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Peripheral Plasma Levels of Human Growth Hormone Releasing Hormone (GHRH) during the Sleep Test in Short Children

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GH releasing-hormone (GHRH) is the hypothalamic hormone which is released into the pituitary portal vessels to stimulate GH secretion [1, 2]. Since the plasma half life of GHRH is short, and there is a long distance from pituitary portal vessels to the general circulation, GHRH levels in the peripheral blood are expected to be very low. GHRH is also produced by the upper gastrointestinal tract [3, 4]. It had, therefore, been presumed difficult to monitor hypothalamic GHRH neuronal activity by measuring GHRH levels in the peripheral blood.

Recent reports have indicated that the sleep-associated GH release is mediated by hypothalamic GHRH release [5, 6]. Some previous reports suggested that the GHRH in the peripheral blood could reflect hypothalamic GHRH neuronal activities stimulated by l-dopa [7], glucose [8] or sleep [9, 10], although plasma GHRH concentrations were measured by RIAs combined with rather unreliable extraction methods [7, 11].

We recently developed a highly sensitive sandwich enzyme immunoassay (SEIA) for GHRH, which allows us to measure circulating GHRH without any extraction procedure [12]. In an attempt to monitor the hypothalamic GHRH neuronal activity in the peripheral blood, we examined the relationship between plasma GH and GHRH concentrations during the sleep test in children of short stature.

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Subjects and Methods

Fourteen children of short stature with less than −2.0 standard deviation (SD) from the mean height for chronological age, 5–13 yr, were tested (Table 1). L-arginine (0.6 g/kg) was infused i.v. for 30 min. L-dopa (10 mg/kg) and clonidine (0.1 mg/m^2) were given p.o. Glucagon (0.03 mg/kg) was injected i.m. The pharmacological GH stimulation tests were started at 0830 h after an overnight fast. Blood was withdrawn at 0, 30, 60, 90, and 120 min for arginine test, l-dopa test and clonidine test, and at 150 and 180 min for glucagon test. All subjects underwent at least three kinds of tests on different days. The sleep test was started at 2000 h, 3 h after dinner. Blood samples for GH and GHRH assay were then taken every 20 min for 3 h, 15 min after they fell asleep. All of the children examined went to sleep immediately after the start of the sleep test, and they did not wake during the test.

Nine boys and five girls were divided into three groups based on the results of the GH-responses to both the pharmacological and physiological tests described above: normal short (NS, six children), GH deficiency type A (GHD-A, three children) and GH deficiency type B (GHD-B, five children) (Table 1). All the subjects were in good health and were not obese or hypothyroid. NS showed normal plasma GH responses to both pharmacological GH-stimulation tests and the sleep test with peak

Table 1. Profiles of the patients

Group	Case	Age Yr	Sex	Height cm	Weight SDS	Weight kg	Clonidine test Basal GH (ng/ml)	Clonidine test Peak GH (ng/ml)	L-dopa test Basal GH (ng/ml)	L-dopa test Peak GH (ng/ml)	Arginine test Basal GH (mg/ml)	Arginine test Peak GH (mg/ml)	Glucagon test Basal GH (ng/ml)	Glucagon test Peak GH (ng/ml)	Sleep test Basal GHRH pg/ml	Sleep test Peak GHRH pg/ml	Sleep test Mean GHRH pg/ml	Sleep test Basal GH ng/ml	Sleep test Peak GH ng/ml	Sleep test Mean GH ng/ml
NS	1	8	M	99.6	−2.9	13.5	1.2	10.2	NE		0.3	7.4	0.4	16.7	10.7	16.5	12.4	2.0	10.7	5.3
	2	7	M	116.0	−2.6	20.0	0.3	26.8	NE		3.2	11.8	0.8	18.6	18.5	24.1	15.2	11.5	13.6	8.5
	3	10	F	109.5	−4.1	16.0	0.3	22.3	1.1	39.7	1.9	19.0	NE		6.3	12.3	9.4	3.8	9.9	5.0
	4	4	F	88.5	−3.1	11.2	1.6	19.0	1.3	17.1	NE		0.6	27.0	14.5	11.9	10.5	17.6	20.6	10.8
	5	5	M	85.0	−2.9	10.2	0.7	12.1	NE		1.7	7.0	1.8	11.5	23.1	20.1	16.2	0.9	19.5	7.9
	6	5	F	96.7	−2.7	12.6	NE		0.4	36.4	5.1	25.7	1.9	44.0	7.8	8.1	7.1	16.5	19.4	11.8
GHD-A	7	11	M	123.8	−3.4	28.0	0.3	7.5	NE		0.3	5.6	0.3	3.4	8.3	18.8	8.6	4.2	8.6	3.2
	8	6	M	96.5	−3.7	13.6	0.3	27.2	NE		0.8	15.6	1.2	20.6	7.4	95.0	15.2	4.4	4.4	1.4
	9	11	F	119.4	−3.0	22.3	0.4	3.4	NE		0.4	2.0	0.4	5.2	44.8	44.8	31.4	0.4	6.1	2.1
GHD-B	10	8	M	100.1	−2.9	14.0	1.7	3.8	NE		0.5	7.0	3.6	11.4	10.2	10.2	6.8	20.3	30.4	10.6
	11	9	F	102.8	−2.6	15.1	0.7	4.7	NE		0.3	0.7	0.3	14.3	5.2	5.6	5.1	17.9	27.9	11.1
	12	12	M	130.0	−3.4	31.8	0.3	6.3	NE		0.3	13.8	0.3	5.4	15.8	19.5	16.7	2.1	14.6	5.1
	13	9	M	114.4	−3.2	19.8	0.5	16.4	NE		0.9	6.2	2.0	5.4	18.0	19.2	10.1	0.9	11.8	5.4
	14	13	M	135.1	−2.7	29.4	0.7	6.7	NE		0.3	3.4	0.4	14.4	10.4	10.4	8.3	0.4	13.2	5.3

