Growth Hormone Immunoreactivity Does Not Reflect Bioactivity

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ABSTRACT

In childhood, the largest secretory burst of GH occurs during nighttime, and consists of a complex mixture of molecular forms of GH that are thought to have different biologic activity. Standard GH assays cannot distinguish between bioactive and biologically inactive GH isoforms. To examine this relationship, overnight GH secretion was assessed by blood sampling every 30 min in 10 short prepubertal children (7 boys and 3 girls) to evaluate both the serum concentration and the biologic activity of GH. Serum GH concentrations were measured by an immunofluorometric assay and its biologic activity by the Nb2 cell bioassay. The 12-h (2000 h to 0800 h) and 6-h (2000 h to 0200 h and 0200 h to 0800 h) GH profiles were analyzed using the Pulsar program. When GH secretory pattern was measured by immunofluorometric assay, the area under the curve above the 0 line, the mean GH concentration, and the mean height of the secretory peaks were significantly higher during the first than during the second part of the night (29.17 ± 5.93 versus 16.29 ± 1.87 mIU/L, $p < 0.05$; 7.77 ± 1.28 versus 4.83 ± 0.33 mIU/L, $p < 0.05$; 4.61 ± 0.94 versus 2.68 ± 0.27 mIU/L, $p < 0.05$,

respectively). In contrast, GH biologic activity was not significantly different during the two parts of the night. In conclusion, a dissociation between GH bioactivity and immunoreactivity is present in physiologic conditions, indicating that standard GH measurements do not provide any information on the biologic activity of the hormone. Although GH secretion is regulated by complex neuroendocrine mechanisms, the biologic activity of the hormone seems to be independent of them and is most probably regulated by peripheral mechanisms acting on its clearance or bioavailability to the target tissues. (*Pediatr Res* 48: 619–622, 2000)

Abbreviations

IGFBP-3, IGF binding protein 3

IFMA, immunofluorometric assay

Nb2, Nb2 cell bioassay

hGH, human GH

AUCb, area under the curve above baseline

AUCo, area under the curve above 0 line

GH bioactivity in serum is dependent on many factors, including GH isoforms and binding proteins. At least four different isoforms of GH (27 kD, 20 kD, 17 kD, and 5 kD), one or more acidic peptides, and also monomeric, dimeric, and pentameric GH aggregates of different biopotency have been identified. Furthermore, two major GH binding proteins modulate significantly GH availability to target tissues (1). The essential information that one wishes to gain by GH blood sampling is the amount of biologically active, functional GH in the circulation. However, analytical results provide a quantitation of immunoreactive GH molecular forms in the serum, to which fragments, variants, and aggregates also contribute to a variable extent depending on the specificity of the antibodies used in the assay.

GH secretion is pulsatile and regulated mainly by two hypothalamic regulatory hormones, GH releasing hormone and somatostatin. This intermittent hormone release is of physiologic importance as indicated by the asymptotic relation between the amplitude of GH pulses and growth velocity (2). Furthermore, the rate of increase of serum GH concentration during the upstroke of a GH pulse appears to be an important factor in determining the response of target organs (3). For example, in skeletal muscle in experimental conditions, pulsatile GH increases IGF-I mRNA more effectively than continuous GH infusion (4). The aim of the present study was to assess whether pulsatility is a common feature of both spontaneous immunoreactive GH secretion and GH bioactivity, and whether there is a relationship between GH bioactivity and immunoreactivity. Therefore, we analyzed the pulsatile properties of the 12-h nighttime immunoreactive GH concentrations in a group of prepubertal children affected by growth delay, and compared them with those of the biologically active hormone as determined by the Nb2 cell bioassay.

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METHODS

Subjects

Ten prepubertal children (7 boys and 3 girls) were investigated because of short stature or decreased growth velocity. At the time of the investigation, chronological age was 8.2 ± 3.1 y; bone age, 6.9 ± 3.2 y; height SD score, -1.9 ± 0.7 ; and body mass index SD score, -0.7 ± 1.4 . Bone age was assessed by the method of Greulich and Pyle (5), height was expressed as SD score (6), and weight as body mass index SD score (7). All subjects underwent standard GH stimulation tests (arginine and insulin tolerance tests) before the study, which showed a normal GH response (peak > 10 ng/mL). IGF-I serum concentrations were 34.6 ± 10.9 nM and those of IGFBP3 were 66.8 ± 21.39 nM, both not statistically different from those seen in 24 prepubertal age-matched children, in whom IGF-I was 25.9 ± 18.0 nM and IGFBP3, 63.2 ± 22.4 nM. Organic diseases, chromosomal aberrations, and other endocrine dysfunctions were ruled out. Thus, the subjects studied were considered to be affected by constitutional growth retardation. The study was approved by the Clinical Research Committee of the University of Pavia.

Study Protocol

Blood samples for the evaluation of GH were obtained every 30 min, between 2000 h and 0800 h via an indwelling non-thrombogenic catheter inserted into an antecubital vein. After centrifugation, serum samples were stored at -20°C until assayed.

