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Abstract

Firstly, comparisons have been made of the secretion of human growth hormone (HGH) that was induced by insulin, lysine vasopressin and pyrogen injections in order to study whether these substances can be utilized as a rapid test of HGH secretion. In insulin test, a fall of the fasting blood glucose level by 28.6% or more seemed to be sufficient to provoke adequate HGH elevation, and 9.4 ng/ml or higher HGH increment was recognized as being normal, because lysine vasopressin and pyrogen produce varying degrees of side-effects and are less specific and unpredictable in the release of HGH. Secondly, the pharmacologic effects and mechanism of action of exogenous glucagon upon the HGH secretion were studied. In normal subjects after one mg sc glucagon, there was a mean peak blood glucose level of 142. 4±3.l mg/lOO ml at 30 min, HGH levels reached a mean peak level of 22. 6±4. 8 ng/ml at 150 min, and no false negative response was noted. In patients with hypopituitarism, there was no positive response in plasma HGH levels after the sc glucagon. The present study revealed that the rise and subsequent fall of blood glucose are not the sole mechanism responsible for the effct of glucagon on HGH secretion, and that the HGH secretion in response to the sc glucagon was not triggered by cathecholamine via the stimulation of the adrenal medulla.

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STIMULATION TESTS OF HUMAN GROWTH HORMONE SECRETION BY INSULIN, LYSINE VASOPRESSIN, PYROGEN AND GLUCAGON

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Abstract: Firstly, comparisons have been made of the secretion of human growth hormone (HGH) that was induced by insulin, lysine vasopressin and pyrogen injections in order to study whether these substances can be utilized as a rapid test of HGH secretion. In insulin test, a fall of the fasting blood glucose level by 28.6% or more seemed to be sufficient to provoke adequate HGH elevation, and 9.4 ng/ml or higher HGH increment was recognized as being normal, because lysine vasopressin and pyrogen produce varying degrees of side-effects and are less specific and unpredictable in the release of HGH. Secondly, the pharmacologic effects and mechanism of action of exogenous glucagon upon the HGH secretion were studied. In normal subjects after one mg sc glucagon, there was a mean peak blood glucose level of 142.4 ± 3.1 mg/100 ml at 30 min, HGH levels reached a mean peak level of 22.6 ± 4.8 ng/ml at 150 min, and no false negative response was noted. In patients with hypopituitarism, there was no positive response in plasma HGH levels after the sc glucagon. The present study revealed that the rise and subsequent fall of blood glucose are not the sole mechanism responsible for the effct of glucagon on HGH secretion, and that the HGH secretion in response to the sc glucagon was not triggered by cathecholamine *via* the stimulation of the adrenal medulla.

The secretion of human growth hormone (HGH) has been extensively studied in man in recent years. The administration of many different substances has been used to assess the HGH reserve since the demonstration of insulin-induced hypoglycemia as a stimulus of HGH secretion (1). As insulin, lysine vasopressin and pyrogen can be used for the simple test of the hypothalamo-pituitary-adrenocortical axis for the secretion of cortisol, it seems worthwhile to study whether these substances can also be utilized as the rapid test of the hypothalamo-pituitary axis for the secretion of HGH. The first aim of this study is to compare the effects of insulin, lysine vasopressin and pyrogen injection on the release of HGH, and to define a normal range of response, so that similar tests performed in patients with a variety of endocrine and other disorders can be interpreted.

Variable results have appeared in literatures concerning the capability of glucagon to provoke the release of HGH. Plasma HGH levels, although once considered unaffected by glucagon (1, 2), have been found to increase following the subcutaneous injection of glucagon (3-5). At prerent, HGH secretion in response to subcutaneous injection of glucagon is generally accepted. However, the mechanism of glucagon action on HGH secretion is yet unclarified, and only a few reports are available on the application of glucagon test to patients with various diseases. As the second aim, this study was designed to ascertain the pharmacologic effects and mechanism of action of exogenous glucagon upon the HGH secretion.

