in the patient's serum, the less the radioactive HGH has an opportunity to bind with the antibody. A second incubation period with an antibody to the guinea pig antiserum (raised in rabbits) is used to precipitate the bound complex. The bound HGH is separated from the free by centrifugation. A standard curve is prepared in a similar manner, using serial dilutions of an HGH solution of known concentration instead of the patient's serum. The radioactivity of the bound HGH-antibody complex is determined and the amount of HGH in the patient's serum is determined from the standard curve. The double antibody reaction is not time dependent, and thus an unlimited number of patient samples may be assayed under one standard curve. The normal range is 0–6.5 ng/ml. A peak value of HGH greater than 10 ng/ml or an increase over baseline of 6 ng/ml for two consecutive points indicates a normal response to stimulation. During suppression of HGH secretion serum levels should decrease to at least 75% of baseline values and remain decreased from 60 to 180 min after the glucose load.

Case Reports

Case 1. Human growth hormone stimulation studies were performed for a 16-year-old male, 4 ft 7 in. tall, weighing 73 lb. The patient exhibited a bone age of a 14 year old and the physical appearance of an 11 year old. There was no sign of puberty. Both parents were of short stature, the father 5 ft 7 in. and the mother 5 ft 2 in. Insulin-induced hypoglycemia produced a pituitary response as reflected by the increased HGH levels 30 to 90 min after the insulin injection (Table 1). L-dopa stimulation produced a lower and later response. The endocrinologist's diagnosis indicated retarded growth rather than pituitary hypofunction.

Case 2. A 23-year-old male, 5 ft 8 in. tall, weighing 216 lb, was admitted to the hospital with the chief complaint of growing jaw and hands. In addition, the patient complained of frontal headaches lasting as long as 5 hr. His depth perception had decreased, and his vision blurred upon awakening. A glucose suppression test was performed showing nonsuppression of HGH levels (Table 2). The acromegalic patient went to surgery for a total excision of a pituitary adenoma followed by radiation therapy. A post-treatment L-dopa study revealed a low baseline HGH level and no response to stimulation. These values would indicate no residual functioning pituitary gland.

*This kit is no longer available. We are presently using a Dade kit for HGH determination.

TABLE 1. Results of HGH Stimulation Studies—Case 1

Time (min)	Insulin		L-dopa
	Glucose levels (mg/100 ml)	HGH levels (ng/ml)	HGH levels (ng/ml)
0	83	1.9	1.2
15	44	1.7	–
30	29	9.8	0.7
60	61	14.0	8.0
90	79	7.9	8.4
120	89	5.2	1.6

TABLE 2. HGH Studies on an Acromegliac Patient

Time (min)	HGH (ng/ml)	
	Preexcision glucose suppression test	Postexcision L-dopa stimulation study
0	168.0	1.1
15	–	0.6
30	340.0	0.5
45	–	0.4
60	220.0	0.8
90	–	1.8
120	110.0	0.6

TABLE 3. HGH L-Dopa Stimulation Studies for Two Patients With Anorexia Nervosa

Time (min)	HGH (ng/ml)	
	Case 3	Case 4
0	3.0	22.4
60	0.7	20.8
90	1.0	18.8
120	10.4	18.8
180	6.6	23.2

Cases 3 and 4. Shown in Table 3 are the results of L-dopa stimulation studies for two adult females diagnosed as anorexia nervosa. Both patients had typical symptoms of profound weight loss due to an obsessive aversion to food. Patient 3 was a 20 year old who entered the hospital weighing 78 lb, 37 lb below the ideal weight for a female her age and height (5 ft 4½ in.). Patient 4 was a 39 year old who was admitted to the hospital because of a weight loss from 122 lb to 68 lb over a period of years. Her weight on admission was 50 lb below the ideal weight for her age and height of 5 ft 4 in. The HGH studies performed on both patients indicated responsiveness of the pituitary to stimulation. Patient 3 had a normal baseline value with a low and delayed response to L-dopa (elevated HGH levels at 120 and 180 min). Patient 4 had an elevated baseline value which was probably due to her long-standing state of malnutrition. No additional increase in HGH level was induced by ingestion of L-dopa since maximum stimulation had probably already occurred *(3)*.

Discussion

In children hyposecretion of HGH produces retarded growth and hypersecretion produces gigantism. In adults hyposecretion of HGH does not produce any recognizable sign or symptom while hypersecretion results in acromegaly. The normal pituitary gland responds to various stimuli by increasing the circulating level of HGH and responds to high HGH and glucose serum levels by decreasing secretion of HGH. Two recent review articles are recommended for additional information of regulation of growth hormone secretion *(3,4)*.

Insulin-induced hypoglycemia is a consistently effective stimulus for growth hormone secretion and is the most standardized HGH stimulation test *(5,6)*. Attempts have been made to find other methods of stimulation owing to patient discomfort and potential hazard of insulin induced hypoglycemia *(5,7)*. The oral ingestion of L-dopa is the preferred stimulant in our laboratory. The insulin test is employed only as a check should the patient not respond to L-dopa. We have found a positive response to L-dopa in almost all normals. Peak values range from 6 to 40 ng/ml. The peak usually occurs between 60 and 90 min postdose, but can occur as late as 120 min. These findings agree with the values and times most frequently quoted in the literature *(3, 5)*.

References

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Radioimmunoassay Control Sera

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Some laboratories use pooled human sera to determine day-to-day precision of RIA methods. For each type of assay performed, the RIA laboratory must maintain pools for the normal range and for values outside of the normal range. For example, serum pool aliquots of low, normal, and high T_4 values are assayed each time a series of T_4 determinations are made. Patricia M. Weigand, in an article on quality control in radioimmunoassay (*JNMT*, Vol. 3, No. 3, Sept. 1975) has outlined in detail methods for sample pooling.