Arbitrarily, the nighttime values were divided into two periods (2000 h to 0200 h and 0200 h to 0800 h) that were studied separately. The study was performed within 2 mo from the GH pharmacologic stimulation tests.

Serum GH Concentrations

By IFMA. Serum GH values were measured using a time-resolved IFMA (Delfia; LKB-Wallac, Turku, Finland) based on the direct sandwiching technique in which two MAb are directed against two separate, but unspecified, antigenic determinants on the hGH molecule. The IFMA is specific (detection limit 0.03 mIU/L) for the 22-kD form of hGH and has a low cross-reactivity with other GH molecular variants or pituitary hormones. The standards provided in the kits were calibrated against the First International Reference preparation of hGH 80/505, in which 1 mg = 2.6 IU of hGH. The intra- and interassay coefficients of variation were 2.1 to 5.0% and 4.2 to 6.3%, respectively (8, 9).

By Nb2 cell bioassay. The bioassay was performed according to the method of Tanaka *et al.* (10), modified by Walker *et al.* (11) with further minor changes, as previously described (12). The intra- and interassay coefficients of variation were 6% and 7.6%, respectively. The detection limit was 0.1 ng/mL.

IGF-I Measurement

IGF-1 was measured by double-antibody RIA using immunochemicals and tracer provided by Medgenix (Fleurus, Bel-

gium). The sensitivity of the assay was 150 pg/mL; the intra- and inter-assay coefficients of variation were 6% and 7.5%, respectively. To avoid interference from binding proteins, samples were treated with acid ethanol.

IGFBP-3 Measurement

IGFBP-3 was measured by immunoassay using reagents and tracers provided by Bioclone Australia Pty Ltd. (Narrickville, NSW, Australia). The sensitivity of the assay was 3.5 ng/mL; the intra- and interassay coefficients of variation were 4.25% and 6.6%, respectively.

Pulse Analysis

The Pulsar program was used to quantify the pulse properties of the GH time series (13). The samples were analyzed for mean 12- and 6-h serum hormone concentrations, AUCb, AUCo, number of significant pulses, mean pulse height, amplitude, area, length, and interpulse interval. The cut-off parameters G1–5 were set at 5, 3, 2, 1.5, and 1 times the intraassay SD as criteria for accepting peaks 1, 2, 3, 4, and 5 points wide, respectively. The smoothing time was set at half the total profile time, that is 6-h (12 points), and 3-h (6 points).

Statistical Analysis

Values are reported as mean $\pm$ SEM. Statistical significance was assessed by *t* test or Wilcoxon signed rank test, as appropriate.

RESULTS

GH secretion assessed by IFMA and GH bioactivity assessed by Nb2 assay were both found to be pulsatile (Fig. 1). Nighttime 6-h mean AUCb, AUCo, number of pulses, amplitude, height, length, area, interpulse interval, frequency, and mean concentration of GH assessed by both methods are

Figure 1. Twelve-hour immunoreactive (IFMA GH, dark gray area) and bioactive (biologic GH, light gray area) GH concentrations. The solid line represents the median of immunoreactive GH and the dotted line of the bioactive GH. The shaded areas delineate the lower and upper quartiles.

Table 1. Properties of overnight pulsatile GH measured by an IFMA and by the Nb2 cell bioassay

Property	GH IFMA (mIU/L)		GH Biological Activity (ng/mL)	
	2000–0200 h	200–0800 h	2000–0200 h	0200–0800 h
AUCb	9.67 ± 2.17	12.29 ± 2.01	1.11 ± 0.52	1.44 ± 0.34
AUCo	29.17 ± 5.93	16.29 ± 1.87*	7.37 ± 2.15	8.22 ± 1.76
Number of Pulses	3.14 ± 0.14	3.57 ± 0.20	3.00 ± 0.25	3.83 ± 0.60
Pulse amplitude	4.34 ± 0.72	4.62 ± 0.87	0.52 ± 0.16	0.64 ± 0.09
Pulse height	7.77 ± 0.1.28	4.83 ± 0.33*	1.61 ± 0.46	1.69 ± 0.32
Pulse length (hr)	1.11 ± 0.09	1.02 ± 0.07	0.94 ± 0.09	0.92 ± 0.12
Pulse area (IU or ng/ml.min)	2.68 ± 0.49	2.83 ± 0.52	0.31 ± 0.13	0.28 ± 0.05
Interpulse interval (hr)	2.25 ± 0.17	1.89 ± 0.09	1.76 ± 0.23	2.07 ± 0.36
Frequency (hr)	0.57 ± 0.02	0.62 ± 0.02	0.56 ± 0.06	0.64 ± 0.09
Mean	4.61 ± 0.94	2.68 ± 0.27*	1.22 ± 0.35	1.36 ± 0.29

* $p < 0.05$, 0200–0800 h vs 2000–0200 h.

reported in Table 1. AUCb, AUCo, and mean concentration of bioactive GH were higher during the second half of the night compared with the first half, although the difference did not reach statistical significance. The mean number, amplitude, height, and length of pulses as well as the mean pulse area and frequency remained constant throughout the nighttime period.