TABLE 1 PLASMA HGH AND BLOOD GLUCOSE CONCENTRATIONS AFTER

Diagnosis	No. of patients	Plasma HGH (ng/ml)					
		0 min	30 min	60 min	90 min	maximum	maximal increment
normal subjects	40 Maen	2.7	10.1	29.1	17.8	29.6	27.0
	SEM	0.3	1.7	2.0	1.5	1.9	1.9
normal males	19 Mean	3.4	10.7	31.6	20.9	32.2	29.1
	SEM	0.4	2.3	3.1	2.5	3.0	3.0
normal females	21 Mean	2.2	9.5	26.9	14.7	27.3	23.3
	SEM	0.4	2.3	2.4	1.4	2.4	2.4
acromegaly	6 Mean	82.5	104.0	110.2	99.3	115.3	32.8
	SEM	14.9	19.8	20.6	20.7	19.3	15.6
panhypopituitarism Sheehan's syndrome	4 Mean	1.4	2.1	4.2	3.3	4.3	3.0
	SEM	0.2	0.8	2.4	1.8	2.3	2.2
postoperative hypothalamopituitary tumor	12 Mean	1.4	1.5	1.3	1.4	1.6	0.3
	SEM	0.2	0.2	0.1	1.0	0.2	0.1
pituitary dwarfism	10 Mean	1.1	1.2	1.0	0.9	1.4	0.4
	SEM	0.2	0.3	0.2	0.2	0.2	0.1
primordial dwarfism	5 Mean	3.9	19.3	23.3	13.7	24.7	20.9
	SEM	1.3	3.4	4.6	2.9	3.9	3.8
hypogonadotropic hypogonadism	8 Mean	2.3	9.8	11.7	8.8	13.9	11.7
	SEM	0.3	2.8	1.6	2.4	2.6	2.4
idiopathic diabetes insipidus	10 Mean	1.0	4.6	8.8	6.2	9.5	8.5
	SEM	0.3	2.2	3.2	2.4	3.1	3.3
hyperthyroidism	9 Mean	4.7	7.1	14.1	10.4	15.8	11.9
	SEM	2.3	1.0	2.9	2.4	3.0	3.6
hypothyroidism	5 Mean	2.3	3.3	3.5	3.2	4.1	2.4
	SEM	0.8	0.8	1.3	0.9	1.3	1.1
Cushing's syndrome	7 Mean	0.8	3.8	4.0	1.1	4.4	3.6
	SEM	0.2	2.1	2.0	0.1	2.1	2.0
diabetes mellitus	15 Mean	3.2	9.1	11.9	8.4	15.7	12.5
	SEM	0.5	3.1	2.0	1.6	2.6	2.6

MATERIALS AND METHODS

All tests were begun at 9 AM after an overnight fast. The subjects were kept in the horizontal position for 30 min before withdrawal of the first blood sample to minimize the effect of previous exercise. Serial blood specimens were sampled through an indwelling venous catheter or venipuncture using heparinized syringes. The plasmas were immediately separated and frozen until HGH was assayed.

1. *Insulin test*

A total of 131 tests were performed on 40 normal subjects and 91 patients whose clinical diagnoses are summarized in Table 1.

IV ADMINISTRATION OF REGULAR INSULIN (0.1 U/kg).

Blood glucose (mg/100ml)					% decrement of blood glucose
0 min	30 min	60 min	90 min	minimum	
103.6 2.5	57.1 2.3	79.6 2.3	89.9 2.6	55.5 2.2	47.0% 1.8%
103.9 2.4	57.4 2.6	83.4 2.3	90.1 2.2	57.1 2.5	44.9% 2.5%
103.2 4.2	56.8 3.8	76.1 3.7	89.8 4.7	54.0 3.5	48.9% 2.4%
168.0 49.0	141.3 55.0	150.0 44.1	140.0 31.2	121.3 38.7	29.4% 1.8%
94.8 14.5	52.0 18.7	70.0 9.1	80.7 9.5	45.5 13.2	53.9% 6.9%
96.9 4.7	53.1 2.9	75.2 6.1	77.3 5.2	53.1 2.9	46.4% 1.7%
88.1 4.2	49.7 3.2	73.6 4.7	74.7 5.2	49.7 3.2	43.1% 3.6%
114.8 14.1	54.0 3.3	76.8 8.2	84.0 10.0	54.0 3.3	50.1% 5.3%
103.8 4.2	66.5 6.3	87.8 5.9	93.3 5.9	66.5 6.3	36.5% 4.5%
109.6 4.0	56.3 5.7	85.9 4.7	109.2 14.1	50.1 7.7	48.7% 4.8%
102.0 5.4	61.6 5.4	83.8 5.8	94.4 5.3	61.6 5.4	39.7% 3.9%
92.0 4.4	50.4 4.2	70.4 7.9	69.5 8.3	49.6 3.9	46.0% 3.4%
113.5 18.5	78.7 17.2	87.2 7.1	95.3 3.8	66.3 6.8	37.7% 6.6%
185.7 17.4	132.1 17.8	123.1 14.7	122.6 13.1	112.4 16.3	41.2% 4.5%

After a basal blood sample was drawn, the 0.1 U/kg BW of regular insulin was injected intravenously (iv). Blood samples were then taken at 30 min intervals for 90 min. Blood glucose and plasma HGH levels were measured in these specimens.

