Preservation of Growth Hormone Secretion in Response to Growth Hormone–Releasing Peptide-2 During Prednisone Therapy

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Children who require long-term glucocorticoid treatment often demonstrate poor growth. Growth hormone (GH) secretion is decreased during glucocorticoid treatment, and this decrease may be due to a relative excess of the hypothalamic hormone somatostatin (SRIF). GH-releasing peptide-2 (GHRP-2) is a GH secretagogue that acts via multiple mechanisms at multiple sites. One of its proposed mechanisms is the ability to bypass SRIF blockade of GH secretion. We measured the ability of GHRP-2 to release GH before and during prednisone therapy (20 mg orally three times daily for 4 days). The degree of preservation of GH secretion and the pattern of GH release in response to GHRP-2 were compared with those observed in response to arginine, a known SRIF inhibitor. GH release in response to GHRP-2 and arginine was measured in the same eight subjects before and during prednisone therapy. Before prednisone, peak GH levels in response to arginine and GHRP-2 were 8.8 ± 2.8 and 80.8 ± 21.2 μg/L. During prednisone therapy, the peak GH level in response to arginine and to GHRP-2 was 20.1 ± 8.3 and 71.3 ± 18.4 μg/L, respectively. The difference in peak values before and after prednisone was not significant. The time to the peak GH level during prednisone therapy occurred sooner for both arginine and GHRP-2. The pattern of GH release to arginine and to GHRP-2 was not identical, and the mean area under the curve for GH release to GHRP-2 decreased significantly with steroid treatment ($P = .04$), suggesting that GHRP-2 acts by mechanisms additional to the removal of SRIF inhibition. GHRP-2 elicited a 10-fold greater GH response than arginine at baseline, and the GH response was threefold greater versus arginine even in the face of prednisone therapy. GH release occurred earlier for both arginine and GHRP-2 during steroid treatment. We propose that this may suggest an increased storage phenomenon due to the blockade of GH secretion by glucocorticoids and then a sudden release with SRIF inhibition. If GHRP-2 can indeed counteract the inhibitory effect of glucocorticoids on GH secretion, then a new form of therapy may be available to support growth in children who must receive long-term steroid treatment.

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SUBOPTIMAL GROWTH in children who receive long-term glucocorticoid therapy has long been a concern for physicians who prescribe these steroids as antiinflammatory agents. There are many mechanisms by which glucocorticoids have been shown to suppress growth, including effects at the hypothalamic-pituitary level and at the epiphyseal growth plate.[1] Growth effects vary with the different preparations of glucocorticoids, in steroid treatment at pharmacologic or physiologic doses, with the duration of therapy, and in studies performed on humans or other animals. In humans, alterations of hypothalamic hormone secretion and thereby pituitary growth hormone (GH) release have been attributed to glucocorticoid therapy. Kaufmann et al[2] reported decreased GH secretion in response to GH-releasing hormone (GHRH) in normal subjects who received a typical short course of antiinflammatory glucocorticoid therapy. This lack of response to GHRH during prednisone therapy was attributed to a relative somatostatin (SRIF) excess induced by the glucocorticoid therapy. Such an elevation in SRIF tone would limit the response of GH to most provocative agents, since synchrony between GHRH and SRIF is required for GH release.[3]

The GH-releasing peptides (GHRPs) are synthetic hexapeptides that are different from most other GH-releasing agents. Their mechanism of action does not appear to occur via simple stimulation of GHRH or inhibition of SRIF; instead, the action of GHRPs may involve both of these hypothalamic hormones and perhaps an unidentified hypothalamic factor that modulates hypothalamic-pituitary interaction.[4-6] GHRPs also bind to the pituitary gland and may have direct effects on GH release.[7] The mechanisms used to signal the release of GH by GHRP are complex and interwoven. Their ability to release GH is exceptional even in conditions characterized by increased SRIF tone and relative suboptimal GH secretion such as obesity[5,8] and aging.[9,10]

In this study, we examined GH secretion patterns before and after a typical short course of antiinflammatory prednisone therapy. We measured the GH response to arginine, a known SRIF inhibitor, before and after 4 days of prednisone 20 mg orally three times per day. Then, in the same patients, we measured the GH response to GHRP-2 before and after administration of prednisone. The purpose of this study was to determine the degree of correction of GH release after prednisone attributable to a SRIF-inhibitory effect of GHRP. We assessed this by comparing the timing and extent of restoration of GH release following GHRP and arginine. Differences in the two corrected patterns may reflect the multiple mechanisms of action of GHRP, including effects on GHRH release, direct hypothalamic effects, and direct pituitary effects.