Mean GH AUCo, height of the secretory pulses, and mean GH concentration assessed by IFMA were significantly higher during the first 6 h, in comparison with the values obtained in the following period (29.17 ± 5.93 versus 16.29 ± 1.87 mIU/L, $p < 0.05$; 4.61 ± 0.94 versus 2.68 ± 0.27 mIU/L, $p < 0.05$; 7.77 ± 1.28 versus 4.83 ± 0.33 mIU/L, $p < 0.05$, respectively). The mean number, amplitude, length, area, and frequency of secretory pulses remained constant throughout the night.

The GH IFMA/GH Nb2 ratio was significantly higher during the first 6 h of the night, in comparison with the values calculated for the following hours (4.1 ± 0.51 versus 2.57 ± 0.33 , $p < 0.05$; Fig. 2).

DISCUSSION

The results of the present study indicate that the pattern of GH secretion assessed by an IFMA is different from that

obtained by measuring the hormone biologic activity. During the initial hours of sleep, GH secretion measured by an IFMA showed a significant surge, whereas biologic activity was slightly increased during the second half of the night without showing, however, any significant modification throughout the entire period of observation. Thus, GH biologic activity remained constant throughout the night, whereas GH immunoreactivity exhibited a significant variation during the study period. A dissociation between GH bioactivity and immunoreactivity has previously been reported after pharmacologic stimulation. In one study, acute stimulation of GH secretion was accompanied by greater rises in bioactivity than immunoreactivity (14). In another report, after arginine and insulin stimulation, the responses of GH measured by RIA were 8-fold greater than those measured by bioassay, suggesting that the GH isoforms released by pharmacologic stimuli are not highly bioactive (15). Moreover, also in Turner syndrome, GH bioactivity has been described to be lower than that expected for immunoreactivity (16). To our knowledge, this is the first report of a dissociation between GH bioactivity and immunoreactivity measured in overnight samples and in physiologic conditions.

The IFMA assay recognizes mostly the 22-kD form in its bound and unbound form (*i.e.* total circulating GH), whereas the Nb2 cell assay evaluates GH bioactivity at the target tissue level, *i.e.* the effect of the different GH isoforms, modulated by the binding proteins. A limitation of this assay is that it measures lactogenic, and not somatogenic GH bioactivity. However, lactogenic GH bioactivity, evaluated after s.c. GH administration, shows a good correlation with the growth response during the first year of GH therapy (17), and thus can be used as an indirect measurement of somatogenic activity.

The dissociation between GH bioactivity and immunoreactivity might be interpreted as the result of a different clearance of the various GH molecules. During the period of maximal immunoreactive GH surge, observed during the first part of the night, the 22-kD form could be cleared more rapidly from the circulation than the 20-kD form (18) or other oligomers (19, 20), allowing the 20-kD form to circulate for a longer period of time. The latter, because of its long half-life (18), was shown to have a good growth-promoting activity in the rat (21) despite a decreased affinity for GH receptors (22). The 20-kD isoform is recognized by the Nb2 cell assay (23), and thus could account for the sustained GH bioactivity after the IFMA GH

Figure 2. Twelve-hour immunoreactive (IFMA GH, dark gray area) and bioactive (biologic GH, light gray area) GH concentrations. The solid line represents the median of IFMA GH/biologic GH ratio computed at each time point. The shaded areas delineate the lower and upper quartiles.

surge. However, it has recently been shown that the percentage of the 20-kD isoform does not change after pharmacologic stimulation or during nocturnal sleep (24).

Another possible explanation of our results is that the two assays might recognize different dimers of GH (25). It has been shown, in fact, that an acidic 20-kD/22-kD heterodimer of GH is 2.5 times more potent than the 22-kD molecule alone in stimulating Nb2 cells, although these two variants show a similar immunoreactivity (26).

Alternatively, during the first part of the night there might be an increment of both the GH bound to binding proteins and its free form, which are both recognized by the two assays. Later on, when the IFMA GH troughs are observed, the dissociation of GH from the binding proteins could maintain a relatively constant level of free GH that, in turn, maintains GH bioactivity constant. GH dissociation, in fact, was shown to maintain high levels of GH delivered to target tissues during secretory troughs (1).

In conclusion, a dissociation between GH bioactivity and immunoreactivity is present in physiologic conditions, indicating that standard GH measurements do not provide any information on the interaction between the hormone and its receptors in target tissues and on the effect of the different GH isoforms. This dissociation would explain why some short children grow poorly despite the presence of normal immunoreactive GH levels, and why in tall children IGF-I levels are higher than in normal-height peers (27) in the absence of high GH levels.

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