2. *Lysine vasopressin (LVP) test*

A total of 33 tests were carried out as controls in 33 volunteers and hospitalized people who had no clinical or biochemical evidence of endocrine, hepatic, or renal dysfunction. The four units of synthetic lysine vasopressin (Sandoz) were injected iv. Blood samples were drawn at 0, 15, 30 and 60 min.

In order to compare the effect of LVP and insulin on the release of HGH, 27 subjects out of them also received the insulin test.

3. *Pyrogen test*

As controls, 16 tests were performed in 16 volunteers and hospitalized individuals who had no clinical or biochemical evidence of endocrine, hepatic, or renal dysfunction. After a basal blood sample was drawn, the 0.005 μg/kg BW of "pyrogen E" (Organon) was injected iv. Additional blood samples were taken at 120 and 180 min after injection of pyrogen.

In order to compare the effect of pyrogen and insulin on the release of HGH, 13 subjects out of them had also undergone the insulin test.

4. *Glucagon test*

The subjects were 8 normal males, 6 normal females, 6 patients with hypopituitarism, 6 with diabetes mellitus, one adrenalectomized bilaterally due to Cushing's syndrome, and two with Addison's disease.

After a basal blood sample was drawn, one mg of crystalline glucagon (Novo Industri A/S, Copenhagen, Denmark) was injected subcutaneously (sc). Additional blood samples were drawn at 30, 60, 90, 120, 150 and 180 min after injection of glucagon. Blood glucose and plasma HGH levels were measured in these specimens.

In order to investigate the specificity and the effect of the dose of glucagon on the secretion of HGH, subcutaneous injection of physiological saline and one mg and two mg glucagon were repeatedly carried out in one normal male subject.

In four normal males, oral administration of two, two and four mg dexamethasone was carried out at 1 PM, 7 PM and 11 PM respectively on the previous day, in order to study the influence of dexamethasone on the glucagon-induced HGH secretion.

5. *Assay methods*

Blood glucose was determined by an auto-analyzer using ferricyanide procedure (6), and plasma HGH by radioimmunoassay using a double antibody separation procedure.

RESULTS

1. *Insulin test*

Table 1 shows the levels of HGH and blood glucose obtained before and

after insulin hypoglycemia in all subjects.

Normal subjects showed a mean fall in blood glucose after insulin from the fasting level of 103.6±2.5 mg/100 ml to 57.1±2.3 mg/100 ml at 30 min, followed by a return to preinsulin levels. The mean per cent decrement in blood glucose was 47.0% of the fasting level. In normal subjects, maximum HGH levels following insulin hypoglycemia ranged from 11.3 to 60.0 ng/ml with a maximum mean of 29.6 ng/ml. Although the highest levels usually occurred at 60 min, two and three out of 40 normal subjects peaked at 30 and 90 min, respectively. Although no significant correlation ($\gamma = +0.097$, $P > 0.5$) was obtained between the absolute minimal blood glucose levels and the HGH increments (Fig. 1), significant correlations ($\gamma = +0.631$, $P < 0.001$, $y = 0.6x - 1.2$) were observed between per cent decrement in blood glucose and the HGH increments (Fig. 2). This indicates that the HGH response may

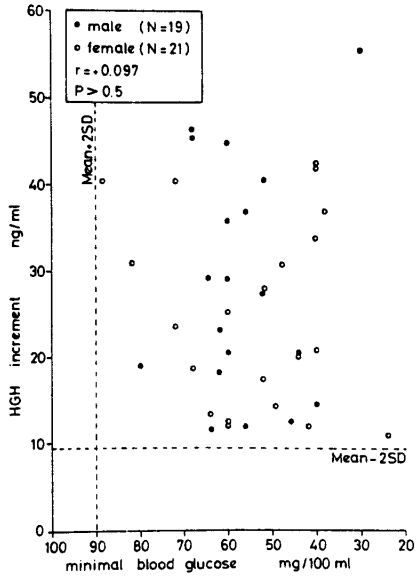

Fig. 1. Comparison of minimal blood glucose levels and HGH increments following insulin in 40 normal subjects. Each dashed line represents two SD below or above the logarithmic mean.

