0021-972X/98/$03.00/0
Journal of Clinical Endocrinology and Metabolism
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Vol. 83, No. 7
Printed in U.S.A.

Effects of Eight Months Treatment with Graded Doses of a Growth Hormone (GH)-Releasing Peptide in GH-Deficient Children*

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ABSTRACT

Stimulation of pituitary GH secretion with administered GHRH can be effective therapy for those GH deficient (GHD) patients whose disorder results from insufficient endogenous GHRH secretion. We have previously shown that most such patients also respond acutely to the GH-releasing peptides (GHRP's), which have a different mechanism of action from GHRH, with release of GH. In this study we tested whether the GH response to a newer GHRP, GHRP-2, would be sustained over time. Six prepubertal children with GHD and growth failure received stepwise increasing sc doses of GHRP-2, at 0.3, 1.0, and 3.0 μg/kg/day, in successive 2-month treatment periods, with monitoring of overnight 12 h episodic GH secretion and toxicity measures at the end of each period. During a fourth 2-month period, they received 3 μg/kg GHRP-2 together with 3 μg/kg sc GHRH. Serum levels of IGF-I and IGFBP-3 were also measured, and stadiometer height measurements were recorded. GHRP-2 administration produced a dosewise increase in overnight GH secretion. GH profiles showed that the effect of GHRP-2 injections was relatively brief, with little effect upon GH secretion later in the night. Serum levels of IGF-I and of IGFBP-3 did not increase. Growth velocity was higher during GHRP-2 treatment than during pretreatment and post-treatment evaluations. There were no side effects or toxicities observed. Thus GHRP-2 is well tolerated and is able to stimulate GH secretion. Formulations or routes of administration that allow for a longer duration of action will likely be needed to use GHRP-2 in therapy. (*J Clin Endocrinol Metab* **83:** 2355–2360, 1998)

GROWTH hormone (GH) deficiency can arise from a variety of causes, but many patients with idiopathic isolated GH deficiency (GHD) have a deficiency of hypothalamic GH-releasing hormone (GHRH) or interruption of the flow of GHRH to the pituitary (1, 2), rather than an instrinsic pituitary defect, and are capable of responding to administered synthetic GHRH with a rise in GH (3, 4). The response may initially be low or undetectable due to chronic GHRH deficiency and somatotroph atrophy, but it usually rises after repeated GHRH stimulation (5). This has led to the suggestion that GHRH could be used as the basis for an alternative treatment of GHD, and a number of clinical trials have demonstrated that repeated administration of GHRH can accelerate the growth of GHD children (6–11). A recent large series found a growth response in 74% of 80 patients after 6 months of GHRH treatment, although the magnitude of the growth response was less than that seen with conventional doses of GH (12).

Compared with GH, treatment with GH secretagogues has several potential advantages. The moderating effect of insulin-like growth factor-I (IGF-I) feedback on the somatotroph could buffer against overtreatment. Unlike administered GH, GH secretion remains pulsatile during GHRH administration, presumably because of variability in endogenous secretion of somatostatin, although current GHRH treatment regimens do not take full advantage of this phenomenon because of the relatively short duration of action of sc GHRH injections.

The GH-releasing peptides (GHRP's) are another category of GH secretagogues that offer some of the same potential advantages but also have unique features that differ from GHRH. GHRP's are small synthetic peptides originally derived from enkephalin, which have no residual opioidergic effects, but are relatively specific stimulators of GH secretion *in vitro* and *in vivo* (13–15). GHRP's have no structural similarity to GHRH; they bind to entirely different receptors and exhibit a strong synergy with GHRH in the release of GH (16, 17). It is believed that they may be analogs of a still unidentified endogenous ligand, and they appear to act at both hypothalamic and pituitary sites (18). The small size and the presence of D amino acids in their structure, which makes them resistant to peptidases, allow them to be active by oral administration. Since the description of the original hexapep-

Received January 16, 1998. Revision received March 30, 1998. Accepted April 8, 1998.