SUBJECTS AND METHODS

Subjects and Study Design

Eight normal healthy men were studied in the General Clinical Research Center (GCRC) of Emory University Hospital. The subjects had an age range of 20 to 42 years, and all had a normal body mass index. None of the subjects were taking regular medications or had significant medical illness. The protocol was approved by the Emory

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University School of Medicine Institutional Review Board, and written informed consent was obtained from each subject.

The subjects were randomly assigned to one of two groups. The group assignment determined the sequence of provocative GH testing. Group A underwent arginine stimulation on day 1 and GHRP-2 stimulation on day 2 followed by prednisone therapy and then arginine stimulation on day 6 and GHRP-2 stimulation on day 7. Group B received GHRP-2 stimulation on day 1 and arginine stimulation on day 2 followed by prednisone therapy and then GHRP-2 stimulation on day 6 and arginine stimulation on day 7. All subjects were required to fast after midnight prior to the day of testing. Subjects were admitted to the GCRC at 7:00 AM, and an intravenous line for blood sampling and medication administration was placed in the forearm at least 1 hour before administration of the GH secretagogue. On days 1, 2, 6, and 7, a blood sample was taken for serum GH measurement at −15 minutes. On days when arginine was used as the stimulus for GH release, arginine HCl was infused at a dose of 30 g intravenously over 30 minutes. Blood was sampled for serum GH measurement at 15, 30, 45, 60, 90, and 120 minutes from the start of the infusion. When GHRP-2 was infused as the stimulus for GH release, a dose of 1.0 μg/kg was administered as an intravenous bolus, and blood was sampled for GH measurement at 15, 30, 45, 60, 90, and 120 minutes after the GHRP-2 bolus. Prednisone was administered as 20 mg orally three times per day at 7:00 AM, 3:00 PM, and 11:00 PM. The first dose was taken at 3:00 PM on day 2 and the last dose at 7:00 AM on day 7, the last day of the study.

GHRP-2 was synthesized by Kaken Pharmaceutical (Japan). Serum GH levels were measured by immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA). The sensitivity of the assay was 0.2 μg/L, the intraassay coefficient of variation was 4.22%, and the interassay coefficient of variation was 7.2%. Samples were assayed in duplicate.

Results are presented as the mean ± SEM for a sample size of eight per group (n = 8). Paired *t* tests were used for significance testing, with a *P* value less than .05 considered statistically significant. In addition, the Wilcoxon signed-rank test, the nonparametric equivalent of the paired *t* test, was also used to assess significance.

RESULTS

The order of stimulation testing (arginine before GHRP-2 or GHRP-2 before arginine) did not affect the GH response. *P* values calculated using the paired *t* test and Wilcoxon signed-rank test were similar in all analyses. Therefore, only paired-test *p* values are provided.

The GH response to arginine is depicted in Fig 1. At baseline and before prednisone, the mean peak GH level in response to arginine was 8.8 ± 2.8 μg/L, and this peak value occurred 60 minutes after beginning the arginine infusion. When the same subjects received prednisone, the peak GH value after arginine infusion increased to 20.1 ± 8.3 μg/L, and this peak value occurred 45 minutes after the start of the infusion. The mean peak GH response following the combination of prednisone treatment and arginine stimulation was not significantly increased versus arginine alone (paired *t* test, $P = .21$).

Figure 2 depicts similar data for the response to GHRP-2. At baseline and before prednisone therapy, the peak GH level in response to GHRP-2 was 80.8 ± 21.2 μg/L, and this peak occurred 45 minutes after the GHRP-2 bolus. In the same subjects on prednisone, the peak GH level was only slightly diminished at 71.3 ± 18.4 μg/L, and this peak value occurred 15 minutes after the intravenous bolus of GHRP-2 (paired *t* test, $P = .46$).