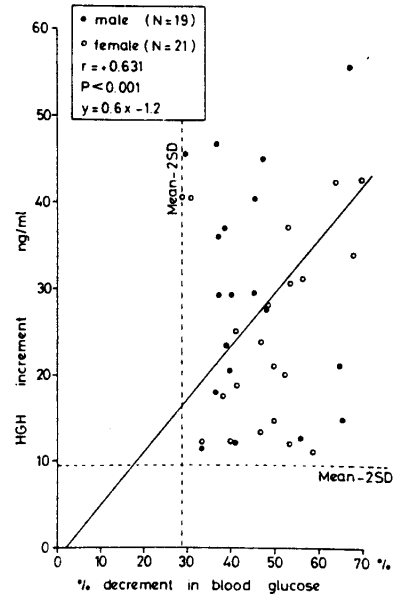

Fig. 2. Correlation between per cent decrements in blood glucose and the HGH increments following insulin in 40 normal subjects. Each dashed line represents two SD below the logarithmic mean.

be related to the per cent blood glucose decrement rather than to the absolute minimal blood glucose levels. Because of the skew distribution, the data were analyzed after logarithmic transformation for definition of the normal

range. The 95% confidence limits were considered to be the logarithmic mean ± 2 SD. By this analysis, the mean per cent blood glucose decrement of 40 normal subjects following insulin-induced hypoglycemia was 45.8%, with 95% confidence limits of 28.6 to 73.5%, and the mean HGH increment was 24.4 ng/ml, with 95% confidence limits 9.4 to 62.7 ng/ml. Namely, the fall of the fasting blood glucose level by 28.6% or more seemed to be sufficient to provoke an adequate HGH elevation, and 9.4 ng/ml or higher HGH increment was recognized as being normal. This criterion should be applied to LVP test and pyrogen test.

Six patients with acromegaly had fasting HGH levels ranging from 45.0 to 160.0 ng/ml. Although HGH increase was noted in three out of six subjects following insulin, no HGH increase was noted in three subjects.

The panhypopituitarism group had no change in plasma HGH levels following insulin hypoglycemia. The blood glucose response to insulin administration was similar to that seen in the normal subjects.

Although five patients with primordial dwarfism had a normal increase in HGH, 10 patients with pituitary dwarfism failed to show any change in HGH with insulin-induced hypoglycemia.

In 8 patients with hypogonadotropic hypogonadism, the mean HGH increment following insulin was in the normal range but it was significantly lower ($P<0.01$) than that in normal subjects.

Despite a very similar fall in blood glucose following insulin in the 10 patients with idiopathic diabetes insipidus as shown in Table 1, the corresponding change in plasma HGH levels was from a basal mean value of 1.0 ±0.3 ng/ml to only 8.8±3.2 ng/ml at 60 min.

In 9 patients with hyperthyroidism, the mean HGH increment was in the normal range but it was significantly lower ($P<0.01$) than that in normal subjects. On the other hand, the hypothyroidism had a small increase in HGH levels following insulin.

In 7 patients with Cushing's syndrome, a markedly impoverished HGH response to insulin was seen.

The mean HGH increment of diabetics following insulin hypoglycemia was in the normal range but it was significantly lower ($P<0.05$) than that in normal subjects.

Transient hypoglycemia symptoms usually occurred about 40 min after administration of insulin. These consisted of drowsiness, excessive perspiration, and hunger.

2. *Lysine vasopressin (LVP) test*

The iv injection of LVP induced 9.4 ng/ml or higher HGH increment in

only 9 of 33 control subjects tested (Fig. 3).

A comparison of HGH increments obtained following insulin and LVP is shown in Fig. 4. Despite 20 out of 27 control subjects tested respond to insulin test, only six of 27 subjects respond to LVP test. There is no significant correlation ($\gamma = -0.131$, $P > 0.5$) between HGH increments obtained from insulin test and those from LVP test.

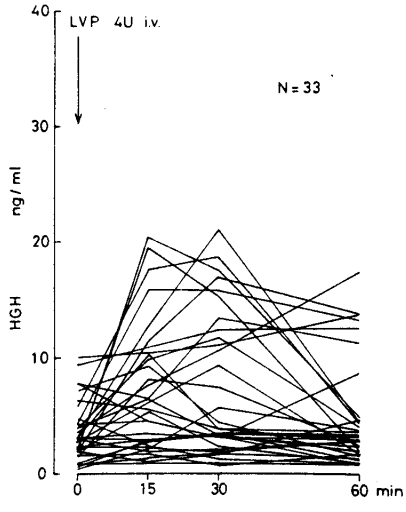

Fig. 3. Plasma HGH response to iv administration of four U LVP in 33 control subjects.

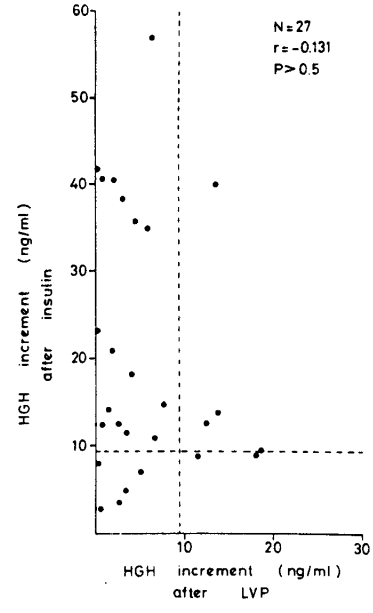

Fig. 4. Comparison of the HGH increments following insulin and LVP in 27 control subjects. The dashed line represents the normal threshold (9.4ng/ml), which is adopted in the present paper.