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* This work was supported by FONDECYT Grant 1940543, Tulane University, the Beinecke Foundation, and the Department of Veterans Affairs. This work was presented in part at the 77th Annual Meeting of The Endocrine Society, Washington, DC, 1995; at the IX Latin American Pediatric Endocrine Society meeting, Ica, Perú, 1995; and at the meeting of the Western Society for Clinical Investigation, Carmel, California, 1996.

tide GHRP by Bowers *et al.*(19), several other GHRP's with higher potency have been described, and Patchett *et al.* (20) have described nonpeptidyl compounds that act in a similar manner.

These features make GHRP's another very attractive potential treatment for GH deficiency. Because they may act in part at hypothalamic sites, and some hypothalamic responses are impaired in most patients with GHD, it was unclear whether GHRP's would be as effective in GHD as in normal subjects or in aging subjects. To clarify this, we tested the responses of 22 prepubertal children with GHD to the acute iv administration of the second-generation peptide GHRP-1, to GHRH (1–44) NH2, and to the two secretagogues together (21). We found that GHRP-1 stimulated a significant rise of GH in the majority of these patients.

Because acute administration of GHRP stimulates an acute rise in GH, repeated administration of a GHRP could in principle stimulate a repeated rise in GH, with the potential for increased growth. However, short-term studies have shown that, when GHRP is administered repeatedly, the GH response diminishes markedly, and it is not clear whether a meaningful rise in GH would be sustained over extended periods of time. In this study, we examined the effects of chronic sc administration of a third-generation GHRP, GHRP-2, on GH secretion in a group of prepubertal GHD children. Because there was no previous experience with the safety or dose-response characteristics of this material, an open-label escalating dose protocol was used, with three successive 2-month treatment blocks. In a final 2-month treatment period, the combination of GHRP-2 with GHRH was administered together. Growth measurements were also recorded before and during treatment.

Subjects and Methods

Six previously untreated prepubertal children (four boys and two girls) with GHD participated in the protocol. For the purposes of this study, GHD was defined by GH responses of less than 7 μg/L to at least two standard provocative tests, and a growth velocity of 4 cm/yr or less over at least the preceding 6 months (22). Some of our patients had normal serum IGF-I and IGFBP3 concentrations, so they do not appear to have classical GHD. Clinical features of the patients are shown in Table 1. Three of the subjects (numbers 3, 5, and 6) had participated in our earlier study of acute GH responses to single doses of GHRP-1 (21); their enrollment was based upon availability and not upon their acute GH responses. The study protocol was reviewed and approved by the University of Chile institutional review board; children gave written assent, and informed consent was obtained from parents.

GHRP-2 (D Ala-D Nal-Ala-Trp-D Phe-Lys-NH2) was synthesized by Kaken Research Laboratories (Kyoto, Japan) and distributed to patients' families as a sterile solution. GHRH (1–29) NH2 (Geref (R)) was obtained from Serono Laboratories. Parents were instructed in sc injection technique using insulin syringes. After baseline assessment, patients were treated for successive 2-month periods with daily bedtime injections of GHRP-2 in doses of 0.3, 1.0, and 3.0 μg/kg. During a final 2-month period, patients received both 3.0 μg/kg GHRP-2 and 3.0 μg/kg GHRH at separate injection sites. Patients and parents were instructed to report any side effects and remained in frequent contact with study personnel.

Before treatment and at the end of each treatment period, patients were admitted to the clinical study unit at the University of Chile for overnight sampling for GH (q 20 min $\times$ 12 h). Samples were drawn fasting in the morning for plasma levels of IGF-I and IGFBP-3, and the following toxicity measures: white blood cell count, hemoglobin, total protein, albumin, creatinine, calcium, phosphate, SGOT, SGPT, alkaline phosphatase, cholesterol, triglycerides, T4, TSH, and cortisol, using standard assay procedures. Patients were weighed, and the average of 10 stadiometer heights was recorded at a standard time of day. This measurement has a precision (SD) of 0.3 mm. Bone ages were read blinded from hand films obtained before and at the end of the eight months of treatment, using the standards of Greulich and Pyle.

