Plasma Growth Hormone Responses to Growth Hormone-Releasing Factor in Normal Children and Children with Short Stature

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Abstract

Plasma GH responses to GRF have been studied in 12 normal prepubertal children (6 boys and 6 girls) with normal height, 6 normal young adults and 90 patients with short stature due to various causes. Mean peak GH responses in normal boys and girls were 36 ± 9 ng/ml ($M \pm SE$) and 33 ± 5 ng/ml respectively. Among 25 patients with idiopathic severe pituitary dwarfism, 16 cases showed peak GH to GRF less than 5 ng/ml, 3 cases between 5 and 10 ng/ml and 6 more than 10 ng/ml. There was a negative correlation between chronological age and peak GH responses to GRF in these 25 pituitary dwarfism. However, no correlation was observed in patients with partial idiopathic dwarfism (6 cases), normal short children (31 cases) and normal children. GH responses to GRF were blunted in organic pituitary dwarfism and primary hypothyroidism, but normal in deprivation dwarfism and Turner's syndrome. The responses in normal short children were variable, some children showed blunted but some normal. Acute exogenous GH administration suppressed the responsiveness of somatotrophs to GRF, but this was easily reversible even after chronic administration of GH for one year in normal short children who showed increased growth rate during the GH treatment.

Since growth hormone-releasing factor (GRF) was isolated and characterized (Guillemin *et al.*, 1982; Rivier *et al.*, 1982), many clinical and basic reports on GRF have been published (Frohman and Jansson, 1986). Synthetic GRF is now available for clinical testing as a method of assessing GH secretion in physiological and pathological conditions, and it is thought that direct stimulating effect of GRF on somatotrophs is faster and greater than other secretagogues (Laron and Bauman, 1986). However,

much still remains to be clarified with respect to the clinical usefulness of GRF as diagnostic agent.

We will present some results of our clinical experiences on GRF test in normal children with growth retardation.

Subjects and Methods

Subjects examined in our study are illustrated in Tab. 1. Twelve normal children with normal height consisted of 6 prepubertal boys (5-11 years) and 6 prepubertal girls (4-9 years). Thirty-one short children with normal GH secretion to secretagogues other than GRF were

* This study was presented at the International Symposium on GRF, GH and Somatomedin (Tokyo) 1986.

Table 1. List of subjects examined.

Diagnosis	No. of Cases
Normal Children (Normal Height)	12
Normal Adult	6
Idiopathic Pituitary Dwarfism	
severe*	25
partial**	6
Organic Pituitary Dwarfism	
severe*	9
partial**	2
Primary Hypothyroidism	4
Deprivation Dwarfism	5
Turner's Syndrome	6
Delayed Adolescence with Short Stature	2
Normal Short Children	31

* Severe : Peak GH Responses to Provocative Tests Other Than GRF ≤ 5 ng/ml (RIA)

** Partial : $5 \text{ ng/ml} < \text{Peak GH} \leq 7 \text{ ng/ml}$

named as normal short children. Severe GH deficiency was defined as peak GH responses to provocative tests other than GRF was less than 5 ng/ml and partial GH deficiency as between 5 and 7 ng/ml.

A single bolus of $1 \mu\text{g/kg}$ of synthetic human

GRF-44 was administered iv, and plasma GH was measured by radioimmunoassay (RIA) during a 2 hours sampling period. Plasma GH was also determined by high sensitive immunoradiometric assay (IRMA: Sucrosep hGH from Boots-Celltech Diagnostics Lt.) in several patients with severe GH deficiency. Synthetic GRF-44 which we used was kindly supplied mainly by Sumitomo Pharmaceutical Company (Osaka, Japan). Statistical analysis was conducted by Student's *t*-test.

Results

Normal children

The Fig. 1 shows GH responses to GRF in the normal prepubertal boys and girls. Mean peak GH levels were $36 \pm 9 \text{ ng/ml}$ ($M \pm SE$) and $33 \pm 5 \text{ ng/ml}$ respectively. Time of peak GH in boys was between 10 and 20 minutes after GRF injection, and between 10 and 45 minutes in girls.

There is no significant difference between these two normal groups ($p < 0.05$). And these values were similar to those of 6 normal young adults examined, who showed

Fig. 1. Plasma GH responses to synthetic human GRF-44 ($1 \mu\text{g/kg}$, iv) in normal prepubertal boys (left) and girls (right).