Alternately, control human sera for a wide range of radioimmunoassays are available commercially. Table I lists suppliers of vials of lyophilized human sera containing multiconstituents. For example, the reference sera made by Nuclear Medical Systems (NMS) provide known amounts of over 20 different compounds in single vials. NMS-I Reference Serum contains normal levels of the indicated constituents and NMS-II contains elevated levels. The control sera manufactured by Ortho Diagnostics are available in a series of two pairs. Ortho RIA Control Serum I contains normal levels of the constituents indicated with an O(I) and Ortho II contains abnormal levels of the same constituents. Ortho III and IV contain, respectively, normal and abnormal levels of nine constituents indicated with an O(III). Lederle Diagnostics manufactures two control sera, one with constituents at normal levels and one which has them at abnormal levels. The control sera supplied by Pharmacia and Wien are in single vials containing concentrations within the physiological range of the constituents indicated in Table I.

The expected values provided by the supplier for a given vial of serum should be used only as a guide. The

TABLE 1. Available Control Human Sera for RIA

Constituent		Constituent	
Aldosterone	N,W	Gastrin	N
a-Amylase	P	Gentamicin	N,O(III)
Angiotensin I	N	HCG B subunit	N,O(III)
B_2-micro	P	HGH	N,O(I),L
CEA	N	hPl	O(III),P
Cortisol	N,O(I),W,L	IgE	P,N
Digoxin	N,O(I),L	Insulin	N,O(I),P,L
Digitoxin	O(III),W	LH	N
Estradiol	N,O(III),W	Progesterone	N,O(III)
Estrogen	N	Prolactin	N
Estrone	N,O(III)	Testosterone	N,O(III),W
Estriol	N,O(III)	T_3 and T_4	N,O(I),W,L
FSH	N	TSH	N,P
Folate (folic acid)	N,O(I),L	Vitamin B_{12}	N,O(I),P,L

N—Nuclear Medical Systems, Inc., Newport Beach, CA 92660.
O—Ortho Diagnostics, Inc., Raritan, NJ 08869.
P—Pharmacia Laboratories, Inc., Piscataway, NJ 08854.
W—Wien Laboratories, Inc., Succasunna, NJ 07876.
L—Lederle Diagnostics, American Cyanamid Co., Pearl River, NY 10965.

values obtained in the laboratory will depend on the method used for the assay and may vary from the expected values. Each laboratory must establish its own values for a given control serum. Once values have been determined, it is expeditious to continue to use controls from the same lot number as long as the supply lasts. For this reason, when ordering control sera, the lot numbers should be designated. Some companies will even allow customers with standing orders to reserve a year's supply of a specific lot number.

While the purchase and use of commercial control sera may be an additional expense, they are much more convenient than maintaining laboratory control pools. Additionally, some companies have made control serum vials an integral part of the supplies for their radioimmunoassay methods. One major advantage in using commercial control sera is that the laboratory is no longer responsible for maintaining quality control of the reference pools.

Comparison of Commercially Available TSH RIA Methods which Can Be Performed in 24 hr

	Abbot	Beckman	ICN	NMS	Pharmacia
Standards					
Range in μIU/ml	0-40	2-100	0-100	1-60	1.5-50
Recommended number	6	6	10	7	5
Concentrations supplied	6	6	3	7	5
Control serum supplied	None	1	None	3	None
Lyophilized reagents	No	Yes	Yes	No	Yes
Sample size (μl)	100	200	200	100	100
Micropipetting steps	3	4	5	4	3
Incubation periods	2	3	2	3	2
Separation methods	PEG*		Second Antibody		Solid phase**
Suggested normal range (μIU)	1-5	1-10	1-9	1-10	1.5-7.2
Equipment	Scintillation counter, centrifuge, freezer, refrigerator				
Vortex	Yes		Yes		Yes
Water bath (37°C)	Yes	Yes		Yes	
Magnetic stirrer, aspirator					Yes
Micropipettes (μl)	100, 300	100, 200	50, 100, 200 300, 400	100, 200	100, 500

Polyethylene glycol

†*Sephadex Anti TSH complex*

Abbott Diagnostics Division, North Chicago, IL 60064
Beckman Instruments, Inc., Fullerton, CA 92634
ICN Medical Diagnostic Products, Portland, OR 97208
Nuclear Medical Systems, Inc., Newport Beach, CA 92660
Pharmacia Laboratories, Inc., Piscataway, NJ 08854

RIA Questions and Answers

1. We use several kits with a tritiated label. Periodically, one sample of a set of duplicates requires quench correction. Our beta counter appears to be working fine and our technique is consistent. Any suggestions?

   You are probably using plastic liquid scintillation vials. Occasionally, a vial will have a slight yellow color due to poor quality control on the part of the manufacturer, causing your sample to quench. Once corrected, both answers should agree.

2. Our clinicians have been complaining that our T_4s are too low. Our standards are fine and we can't find anything wrong with the technique, our counter, or our pipettes.

   You state that you use a T_4 procedure requiring extraction efficiency. We had a problem similar to this some years ago. We finally determined that the 95% ethyl alcohol was evaporating, resulting in a change of the extraction efficiency from its initial value. (The standards are unaffected, since they do not require extraction.) Either make up the 95% alcohol in smaller volumes, so that it will be used faster, or do periodic checks on its efficiency. Also, run serum-based controls to keep a better check on problems such as this.

If you have any questions regarding RIA procedures, please write to Patricia Weigand, Nuclear Medicine Service, VA Hospital, Philadelphia, PA 19104. Selected questions and answers will be published in future issues of the JNMT.