Serum GH concentrations were measured by a double-antibody RIA (Diagnostic Product Corp., Los Angeles, CA) with an intraassay coefficient of variation of 6%, an interassay variation of 9% at a measured level of 10 μg/L, and a least detectable concentration of 0.8 μg/L. All samples from each series were run in a single batch, except that values over 30 μg/L were reassayed after dilution. IGF-I was measured by RIA after acid-ethanol extraction, using a reference standard purchased from Bachem (Torrance, CA) and an antiserum (NIH UB2–495) donated by the National Hormone and Pituitary Program (Rockville, MD). This assay has an intrassay coefficient of variation of 7.5% and an interassay coefficient of variation of 11.1% at measured level of 160 ng/mL, and a lowest detectable concentration (95% B/B0) of 3 ng/mL. IGFBP-3 was measured by IRMA using reagents purchased from DSL (Webster, Texas); the assay has an intrassay coefficient of variation of 1.9% and an interassay coefficient of variation of 3.2% at a measured level of 2.2 mg/L, and a detection limit of 0.05 mg/L. All samples for IGF-I and IGFBP-3 from a single patient were run together in the same assay batch.

The significance of changes in response variables was assessed with analysis of variance followed by paired *t*-testing. Characteristics of overnight episodic GH secretion were assessed using the Pulsar algorithm (23).

Results

After 2 months on each dose level, GH rose acutely in response to the GHRP-2 injections, and the magnitude of this immediate response was dose-related. An example of overnight pulsatile GH secretion is shown in Figs. 1 and 2, and responses for all subjects are shown in Fig. 3. Episodic GH secretion during the remainder of the night was generally unaffected by bedtime GHRP-2. The combination of GHRP-2 and GHRH during months 6–8 evoked a significant acute GH response (Fig. 2), but even this large effect was of relatively short duration. Thus in the aggregate, the maximal overnight GH and GH peak amplitude showed a progressive

TABLE 1. Clinical characteristics and hormonal data of the patients studied

Patient No./Sex	Age, yr	Bone Age, yr	Height SDS	Pre-Rx growth velocity, cm/yr	BMI	IGF-I μg/L[a]	IGFBP-3 mg/L[b]	Peak GH, insulin, μg/L	Peak GH, clonidine, μg/L	Peak GH, GHRP, μg/L	Peak GH GHRH, μg/L	Head CT
1/F	12.4	7.8	−5.7	3.0	18.3	46.0	2.1	2.4	5.1	13.0	ND	N
2/M	6.2	4.5	−3.8	2.8	17.0	<3.0	0.8	1.8	3.7	6.6	ND	N
3/M	9.6	7.5	−1.4	4.0	16.5	158.0	1.6	4.8	4.5	27.0	27.8	Empty sella
4/M	9.8	7.0	−2.4	2.5	16.0	201.0	2.2	5.3	5.4	4.4	ND	N
5/M	14.3	9.5	−3.9	3.0	19.0	167.0	1.5	2.3	3.6	13.0	10.5	N
6/F	12.2	10.5	−3.5	3.0	20.0	172.0	1.7	2.4	4.4	5.6	9.9	N

SDS, height standard desviation scores relative to NCHS tables; BMI, body mass index; ND, test not done; N, normal.
[a] Normal IGF-I range: boys 83–239 (μg/L), girls 91–315.
[b] Normal IGFBP3 range: boys 1.5–3.2 (mg/L), girls 1.9–3.0.