Most of subjects tested complained of the abdominal colic at 2 to 5 min after iv administration of LVP.

3. *Pyrogen test*

Following the iv injection of pyrogen, the HGH increments were beyond 9.4 ng/ml in only 5 of 16 control subjects tested (Fig. 5).

A comparison of HGH increments obtained following insulin and pyrogen is shown Fig. 6. There is no significant correlation ($\gamma = -0.038$, $P > 0.9$) between HGH increments obtained from insulin test and those from pyrogen test.

Furthermore, the discomfort, such as pyretic response to the patients

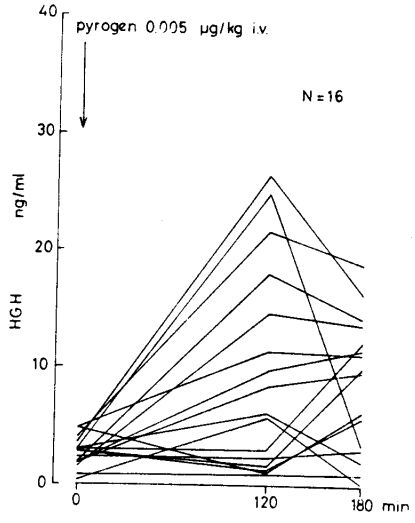

Fig. 5. Plasma HGH response to iv administration of pyrogen (0.005μg/kg) in 16 control subjects.

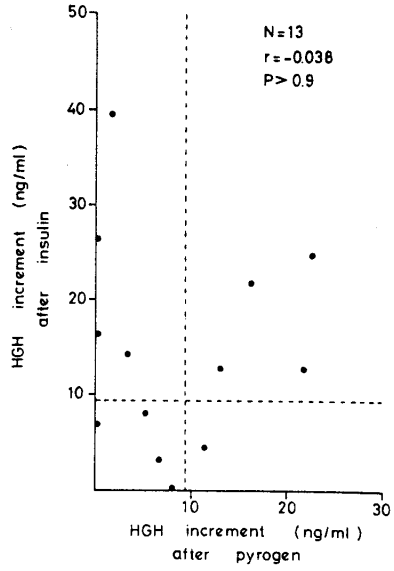

Fig. 6. Comparison of the HGH increments following insulin and pyrogen in 13 control subjects. The dashed line represents the normal threshold (9.4 ng/ml), which is adopted in the present paper.

receiving pyrogen, argues against its use in diagnosis.

4. *Glucagon test*

Table 2 summarizes the HGH values after repeated sc administration of physiological saline and one mg or two mg glucagon in one normal male subject. HGH values failed to change significantly in response to physiological saline. Responses of HGH were similar between administrations of

TABLE 2 REPRODUCIBILITY OF PLASMA HGH RESPONSE TO SC GLUCAGON ON 5 OCCASIONS IN A NORMAL MALE SUBJECT.

glucagon	plasm HGH (ng/ml)							blood glucose (mg/100ml)						
	0 min	30 min	60 min	90 min	120 min	150 min	180 min	0 min	30 min	60 min	90 min	120 min	150 min	180 min
1 mg	0.9	1.2	1.0	1.0	4.9	20.0	17.2	90	150	122	98	86	80	92
1 mg	1.3	2.9	1.8	2.2	1.2	22.5	19.9	104	148	112	94	86	98	104
1 mg	0.7	1.5	1.7	1.2	0.8	38.3	40.0	104	152	138	100	70	80	70
2 mg	1.0	1.0	0.8	1.1	3.9	22.0	20.0	80	148	126	106	84	80	94
2 mg	2.1	2.0	0.9	0.6	1.7	3.2	23.8	94	156	120	100	88	88	92
saline only	1.0	4.4	5.0	3.9	2.7	1.8	2.7	—	—	—	—	—	—	—

one mg and two mg glucagon, indicating that the sc injection of one mg glucagon is sufficient for the stimulation test of HGH secretion.

Table 3 shows the mean values of plasma HGH and of blood glucose at various times after glucagon sc injection, including standard errors of the means. In normal subjects after one mg sc injection of glncagon, there was an increase in blood glucose levels from a mean baseline level of 95.9 ± 1.8 mg/100ml (SE) to a mean peak level of 142.4 ± 3.1mg/100ml (SE) at 30 min, and then blood glucose levels fell slightly below the baseline levels at 90-180 min. HGH levels reached a mean peak level of 22.6 ± 4.8 ng/ml (SE) at 150 min and remained elevated at 180 min, and they were significantly higher ($P<0.001$) than the mean baseline level of 2.5 ± 0.5 ng/ml (SE). The range of maximal HGH levels after glucagon was 13.2-60.5 ng/ml. In patients with hypopituitarism, blood glucose levels reached a mean peak level of 129.4 ± 9.6 mg/100 ml (SE) at 30 min without significant difference ($0.1>P>0.05$) from normal subjects, but there was no positive response in plasma HGH levels and plasma HGH concentrations at each interval did not differ significantly from those at 0 time.

