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Growth Hormone (GH) Secretion in Primary Adrenal Insufficiency: Effects of Cortisol Withdrawal and Patterned Replacement on GH Pulsatility and Circadian Rhythmicity

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Abstract. **We studied the effects of cortisol withdrawal and patterned replacement upon spontaneous GH secretion and circadian rhythmicity in 7 patients with Addison's disease. Hydrocortisone was administered in physiological daily total dosages, and all resulting plasma cortisol values were 2–15 μg/dl. It was given in 3 pulsatile modes: simulating "physiological" rhythm, "reverse" diurnal rhythmicity and "continuous" pulsatility. All modes of cortisol administration increased mean 24h, GH pulse amplitude and interpulse GH levels. During saline infusions circadian GH rhythmicity was preserved, with GH being at its highest between 2400–0400 h. Administration of hydrocortisone in any mode did not modify circadian GH rhythmicity.** ***We conclude:*** **Cortisol replacement in physiological daily doses increases GH output in patients with Addison's disease by augmenting GH pulse amplitude and interpulse levels. This is likely due to the attenuation of hypothalamic somatostatin (SRIF) secretion by physiologic levels of cortisol. By inference, it implies that cortisol deficiency leads to diminution of GH output with low GH pulse amplitude, likely as a result of an augmented hypothalamic somatostatin secretion. However, circadian rhythmicity of GH secretion is glucocorticoid-independent.**

Keywords. **Addison's disease, hydrocortisone, diurnal rhythm, somatotropin**

Introduction

Glucocorticoids exert powerful effects upon GH synthesis and secretion. Alterations in GHRH and SRIF mRNA's expression [1,2], pituitary GHRH receptors [3], GH gene expression [4] and GH release [5] have been reported in vitro and in animals post adrenalectomy or glucocorticoid administration. In humans, understandably, the information is not as detailed but GH deficiency is a regular consequence of Cushing's syndrome [6,7]. The effects of glucocorticoid deprivation in humans are even less well studied. Isolated ACTH deficiency is accompanied by diminished GH responses to a variety of provocative stimuli and these are normalized by glucocorticoid replacement [8,9]. On the other hand, a dose-dependent inhibition of the GHRH-induced GH release has been observed in patients with Addisons's disease given short-term hydrocortisone infusions [10].

The effects of chronic glucocorticoid deprivation and/or replacement upon spontaneous GH secretion have never been studied in humans. Also, the effects of glucocorticoids upon circadian GH rhythm in humans have not been examined. Interestingly, circadian rhythms of GH and TSH are almost parallel, with relatively low levels throughout the day and a temporary augmentation during early night hours [11]. Since glucocorticoids are believed to stimulate somatostatin (SRIF) secretion [2], one can hypothesize that the circadian rhythm of cortisol secretion is the driving mechanism for both TSH and GH rhythms. In this model, high morning cortisol levels increase SRIF secretion which in turn suppresses both TSH and GH, whereas a nocturnal decrease in cortisol suppresses SRIF secretion with the resultant disinhibition of both TSH and GH. If this hypothesis is correct, patients with Addison's disease should have enhanced daytime TSH and GH release with absent or blunted circadian rhythm and a "physiologic" cortisol replacement should restore circadian TSH and GH rhythmicity and attenuate daytime hormone release. This hypothesis is supported by the existing data on TSH pulsatility: in patients with Addison's disease, daytime TSH levels are tonically elevated, nocturnal rise of TSH is lost and

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replacement of cortisol in a physiological dose and pattern (high morning and low nighttime levels) restores normal circadian TSH rhythm [12]. However, the same doses of cortisol given as a continuous infusion or in a "reverse" pattern (low morning and high nighttime levels) are ineffective in this regard. These data support the model of glucocorticoid-dependent TSH rhythmicity. To test whether it is also applicable to GH rhythmicity, we conducted a study in 7 patients with Addison's disease. GH pulsatility and circadian rhythmicity were assessed both before and during cortisol replacement given in different modes.