Figure 3 shows the GH area under the curve in response to arginine and GHRP-2 before and after glucocorticoid therapy.

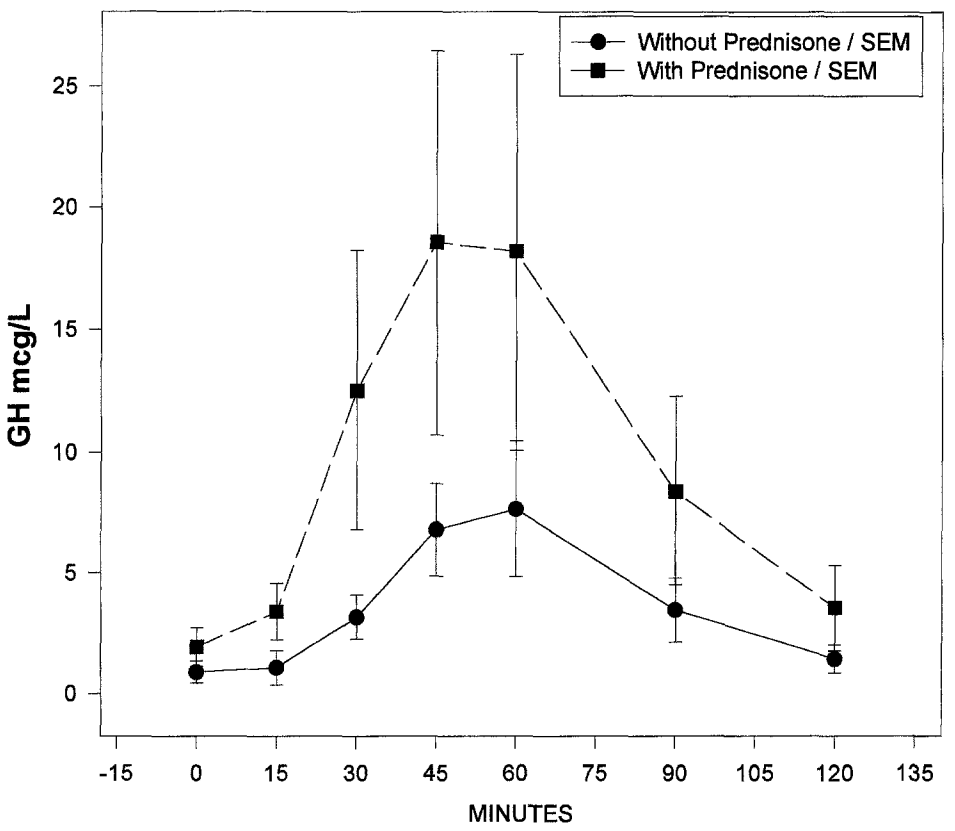

Fig 1. GH response to arginine before and during prednisone treatment. Data are the mean ± SEM at each time point.