Fig. 2. Plasma GH responses to GRF in idiopathic severe pituitary dwarfism. 16 cases with peak GH ≤ 5 ng/ml were precluded in the figure. IGHD: isolated GH deficiency; MPHD: multiple pituitary hormones deficiency.

Fig. 3. Correlation between chronological age and peak GH responses to GRF in idiopathic severe pituitary dwarfism.

the mean peak GH of 48 ± 18 ng/ml.

Pituitary dwarfism

Among 25 cases with idiopathic severe pituitary dwarfism, 16 cases showed peak GH to GRF less than 5 ng/ml, and the pattern of GH responses to GRF in the rest 9 cases are shown in Fig. 2. Of these 9 cases, 6 had the peak GH more than 10 ng/ml. Except one case, plasma GH rose slower and the time of peak GH was later than normal.

Fig. 3 demonstrates the correlation

Fig. 4. Plasma GH profiles in 4 cases with idiopathic severe pituitary dwarfism. GH measured by RIA kit showed blunted responses to GRF, but GH measured by high sensitive immunoradiometric assay showed definite rise after GRF injection. The higher the age of the patients, the less peak GH levels were observed.

between chronological age and peak GH responses to GRF in severe idiopathic pituitary dwarfism. The vertical axis of the Fig. 3 is indicated as log. scale. The correlation coefficient is -0.8 . The correlation is higher in the cases before 15 years old, and lower after that.

Plasma GH was determined by high sensitive immunoradiometric assay (IRMA) in 4 cases with low-responder to GRF. The results are shown in Fig. 4. Plasma GH levels measured by routine RIA kit showed minimal rise in response to GRF, but GH measured by IRMA showed clear rise in response to GRF. The decreasing tendency of peak GH with chronological age was observed.

Fig. 5 shows also the correlation between peak GH responses to GRF and chronological age in normal children, normal short children and idiopathic partial GH deficiency. There was completely no correlation in each group at all.

Idiopathic partial GH deficiency, aged 6 to 14 years, showed normal response to GRF in terms of peak GH levels and the time of peak GH (Fig. 6).

The responses to GRF in organic pituitary dwarfism are shown in Fig. 7. The

Fig. 5. No correlation between age and peak GH responses to GRF was observed in normal children, normal short children and patients with partial GH deficiency.

Fig. 6. GRF tests in idiopathic partial GH deficiency showing almost normal responses.

Fig. 7. GRF tests in organic pituitary dwarfism. Both of the patients with severe and partial GH deficiency showed blunted, but over 5ng/ml responses.

Table 2. List of primary hypothyroidism.

Name	Sex	Age (yr)	TSH (μ U/ml)	T ₄ (μ g/ml)	FT ₄ (ng/dl)	T ₃ (ng/dl)	Peak GH to ITT (ng/ml)
H. M.	F	7 1/12	1,023	<1.0	<0.2	20	8.7
K. K.	M	10 7/12	240	3.5	0.3	80	4.2
F. A.	F	12 1/12	404	<1.0	<0.2	20	3.1
F. F.	M	14 7/12	16	6.8	1.0	110	15.9

Fig. 8. GRF test in primary hypothyroidism before treatment (○—○ girls, ●—● boys) and during treatment (○---○).

results of 6 cases with severe GH deficiency are shown on the upper panel, and 2 with partial ones on the lower panel. All of these 8 cases exhibited peak GH higher than 5 ng/ml. But the pattern of the curves were low and blunted.

Primary hypothyroidism

The GH responses to GRF in 4 cases with juvenile onset primary hypothyroidism were examined. Peak GH responses to insulin induced hypoglycemia (ITT) were severely suppressed in 2 cases, mild in one and normal in one (Tab. 2). GH responses to GRF in these patients are shown in Fig. 8. Minimal GH rises were observed in 3 cases who showed low GH response to ITT. However one case who responded normally to ITT showed normal GH response to GRF, and one non-responder before treatment showed normal after thyroxine treatment.

Turner's syndrome

Fig. 9 shows the pattern of GH responses to GRF in 6 cases with Turner's syndrome aged between 5 and 13 years.

Fig. 9. GRF tests in Turner's syndrome.

Four of them showed normal but 2 showed delayed responses.