SDS, standard deviation score of the mean height for chronological age of Japanese children; NE, not examined.

plasma GH concentrations above 10 ng/ml. GHD-A showed impaired GH response to the sleep test with or without impaired GH responses to the pharmacological tests with peak plasma GH concentrations below 5 ng/ml. GHD-B showed impaired plasma GH responses to at least two of four GH-stimulation tests, but showed a normal GH response to the sleep test. Results of GH stimulation test in each individual are shown in Table 1.

Plasma GH and GHRH were measured by immunoreactive radio-metric assay (IRMA, Eiken, Tokyo) and SEIA [12], respectively, without any extracting procedures. The least detectable values (LVD) for GH and GHRH in plasma were 0.3 ng/ml and 2.0 pg/ml, respectively. Intra- and inter-assay variations were 5% and 8% for GH, and 7% and 12% for EIA, respectively. Values below LVD for GH and GHRH were treated as 0.3 ng/ml and 2.0 pg/ml, respectively.

The Kruskal-Walls test was used to compare the basal, peak and mean GHRH and GH concentrations in the sleep test among three groups. The mean GHRH or GH level was the mean for 10 samples in the sleep test. Since GHRH given iv stimulates GH release with a peak 20 min or 40 min after injection, plasma GHRH and GH concentrations at the same time, 20 min or 40 min after peak GHRH values, to see if there was a relationship between GHRH and GH during the sleep test (correlation coefficient). A significant p value was less than 0.05. The computer program StatView 4 for Macintosh was used.

Result

Most samples of plasma GH and GHRH were above the LDV of each assay throughout the sleep test (GH 98% and GHRH 100%). Sleep associated GH secretion was observed in NS and GHD-B, but not in GHD-A. There were significant differences in both peak GH and mean GH levels among NS, GHD-A and GHD-B in the sleep test (peak/mean levels (mean ± SEM) in NS, GHD-A and GHD-B: 10.3 ± 2.7/8.2 ± 1.1, 3.1 ± 2.6/2.2 ± 0.5 and 12.1 ± 5.4/7.5 ± 1.4 ng/ml, NS or GHD-B *vs.* GHD-A; $P<0.05$). In contrast, there were little variations in plasma GHRH throughout the sleep test in the three groups (Fig. 1). There seemed to be an exceptional coincidental increase in plasma GHRH with GH 40 min after sleep in case 7. There were, however, no coincidental changes in the plasma GHRH concentration associated with GH throughout the sleep test in any other subject examined. No significant temporal correlation was found between simultaneous plasma GHRH and GH (Fig. 2a), nor between plasma GHRH 20 min (Fig. 2b) or 40 min