In patients with diabetes mellitus, blood glucose levels reached a mean peak level of 223 ± 14.2 mg/100 ml at 60 min, followed by a slow decline and returning to the previous level at 180 min. While the blood glucose concentrations at each interval in patients with diabetes mellitus was much higher ($P<0.001$) than in the controls, the peak values of HGH (18.1 ± 3.1 ng/ml, at 150 min) were the same as those of normal controls ($0.4>P>0.3$).

In four normal male subjects, 8 mg of dexamethasone were orally administered on the day before the glucagon test. Blood glucose levels at each interval were significantly higher (at 0-120 min: $P<0.001$; at 150 min: $0.01<P<0.02$; at 180 min: not significant) than in normal male subjects, and high blood glucose levels persisted. However, the HGH secretion showed a pattern similar to that in normal male subjects, without significant difference. On the other hand, plasma cortisol levels were completely suppressed in these cases.

In order to remove the hyperglycemic response alone, 0.09 U/kg regular insulin was injected in a normal male subject along with sc injection of glucagon. As the result, there was no change in blood glucose levels but the HGH secretion was present (Fig. 7). In a patient adrenalectomized bilaterally due to Cushing's syndrome who was under replacement therapy with 37.5 mg/day cortisone acetate and in two patients with Addison's disease, glucagon test gave the same HGH secretion as in normal subjects (Table 4).

Occasionally, some patients complained of an increased sensation of hunger, or of slight nausea at 120 min after injection of glucagon.

TABLE 3 PLASMA HGH AND BLOOD GLUCOSE

Diagnosis	No. of subjects	Plasma HGH 0 min	30 min	60 min	90 min	120 min
normal subjects	14 Mean	2.5	2.3	2.4	5.9	11.9
	SEM	0.5	0.4	0.5	1.8	3.0
normal males	8 Mean	1.9	1.7	1.9	3.4	11.5
	SEM	0.4	0.3	0.4	2.1	4.8
normal females	6 Mean	3.9	3.1	3.1	9.2	12.4
	SEM	0.7	0.8	1.1	2.9	3.8
panhypopitutarism	6 Mean	1.1	1.3	1.2	1.4	1.4
	SEM	0.2	0.2	0.3	0.3	0.2
diabetes mellitus	6 Mean	2.0	2.7	2.9	5.6	14.6
	SEM	1.0	1.0	0.9	1.4	3.8
dexamethasone premedicated normal males	4 Mean	2.8	3.1	9.0	12.3	20.9
	SEM	1.1	1.3	2.5	3.2	7.7

TABLE 4 PLASMA HGH AND BLOOD GLUCOSE RERPONSE TO SC INJECTION OF GLUCAGON IN

Initials	Age	Sex	Diagnosis	Plasma HGH 0 min	30 min	60 min	90 min	120 min
S.K.	33	M	adrenalectomized bilaterlly (Cushing's syndrome)	1.0	4.0	0.1	4.2	14.8
O.M.	55	M	Addison's disease	0.6	1.1	0.5	1.7	2.5
O.H.	39	M	Addison's disease	1.5	1.5	1.4	2.1	5.3

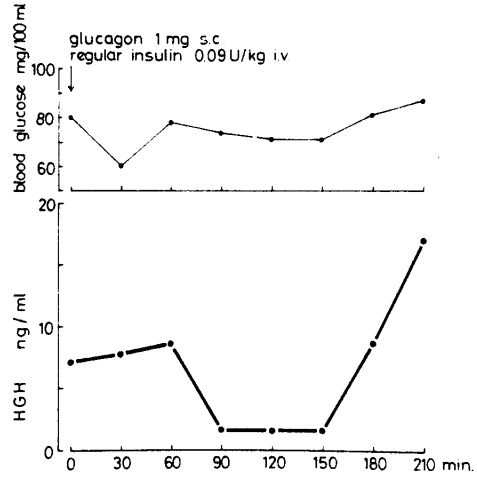

Fig. 7. Serial plasma HGH and blood glucose values before and after receiving a concomitant iv injection of regular insulin (0.09 U/kg) with sc injection of one mg glucagon.

CONCENTRATIONS AFTER SC GLUCAGON.