Deprivation dwarfism

We examined GH secretion in 5 cases with deprivation dwarfism. Fig. 10 shows GH secretion in ITT (on the left), and during sleep (on the right). Four of them showed subnormal GH secretion. GRF test in these 5 cases showed almost normal GH responses (Fig. 11).

Normal short children

The results of GRF test in 31 cases with normal short children are illustrated in Fig. 12. Some of them showed normal, some blunted or delayed and others very low responses. Peak GH responses to GRF (22.3 ± 2.5 ng/ml) were compared with maximum peak GH responses to several secretagogues other than GRF (22.4 ± 2.8 ng/ml). The mean peak GH levels of each responses did not differ significantly, but there was no correlation between the two. We could

Fig. 10. Plasma GH responses to insulin induced hypoglycemia and during sleep in 5 patients with deprivation dwarfism.

Fig. 11. GRF tests in 5 patients with deprivation dwarfism.

Fig. 12. GRF tests in normal short children.

Fig. 13. Peak GH responses to GRF in normal children and short children due to various causes.

Fig. 14. Time of peak GH responses to GRF in normal children and short children due to various cases.

Table 3. Normal short children and patients with constitutional delayed adolescence with slow growth treated hGH for 1 year. 2 cases with delayed adolescence showed blunted GH responses to provocative tests, but normal after testosterone priming.

(1) Normal Short Children

Name	Age (Yr)	Sex	Ht (cm)	SD	BA	Peak GH (ng/ml)		
						ITT	Arg	Sleep
M. H.	4-6	M	94.9	-2.2	2-8	10.6	9.2	3.5
O. T.	6-5	M	98.5	-3.7	3-2	26.9	12.9	8.0
H. K.	7-1	M	100.8	-3.8	4-11	22.9	6.4	—
W. S.	9-4	M	114.6	-3.1	6-0	14.4	12.6	18.5
O. K.	10-0	M	100.8	-5.7	7-0	11.8	4.8	16.8
I. M.	11-9	M	130.4	-2.2	12-9	23.0	20.8	16.4
M. I.	4-9	F	89.5	-3.7	2-6	19.6	30.1	8.8
O. R.	9-3	F	115.9	-2.6	6-6	35.8	12.9	14.6

(2) Delayed Adolescence

I. H.	8-4	M	112.2	-2.6	5-11	4.6 (22.7)*	5.4	3.5
A. D.	12-4	M	127.2	-2.8	8-8	3.9 (30.0)*	5.8	4.2

* Testosterone Priming

not find any correlation between peak GH responses to GRF and plasma Sm-C in these normal short children. Two cases showed high Sm-C levels with low GH response to GRF, a few cases showed low Sm-C but normal GH responses.

Levels and times of peak GH responses to GRF

The results of GH responses to GRF in normal and growth retarded children which we examined are summarized in Fig. 13. Fig. 14 shows the time of peak GH levels in GRF test. When compared with

normal children, not only GH deficiency, but also Turner's syndrome and normal short children showed significantly delayed peak time.

Effects of exogenous hGH on GRF test

We examined GRF test before and after 1 year treatment with pituitary derived GH in 8 cases with normal short children and 2 cases with constitutional delayed adolescence with slow growth. Age, sex, height, bone age and results of GH secretion tests other than GRF test are shown in Tab. 3.

Growth rate before and during GH

Fig. 15. Growth rate before and during hGH therapy (0.5 U/kg/w) in normal short children (NSC) and constitutional delayed adolescence with growth retardation (DAGR).

Fig. 16. GRF tests before and after one year treatment with hGH in normal short children. GRF tests were done after more than 2 days washout period.

Fig. 17. GRF tests in normal short children. Acute GH administration diminished the GH response to GRF.

But the GH response to GRF after more than 2 days washout period, showed normal ever after chronic GH administration for one year.

treatment for 1 year are illustrated in Fig. 15. All of the cases showed increased growth rate during GH treatment. No antibody to GH was found during GH treatment.

No statistical difference ($p < 0.05$) in the GH responses to GRF between before and after the GH treatment was found (Fig. 16). The GRF tests after GH treatment were done after more than 2-days washout period. However, the GH responses to GRF which was done 24 hrs after the 1st GH injection, showed diminished pattern in 4 cases (Fig. 17). But after 1-year GH therapy (0.5 U/kg/w), the results of GRF test which was proceeded after more than 2 days washout interval showed similar pattern to that before the therapy.