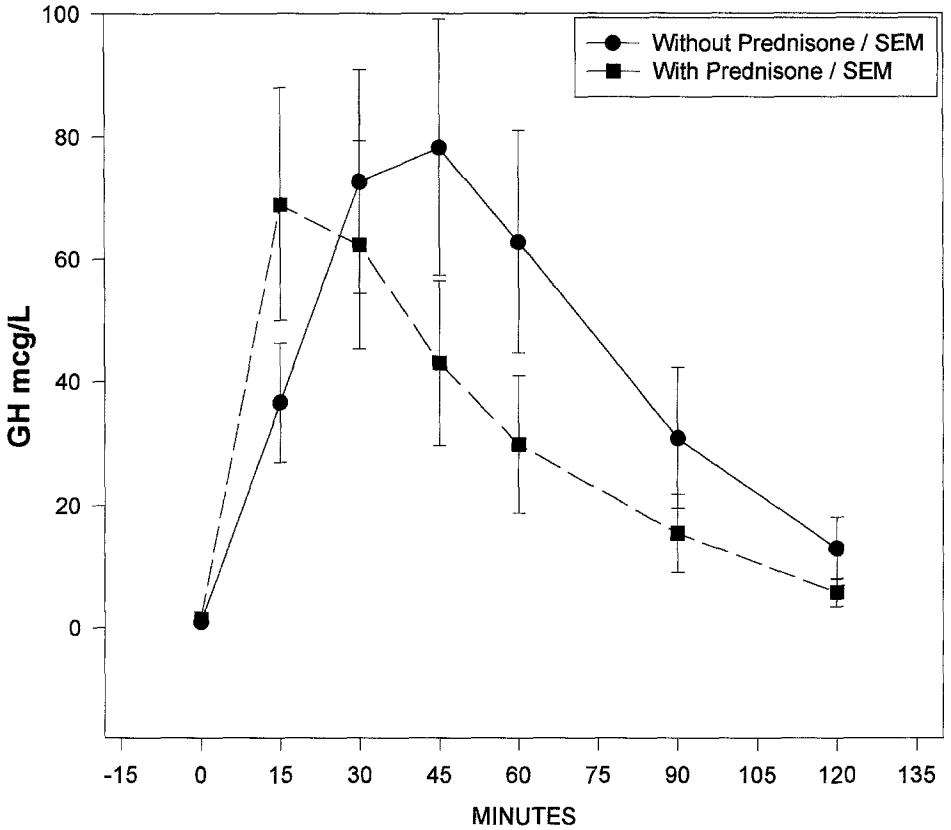

Fig 2. GH response to GHRP-2 before and during prednisone treatment. Data are the mean ± SEM at each time point.

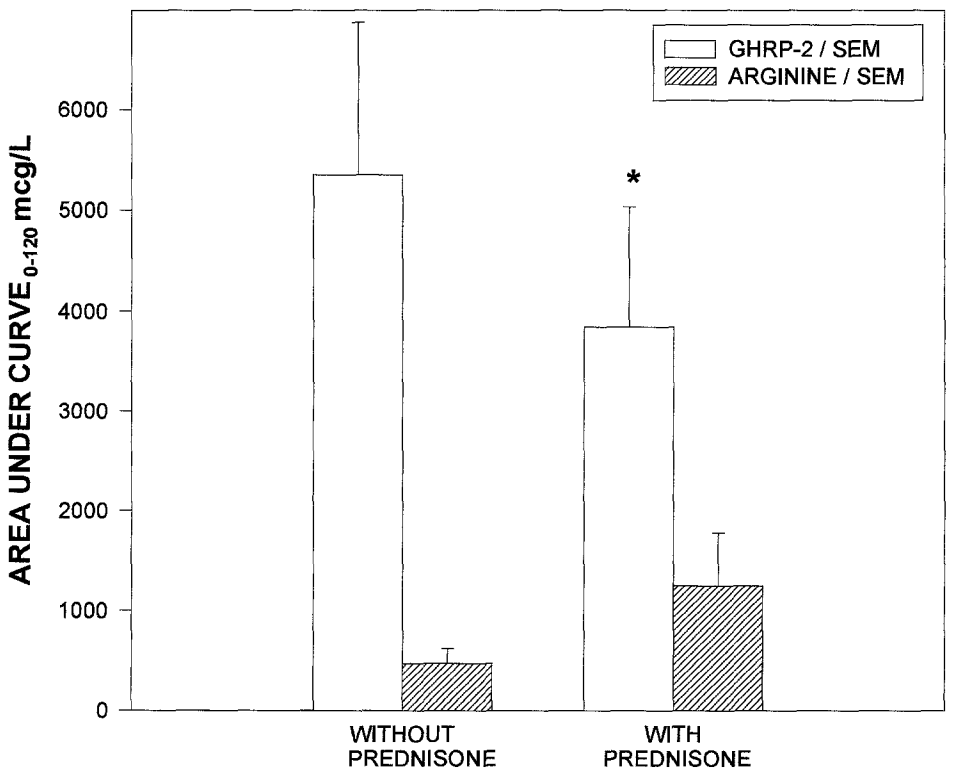

Fig 3. Calculated area under the curve for GH response to GHRP-2 and arginine before and during prednisone. The area under the curve in response to GHRP-2 decreased significantly during prednisone therapy (paired *t* test, $P = .04$).