(ng/ml)		Blood glucose (mg/100ml)						
150 min	180 min	0 min	30 min	60 min	90 min	120 min	150 min	180 min
22.6	17.3	96	142	123	92	84	89	88
4.8	4.1	1.8	3.1	5.2	3.4	2.3	2.1	3.0
27.4	23.3	97	149	120	90	82	89	89
6.8	5.5	2.6	2.6	8.3	4.4	2.0	3.3	4.1
19.4	7.8	94	133	127	95	87	89	87
5.7	3.0	2.3	4.3	5.8	5.6	4.7	2.8	4.6
1.5	1.2	92	129	121	97	78	76	78
0.2	0.1	4.5	9.6	13.4	8.0	4.2	4.5	4.8
18.1	9.9	145	207	223	222	194	168	144
3.1	3.5	6.4	13.6	14.2	22.1	22.3	17.5	15.8
32.0	29.5	120	175	190	167	128	105	91
9.3	9.4	3.7	2.4	8.7	14.1	13.7	8.5	3.8

A PATIENT ADRENALECTOMIZED BILATERALLY AND IN 2 PATIENTS WITH ADDISON'S DISEASE.

(ng/ml)		Blood glucose (mg/100 ml)						
150 min	180 min	0 min	30 min	60 min	90 min	120 min	150 min	180 min
26.0	22.5	84	148	114	92	76	70	68
18.0	17.0	114	144	156	130	98	80	82
28.7	16.2	86	136	130	104	76	56	64

DISCUSSION

Recently, many stimulation tests have been used to assess the HGH reserve. Some of these tests include insulin, lysine vasopressin, pyrogen and glucagon administration.

The stimulatory effect of hypoglycemia upon HGH secretion has been demonstrated many times (7-9) since its first description by ROTH *et al.* in 1963 (1). It has been cited that the magnitude of fall in blood glucose was 50% or more of the fasting level, which was the criterion as being sufficient to provoke adequate HGH elevation (1, 8, 10-11). On the other hand, ROTH *et al.* (12) have demonstrated that a rapid fall in blood glucose, without attaining hypoglycemia, could induce a significant rise in HGH, and KOH *et al.* (13) and GLICK (14) found that the threshold fall of blood glucose to release HGH was between 20 and 30 mg/100 ml. LUFT and CERASI (15) reported that a decrease in blood glucose of at least 10 mg/100 ml was almost always accompanied by a substantial rise in plasma HGH and that the magnitude of rise in HGH was followed by the degree of hypoglycemia obtained. The present

data indicate that the decline of blood glucose level is more significant factor than the absolute level of glucose itself with respect to the release of HGH, *i. e.* the HGH response is related to the per cent blood glucose decrement rather than to the absolute minimal blood glucose levels (Figs. 1 & 2). And then, it was also revealed that the magnitude of fall in blood glucose which was the criterion as being sufficient to provoke HGH secretion for clinical evaluation was 28. 6% or more of the fasting level.

Because of the great variation in the normal response of plasma HGH to insulin hypoglycemia, it is difficult to determine a borderline between normal and subnormal responses. The present results obtained from 40 insulin tests performed on normal subjects demonstrate that 9. 4 ng/ml or higher HGH increment was recognized normal, being in close agreement with GREENWOOD *et al.* (16) and PARKER *et al.* (17). Although a defective HGH response to insulin test has been reported in patients with acromegaly (18), some patients in this study showed a rise in plasma HGH while others failed to do so, in agreement with observations made by ROTH *et al.* (19), GLICK *et al.* (9) and GREENWOOD *et al.* (20). In patients with hypothalamo-pituitary disease, as noted by RABKIN and FRANTZ (21) and KAPLAN *et al.* (22), unresponsiveness was associated with a complete lack of HGH elevation in response to insulin hypoglycemia. The lack of HGH response in the postoperative hypothalamo-pituitary tumor group may indicate that the integrity of neural or vascular connections between the hypothalamus and the pituitary appears to be necessary for responsiveness of HGH secretion to the change in blood glucose. Although conflicting results have been reported on plasma HGH response to insulin-induced hypoglycemia in hyperthyroidism (23-25), it has been generally accepted that patients with hypothyroidism exhibit impaired release of HGH following insulin-induced hypoglycemia (26, 27). The insulin-induced hypoglycemia was less effective stimulus to HGH secretion in patients with Cushing's syndrome than in normal subjects, agreeing with the well known facts that administration of glucocorticoids supresses the HGH response to hypoglycemia (8, 28). The diagnostic value of insulin test outweighs its possible risk. No patients in the present studies have experienced severe side effects; only one complication encountered in present 131 studies was arrhythmia, which responded promptly to the intravenous administration of glucose.