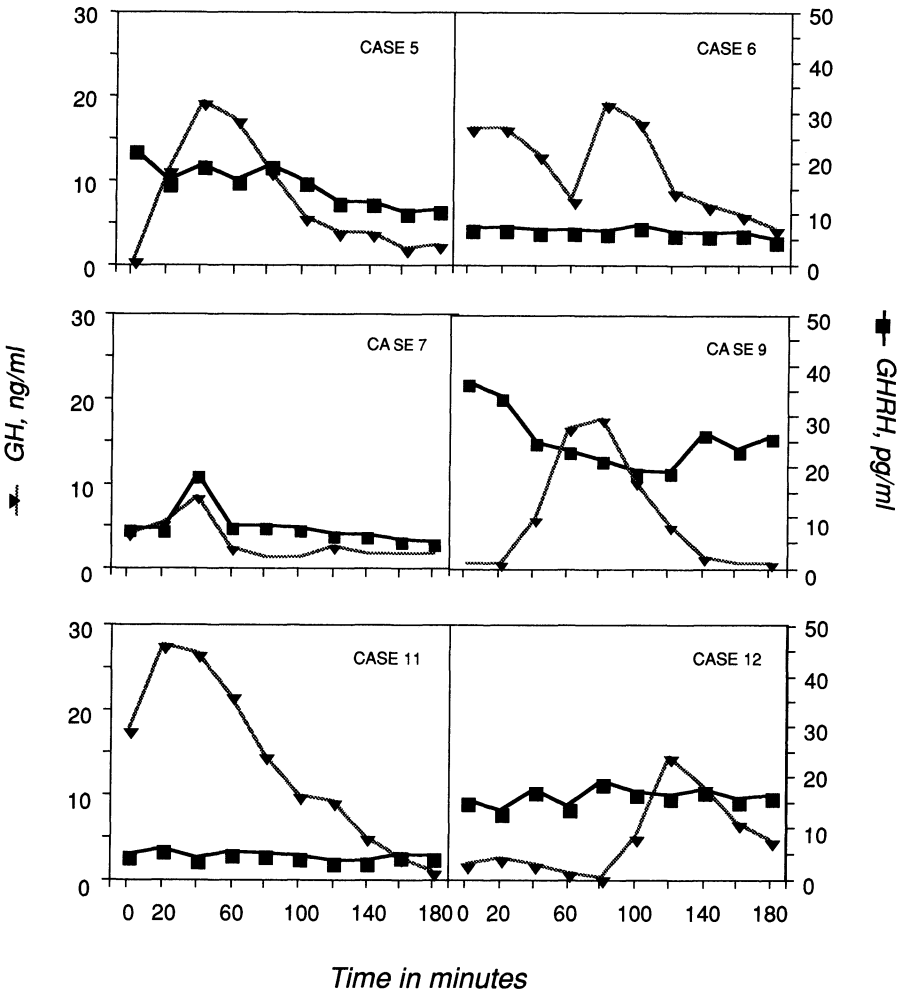

Fig. 1. Plasma GH and GHRH concentrations during the sleep test in representative NS subjects (cases 5 and 6), GHD-A subjects (cases 7 and 9) and GHD-B subjects (case 11 and 12). Closed triangles and rectangles indicate GH and GHRH, respectively.

prior to peak GH and plasma GH levels (Fig. 2c). There was no significant difference in mean plasma GHRH among three groups (NS 11.8 ± 1.4 pg/ml, GHD-A 18.4 ± 6.8 pg/ml, GHD-B 9.4 ± 2.0 pg/ml, P=0.314) (Table 1).

Discussion

In the present study, confirming the sleep-associated GH secretion in NS and GHD-B, but not in GHD-A, we found that plasma GHRH showed little change during the sleep test in any of the three groups. Since there is a 15 to 30 min-delay in GH response to GHRH [13], we tried to find any temporal correlation between plasma GHRH and GH at various times, i.e., the GH concentration at the same time, 20 min or 40 min after GHRH during the sleep test. There were no significant temporal correlation between GHRH and GH. We further demonstrated that mean plasma GHRH concentrations during the sleep test were similar in these groups.