The area under the curve at baseline in response to arginine was 469 ± 147 μg/L, and after prednisone it increased to 1,245 ± 535 μg/L (paired t test, $P < .19$). The area under the curve in response to GHRP-2 before prednisone was 5,350 ± 1,525 μg/L and was more than 10-fold higher than the area under the curve at baseline in response to arginine. After prednisone, the area under the curve for GHRP-2 decreased to 3,844 ± 1,191 μg/L but remained threefold larger than the area under the curve in response to arginine while on prednisone. The area under the curve in response to GHRP-2 decreased significantly during prednisone therapy (paired t test, $P = .04$), whereas the area under the curve increased in response to arginine while on prednisone, but this increase was not significantly different.

DISCUSSION

One of the proposed mechanisms for decreased GH release to provocative agents in individuals on glucocorticoid therapy is an increase in SRIF tone.[2] Since GH release is dependent on a synchronized decrease in SRIF and an increase in GHRH, a persistent elevation of SRIF could result in suboptimal GH secretion and poor growth. Gertz et al[11] were able to demonstrate a partial reversal of glucocorticoid-induced GH suppression by administration of L-692,429, a nonpeptide modified benzolactam that is functionally very similar to GHRP-6. They demonstrated that the mean GH area under the curve in response to 0.2 mg/kg L-692,429 was reduced 60% by the addition of prednisone. When they administered nearly four times the original dose of L-692,429 while the subjects were on prednisone, they were able to restore the values for the GH area under the curve from 40% to 66% but were still unable to normalize GH release fully. In our study, we evaluated the potential of GHRP-2 to restore GH secretion in individuals treated with glucocorticoids. We compared the GH secretory pattern after GHRP-2 with the GH secretory pattern after arginine (a "pure SRIF inhibitor") in individuals before and during prednisone therapy.[12] Similar patterns of GH release to GHRP-2 and arginine would suggest that the inhibition of GH release with glucocorticoid therapy is related to increased SRIF and that its reversal could be accomplished through SRIF antagonism.

Our data support the fact that GH release in response to GHRPs is much larger, both for the GH peak level and the area under the curve, than the response to more typical secretagogues such as arginine or GHRH.[10] In our study, subjects who were not on prednisone demonstrated an almost 10-fold higher mean peak GH level and an 11-fold higher mean value for the GH area under the curve in response to GHRP-2 versus arginine. During prednisone therapy, the peak GH level and area under the curve decreased in response to GHRP-2 and increased in response to arginine. Because of the large between-subject variability, we were unable to detect significant changes in the peak response, and the only significant change was a decrease in the area under the curve for GH secretion in response GHRP-2. It is important to note that during prednisone therapy, the GH response to GHRP-2, albeit decreased, was still three times greater than the GH response to arginine. Interestingly, the time for peak GH secretion was earlier in response to both secretagogues when the subjects were on prednisone. We propose that glucocorticoids, acting via increased levels of SRIF, may inhibit pituitary GH secretion and GH may accumulate build up in the pituitary gland. When the inhibition is removed by a SRIF antagonist, the sequestered GH is rapidly released and peak GH levels are higher and more rapidly achieved because of the sudden release of stored hormone.

Our data suggest that GH secretagogues such as GHRP-2 may be useful in modulating GH secretion in children who have increased SRIF tone in clinical settings such as obesity and long-term glucocorticoid treatment. In our studies, adult subjects responded amply to the secretagogues. Therefore, we anticipate that children, who are known to respond to GH stimulation tests with more vigor than adults, will respond as well. Pihoker et al[13] have studied GH release in response to intranasal GHRP-2 in short children who were evaluated for GH deficiency. They were able to demonstrate peak levels of GH secretion of 31.3 ± 6 μg/L after intranasal administration of GHRP-2 in a subset of short children who previously demonstrated GH responses greater than 20 μg/L to intravenous GHRH or GHRP-2. Future studies of GH-releasing secretagogues administered either nasally or orally may prove clinically beneficial in the normalization of GH release in children with decreased GH secretion due to alterations in the normal balance of hypothalamic hormonal tone.

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