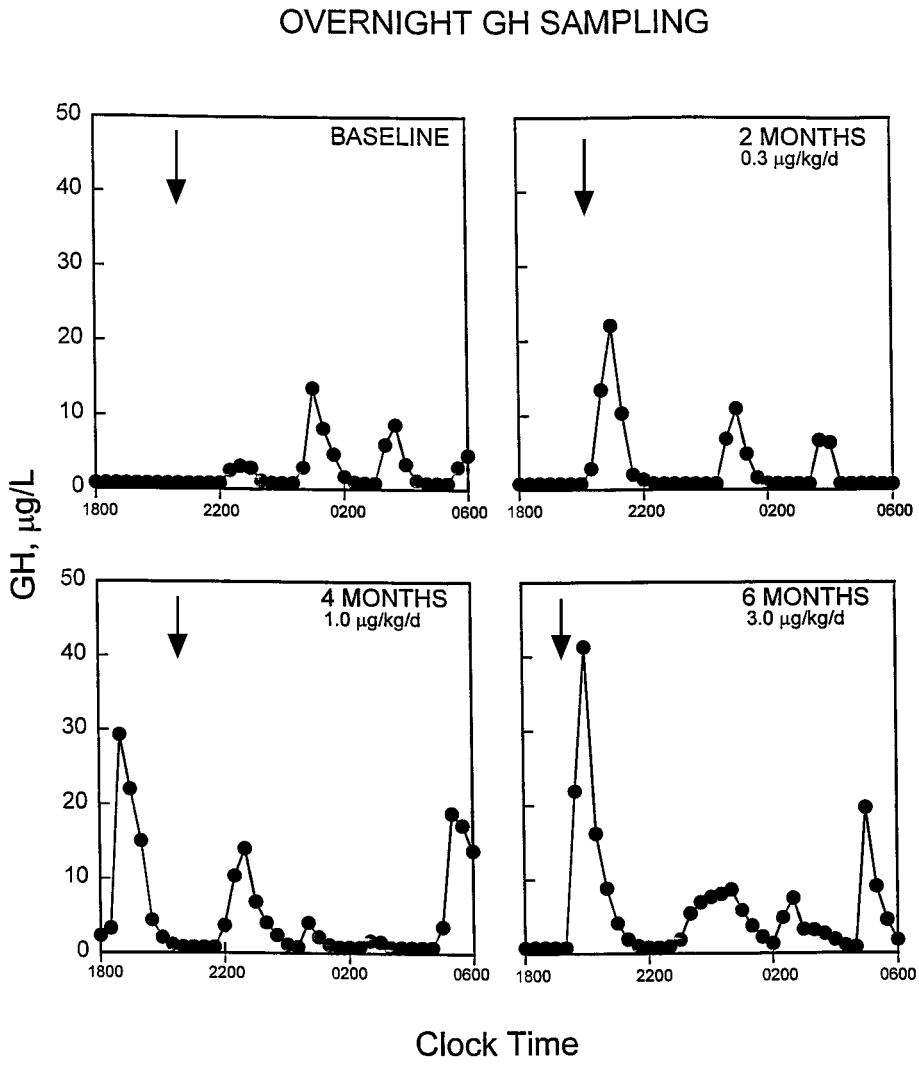

FIG. 1. Nocturnal GH patterns before and during treatment with sc injections of graded doses of GHRP-2 in one of the study subjects (patient 4). Injections were given shortly after the beginning of sampling, at times shown by the *arrows*.

increase at the successively higher doses and a further increment with combined treatment, but the number of nocturnal GH peaks was unaffected (Fig. 3).

Treatment with GHRP-2 was well tolerated by all subjects. There were no significant changes in hematologic or chemical toxicity measures at any of the doses tested, or with the combination of GHRH and GHRP-2 (data not shown). Serum levels of IGF-I and of IGFBP-3 did not increase even at the highest treatment doses (Fig. 4).

Growth velocities were higher during the 6 months of GHRP-2 treatment than during the baseline observation period (5.3 ± 0.8 *vs.* 3.0 ± 0.5 cm/yr, $P < 0.05$), or during the 6 months following discontinuation of treatment (3.3 ± 0.4 cm/yr). Growth velocities during the 2-month period of combined GHRH/GHRP-2 treatment were also increased over baseline, and comparable to those of GHRP-2 alone (5.8 ± 2.3 cm/yr); but the accuracy of measurements over this brief interval is limited. There were no differences in the growth velocities during the individual treatment periods. Bone age did not advance more rapidly than chronologic age, increasing an average of 6 ± 6 (SD) months over the 8 months of treatment.