Hypothalamic GHRH mediates the sleep-associated GH release in man. Repetive or continuous infusion of a specific GHRH antagonist suppresses GH release induced by various pharmacological as well as physiological stimulations [5, 6]. If GHRH concentrations in the peripheral blood were a reflection of hypothalamic

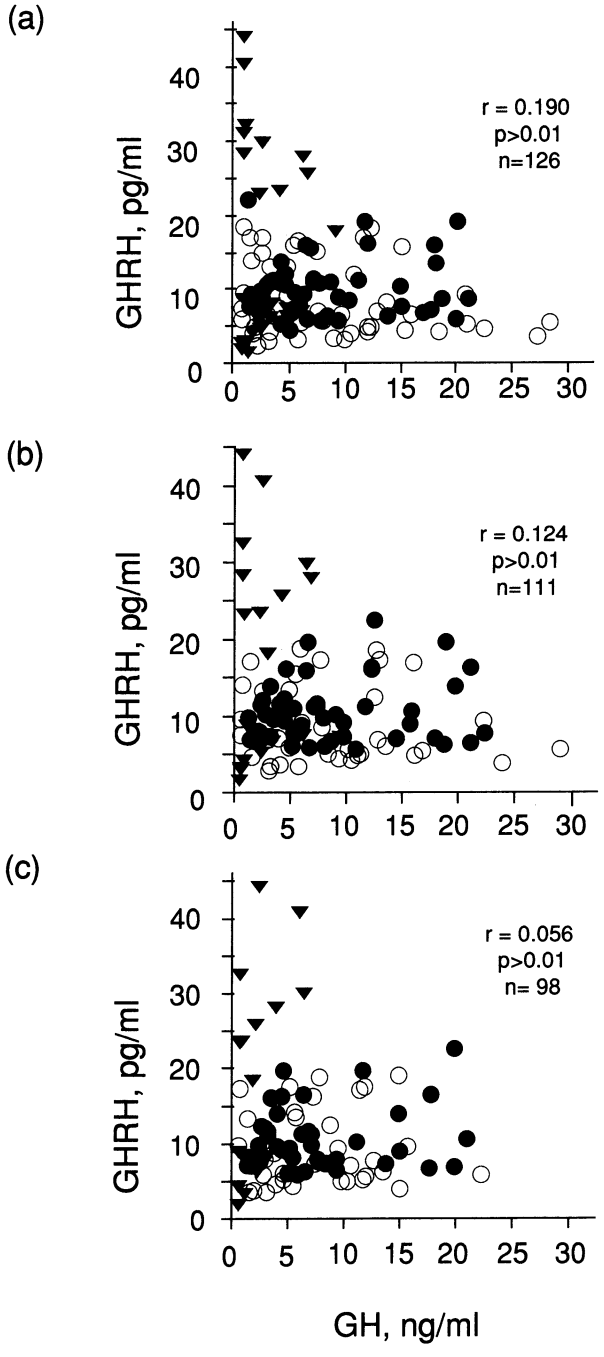

Fig. 2. Temporal correlation between plasma GHRH and GH concentrations during the sleep test. Plasma GH was compared to plasma GHRH at the same time (a), at 20 min (b) or 40 min (c) after the GHRH concentrations in each subject in three groups. Closed circles, closed triangles and open circles indicate individual GH and GHRH levels obtained from the NS, GHD-A and GHD-B groups, respectively.

GHRH neuronal activity, there should have been a difference between normal and GH-deficient children in mean plasma GHRH. The present finding of a lack of a temporal correlation between plasma GH and GHRH in the peripheral blood GHRH during the sleep test is distinct from the previous reports in adults by Saito *et al.* [9] and in children by Ohyama *et al.* [10]. One possible explanation for the difference may be due to the method for measuring plasma GHRH. They measured plasma GHRH levels by RIA combined with a rather unreliable extraction method.

Interestingly there were fluctuations in the plasma GHRH concentration in some patients. One plasma GHRH peak coincided with an increase in plasma GH. The mechanism which underlies these spontaneous changes in plasma GHRH concentrations remains to be elucidated. GHRH is also produced by the gastrointestinal tract, and is released into the circulation after meals [14]. Although the sleep test started at least 3 h after dinner, it is interesting to speculate a possible relationship between gastrointestinal movement and the plasma GHRH concentration during the sleep test. It is supposed that probably the GHRH in the peripheral plasma we measured in this study mostly originates in the gastrointestinal tract.

In conclusion, dynamic secretion of peripheral plasma GHRH does not reflect the sleep-associated GH secretion in children of short stature. It seems difficult to monitor hypothalamic GHRH function by measuring of peripheral plasma GHRH.

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