Although vasopressin has been reported to cause a reliable increase in plasma HGH in monkeys as well as men (29-32), CZARNY *et al.* (33), BROSTOFF *et al.* (34) and KARP *et al.* (35) have reported that LVP caused an increase in plasma corticosteroid levels whereas plasma HGH response to LVP was variable. The present results indicate that an increase in plasma HGH is seldom seen after LVP, and the response in normal subjects is not consistent enough

to be a reliable test for HGH reserve despite the side-effects such as vasoconstriction and abdominal colic. Although it has been reported that bacterial pyrogen stimulated both cortisol and HGH release (36, 37), the present results reveal that HGH levels rise beyond the normal threshold (9. 4 ng/ml) in only five of 16 control subjects tested. Since LVP and pyrogen produce varying degrees of side-effects and they are less specific and unpredictable in the release of HGH, they may be limited in their widespread use for HGH stimulation tests. On the other hand, insulin-induced hypoglycemia generally gave rise to higher HGH levels, and pattern of response was more consistent than those seen in LVP and pyrogen tests. Therefore, insulin-induced hypoglycemia is the better stimulus for routine testing of HGH reserve than LVP and pyrogen.

HGH secretion in response to sc injection of glncagon is generally accepted in recent years (3-5), although some have reported no significant HGH response to iv glucagon (2, 38). CAIN *et al.* (5) demonstrated that one mg glucagon administered either sc in a single dose, or in infusion over 30 min gives consistent and reproducible HGH rises, as compared with a single bolus of glucagon. In the present study, one mg glucagon was administered sc to all subjects except in dose response study. In all the normal subjects, HGH secretion was observed in response to sc injection of glucagon, and no false negative response as descibed by MITCHELL *et al.* (39) was noted. In patients with insufficiency of HGH secretion as shown by orher tests, no HGH response was observable in glucagon test.

The exact mechanism by which glucagon causes HGH release is obscure. While it is conceivable that difference between the sc and iv glucagon administrations is due to a long latent period for its effect, this seems unlikely in view of the short circulating half-life (about 10 min) of glucagon in serum (40, 41), and its rapid stimulation of insulin release and glycogenolysis (42). HGH secretion in response to glucagon, unlike the stimulation by other substances, is characterized by a long interval from glucagon loading to HGH secretion. This might suggest that glucagon stimulates the HGH secretion *via* some intermediate substances or metabolic changes, but no sufficient data are available for confirmation. Some investigators suggest a possibility that HGH secretion in response to sc glucagon is related to the fall in blood glucose from the previously elevated level, rather than a specific response to glucagon (43, 44). This possibility is denied by the following observations: (A) In some studies of HGH release in which the rise and fall in glucose concentration were induced by glucose instead of glucagon, a substantial number of adult subjects showed no increase in HGH (45, 46). (B) In a few normal subjects, both blood glucose and HGH concentrations reached a peak almost

the same time (39, 47). (C) The glucagon-induced rise in plasma HGH failed to be prevented by the coadministration of insulin (Fig. 7). (D) In patients with diabetes mellitus, HGH secretion in response to sc glucagon is similar to that in normal subjects despite persisting hyperglycemia (Table 3). (E) Despite marked hyperglycemia lasting for a long period of time after dexamethasone administration, the timing of response and the magnitude of HGH are similar to those in the untreated group (Table 3). Then, it is unlikely that the rise and subsequent fall of glucose are the sole mechanism responsible for the effect of glucagon on HGH secretion. Such non-participation of blood glucose might indicate that glucagon will be a potentially useful tool in evaluating pituitary HGH reserve in patients with diabetes mellitus.

It has been reported that adrenomedullary secretion was stimulated by glucagon (48-51), and recently glucagon has been used for the provocation test in pheochromocytoma (51). On the other hand, in view of the reports on the invasion of catecholamine in to the hypothalamus (52, 53), it may be possible that HGH secretion in response to sc glucagon is triggered by catecholamine *via* the stimulation of adrenal medulla, although it has been reported that the administration of epinephrine to normal subjects was not followed by elevation of plasma HGH (1). Such a possibility can be denied, since glucagon-induced HGH secretion was observed in a subject adrenalectomized bilaterally and in two patients with Addison's disease (Table 4). DANFORTH *et al.* (38) have described that a rise in the plasma HGH after glucagon is the result of nonspecific stress. However, other stresses such as venipuncture or surgery do not necessarily cause a consistent rise in plasma HGH (54, 55), and the fact that in most instances there was no change in HGH levels for 90 min after administration of glucagon seems to almost deny the stress playing a role in elevating HGH levels. The mechanism of glucagon-induced HGH secretion is being further investigated. Glucagon will gain popularity in clinical practice because of its ease of administration and its consistent action of HGH release.

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