Discussion

It has been already published that peak GH responses to GRF tended to decline with age and most of the normal subjects over 40 years showed low or minimal re-

sponses to GRF (Shibasaki *et al.*, 1984; Pavlov *et al.*, 1986). However, only a few paper regarding responsiveness to GRF in normal children have been published. Our data on the GH responses to GRF in prepubertal children are consistent with other reports published on prepubertal children (Chihara *et al.*, 1985; Pintor *et al.*, 1986), pubertal subjects (Gelato *et al.*, 1986), young adults (Shibasaki 1984; Gelato 1984) and infants (Shimano 1985). Gelato *et al.*, published GH responses to GRF in 90 children of different pubertal stages (Gelato 1986). Only the responses in boys at mid-puberty were somewhat lower, however they discussed that the chronic changes occurring in normal development are quite different from the acute experimental effects, and not all of their observations points towards such a linkage. Therefore, it seems likely that the magnitude of GH response to GRF are quite similar in normal subjects from infant through young adult age. In spite of the GH responsiveness to GRF in puberty being similar to that in prepuberty, plasma immunoreactive GRF concentrations were

reported to be higher in puberty than before it (Argente 1986). On the other hand, plasma Sm-C levels has been reported to be higher in the pubertal subjects. Much studies are necessary to elucidate the relationships between the higher Sm-C levels, the higher GRF levels and the similar responsiveness of GH to exogenous GRF in puberty.

Controversy exists concerning the responsiveness of GH to GRF in patients with pituitary dwarfism. It has been reported that there was a negative lineal correlation between chronological age and peak GH responses to GRF in pituitary dwarfism (Schriock 1984). However, our data suggest that the relationship between peak GH responses to GRF and age is not simple lineal correlation, but seems to be exponential-like one. And even in the low-responders (peak GH responses to GRF ≤ 5 ng/ml), the similar decreasing tendency of peak GH to GRF with age was observed, when plasma GH was measured by high sensitive method. This negative correlation did not exist in normal children, patients with partial GH deficiency who showed normal GH responses to GRF, and normal short children. These results implies the longer the duration of endogenous GRF deficiency, the less the responsiveness of somatotrophs to acute exogenous GRF challenge, in other words, GRF deficiency gradually makes somatotrophs atrophy with time. Therefore, a single bolus iv injection of GRF is not necessarily useful tool to differentiate the disturbance of GH secretion in pituitary gland from that in hypothalamus in the patients with idiopathic pituitary dwarfism. The causes and duration of the organic brain lesions in organic pituitary dwarfism were variable, so we could not determine by GRF test if the lesion situated in hypothalamus or pituitary or both. It has been published that multiple-pulse GRF administrations change the magnitude of GH response (Schriock 1986),

suggesting that diminished capacity of somatotrophs to secrete GH is reversible by long-term pulsatile GRF administrations in the patients with hypothalamic GRF deficiency.

It has been published that patients with primary hypothyroidism show low GH responses to GRF which are resolved in euthyroid state (Frohman, 1986; Williams 1985). We also demonstrated the similar results in children. These results indicates that thyroid hormone deficiency suppress GH production in somatotrophs primarily, which is reversible with thyroxine treatment.

GH secretion in deprivation dwarfism is variable and complicated. Some severe cases show blunted GH secretion to secretagogues and in physiological states. GRF test in deprivation dwarfism has not been published. Our data on GRF test in this syndrome implied that functional abnormality of GH secretion in deprivation dwarfism existed not in the pituitary itself, but in the hypothalamic level.

The GH responses to GRF in normal short children were variable and most complicated, but the mean peak GH levels was significantly lower than normal children, implying that many cases of normal short children may have any abnormal GH regulating mechanism. It is possible that causes of normal short children are heterogeneous ones, therefore the results of GH responses to secretagogues including GRF are variable. In further investigation, we should divide these short children into several groups and analyze the regulatory mechanism of GH. When we consider the GH responsiveness to GRF, we can not exclude the role of somatostatin and the effects of exogenous GH. Liapi (Liapi 1986) reported that a significant negative correlation between the amplitude of the peak GH after GRF injection, and the basal values of somatostatin-like immunoreactivity in short children.

It has been published that exogenous

GH administrations suppress the GH responses to GRF (Nakamoto 1986). Our results also indicate that acute exogenous GH will suppress the responsiveness of somatotrophs to GRF, but this will be easily reversible even after chronic 1 year GH treatment in normal short children.

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