Discussion

These results show that the repeated administration of GHRP-2 is able to produce a rise in nocturnal GH that is sustained after several months of treatment. The effect of each sc injection of GHRP on GH secretion is relatively brief, despite the resistance of the GHRP's to proteolysis (13, 21). In our study, this short duration of action persisted even after 6 months of treatment, with an acute rise in GH lasting less than 2 h. In subjects with measurable endogenous GH secretion, the bedtime injections appeared to have no effect upon episodic GH secretion later in the night (Fig. 1). This contrasts with the effects of GHRP infusions, which can enhance overall pulsatile GH secretion, but are similar to the effects of bedtime sc GHRH treatment (13, 25).

A dose-response relationship is seen in the effects of GHRP-2 upon GH peak amplitude (Fig. 3), which primarily reflects the acute response to the GHRP-2 injections. There is a trend in the overnight mean GH responses, but these changes were not significant for the group as a whole, perhaps because of the brief duration of the GHRP-2 action.

Safety considerations mandated the escalating-dose de-

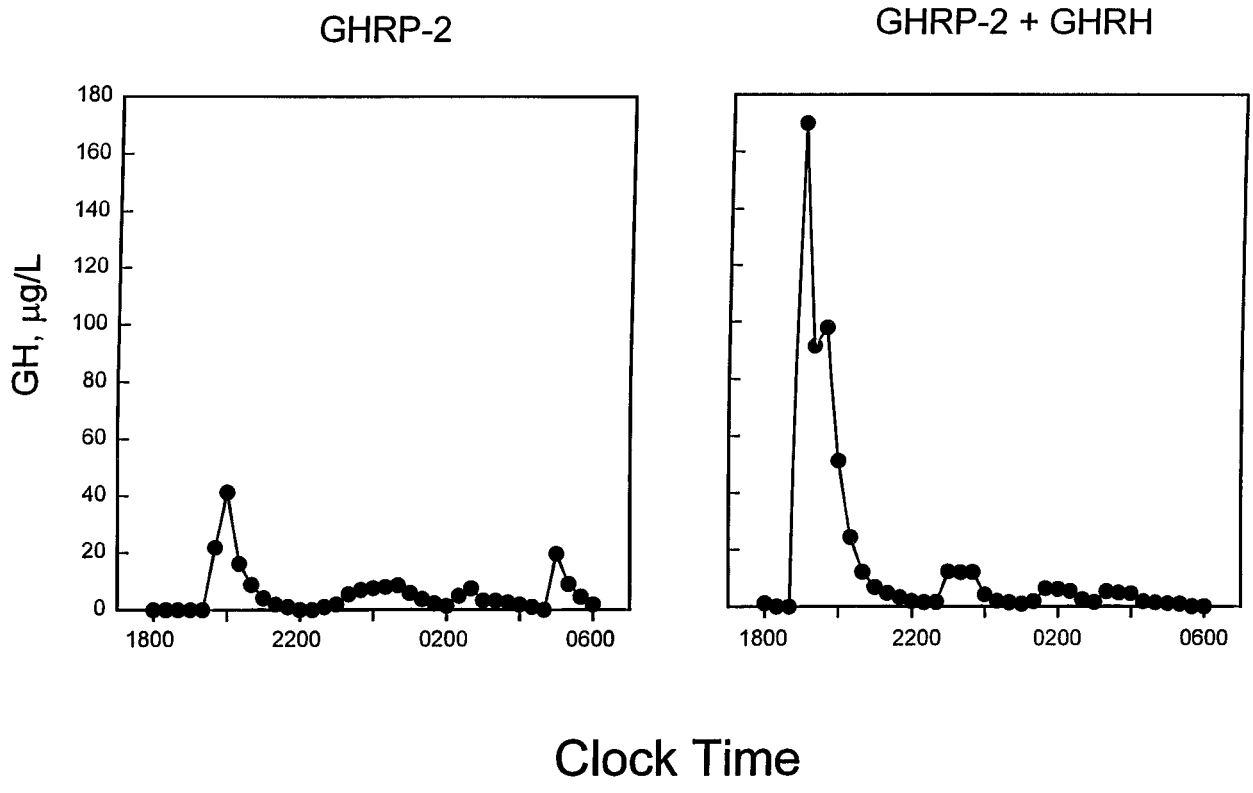

FIG. 2. Nocturnal GH patterns during treatment with sc injections of GHRP-2, 3 μg/kg sc, or with the combination of 3 μg/kg each of GHRP-2 and GHRH(1–29)NH2 in one of the study subjects (patient 4). The mean peak GH response to this dose of GHRH given alone is approximately 15 μg/L; thus there is a much greater than additive response to combined treatment.

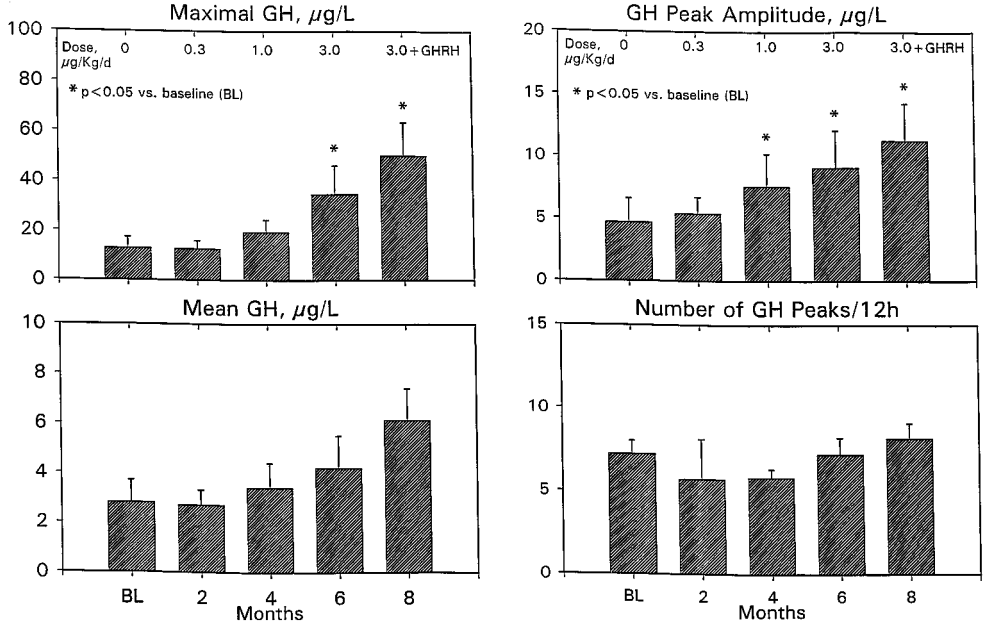

FIG. 3. Characteristics of nocturnal GH secretion during sc injections of increasing doses of GHRP-2 or with the combination of GHRP-2 and GHRH.

sign of the study protocol, but this study design and the absence of a parallel placebo group limit the information that it provides on growth velocities. It is encouraging that growth velocities increased during treatment compared with before or after the treatment periods, but these results do not define an optimal GHRP-2 regimen. The return to a baseline growth rate after administering GHRP, however, may be caused by "catch down" growth and may not be indicative of a beneficial therapeutic effect.

Circulating levels of IGF-I and IGFBP-3 did not increase in our subjects. This result may have been caused by the timing of the sampling for growth factors. While individual effects might be blurred in the mean levels shown in Fig. 4, treatment effects were analyzed using paired statistics, and there

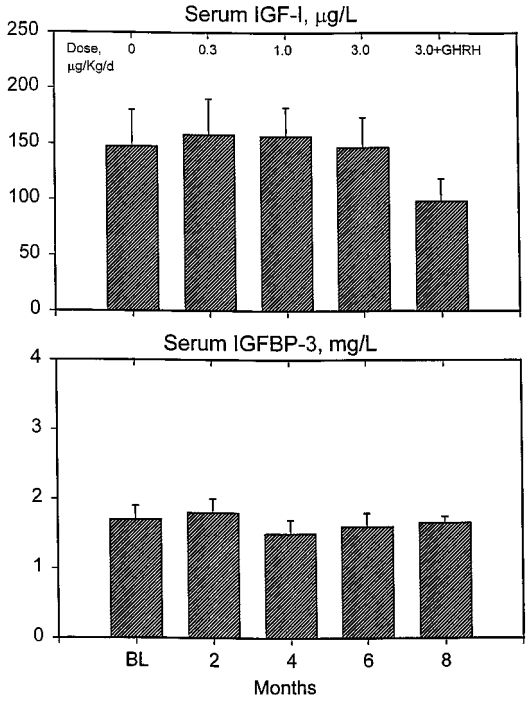

FIG. 4. Serum levels of IGF-I (+ SEM) and of IGFBP-3 during treatment with graded sc doses of GHRP-2 and the combination of GHRP-2 and GHRH(1–29)NH2.

were no significant differences. Although it might be argued that this is evidence for a qualitative difference in the mechanism of action of GHRP-2 upon growth, we believe that a more likely explanation is simply that the treatment effects observed here are submaximal. The annualized growth velocities observed are lower than the first 6 months' growth velocities obtained with standard doses of GH, and as noted the effect of each injection was relatively brief.

Laron *et al*. (27) reported that three times daily intranasal administration of a related GHRP both accelerated growth velocity and increased serum levels of IGF-I in a group of short children. While their subjects may not be directly comparable to our patients, their experience indicates that GHRP's in sufficient doses and frequency do act to stimulate IGF-I. Similar results have been reported by Pihoker *et al.* (28).

Even the highest dose of GHRP-2 that we used, 3 μg/kg, was well tolerated, and there were no toxicities observed. Whether antibody formation or allergic reactions will be a significant problem requires longer studies with larger numbers of subjects. Given evidence for submaximal effects, higher doses could safely be studied. However, duration of action may be as great a limitation on the efficacy of GHRP-2 as is dose; even the effect of combined GHRP-GHRH treatment, which induced a supraphysiological release of GH, was of relatively short duration.

In this protocol we administered GHRP-2 by sc injections, in part because of limited supplies and limited safety information for children by other routes at the time of the study, in part to allow direct comparison with our previous studies using sc GHRH. Among the potential advantages of GHRP-2 treatment is the possibility of oral administration, because of its small size and resistance to digestive proteolysis, although the doses employed are necessarily much higher. This is usually viewed as a matter of convenience and patient acceptance, but the results of our study suggest other potential advantages to that route. Given subcutaneously, GHRP-2 has only a brief effect and does not elevate pulsatile GH secretion through the night. A sustained-release formulation might have such an effect, but it is also possible that oral GHRP-2 will have a longer duration of action based upon the kinetics of absorption, and sustained-release oral preparations may be easier to formulate than long-acting injections.

The capacity of GHRH to markedly enhance the response to GHRP-2 suggests an approach to augment the effect of treatment to either agent. We have previously shown that an agent that enhances the acute GH response to GHRH can increase the growth velocity response to chronic GHRH treatment. The β1-adrenergic antagonist atenolol, which increases GH responses to GHRH, presumably by suppressing somatostatin, increased the first-year growth velocity response to treatment with sc bedtime GHRH (11). Studies of the effects of combined treatment with GHRH and GHRP would be of great interest in connection with studies of either agent given alone.

Acknowledgments

We thank Mr. Stephen Galt and Ms. Shannon Reed for assistance with statistical analysis and preparation of figures.

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