Subjects and Methods

Seven subjects (4 men, 3 women) with a long-standing Addison's disease were recruited for the study. Their mean age was 42 years (range 29–67) and their mean BMI was 29 kg/m2 (range 22–43). The protocol was approved by the IRB at the Oregon Health Sciences University and a written consent form was signed by all subjects before entering the study. Clinical data and detailed description of the protocol have been published previously [12,13]. In brief, on four separate occasions at least one month apart, the subjects were hospitalized in the CRC at the Oregon Health Sciences University. Regular hydrocortisone replacement was stopped 24 hours prior to admission but other replacements (Florinef in all, thyroxine in 2, estrogen/progesterone in two) were continued. Starting the next morning, either normal saline or hydrocortisone (19 mg/day) were infused for 48 hrs. Hydrocortisone was given as a.) 11 equal, 20 minute long infusions given at 130 min intervals ("continuous"), b.) as patterned short-term infusions to mimic the "physiological" rhythm, i.e. same total dose and number of pulses as the "continuous" study, but with the timing and variable pulse amplitude designed to replicate normal 24h cortisol profiles; or c.) as a "reverse" rhythm (same dose and number of pulses but the diurnal rhythm reversed). The exact schemata of different modes of hydrocortisone administration were shown in a previous publication [13]. In two subjects, "physiological" and "reverse" studies were done using 38 mg hydrocortisone per day, in order to achieve normal cortisol levels as explained previously [13]. Blood sampling was done on the second infusion day, for 24 hrs (0800–0800h) every 15 minutes. Samples were initially assayed for cortisol, leptin, and TSH and the results were already reported [12, 13]. After that, samples were transferred on dry ice to the University of Michigan, and GH concentrations were measured there. We utilized the chemiluminometric GH assay (Nichols, San Juan Capristrano, CA) with assay sensitivity 0.01 μg/L, intraassay variability <10% in the relevant assay range and interassay variability <10%. All samples from the same individual were assayed in duplicate, in the same assay.

Analysis

Discrete parameters of GH pulsatility were assessed by Cluster algorithm [14]. False-positive pulse detection was assumed when the program identified a pulse with the amplitude <0.03 μg/L (14). In 5 subjects, all serum samples were available for the analysis. In 1 subject, 16 consecutive samples corresponding to 0400–0800h were missing from the baseline (saline) series. His pulse frequency was adjusted upward by 1/6, and the 0400–0800h block was missing from the rhythmicity analysis (see below). In another subject, a total of 26 samples were missing from the baseline series, both nonconsecutively and in clusters. In this series, we calculated mean GH concentrations only, but did not perform evaluations of pulsatility or circadian rhythmicity. To evaluate circadian GH rhythmicity, we calculated mean GH concentrations in blocks (0800–1145h; 1200–1545h . . . etc.), creating six 4-hour blocks per each study. Area versus time curves (AUC) for the 4-hour blocks were calculated using trapezoidal rule. Data were analyzed by a repeated measures ANOVA with subsequent Tukey-Kramer procedure. The use of PROC. MIXED in SAS package allowed for the use of sets with missing data. When the data were not normally distributed, they were logarithmically transformed prior to analysis. Statistical significance was assumed at $p < 0.05$ level.

Results

Plasma cortisol (Figure 1)

During saline infusion all cortisol values were below 1 μg/dl. During "continuous" cortisol administration, plasma cortisol concentrations fluctuated periodically, between 6 and 12 μg/dl, as would be expected from 20 min long, Q130 min intravenous infusions. During "physiological" patterned infusions, plasma cortisol concentrations were between 9 and 15 μg/dl in the morning (peak between 0830 and 0930 h) and were low (2–3 μg/dl) between 2200 and 0400 h. During the "reverse" study, plasma cortisol concentrations were at 10–14 μg/dl between 1830 and 0145h (peak at 2100h) and were low (2–4 μg/dl) between 0915 and 1700 h. In all samples plasma cortisol levels fluctuated within the physiologic range observed in healthy humans, i.e., 2–15 μg/dl.

Growth hormone

Examples of GH profiles in 2 subjects during all 4 steps of the protocol are shown in Fig. 2. Discrete parameters of GH pulsatility as defined by Cluster algorithm are shown in Fig. 3. There was a significant increase in mean GH concentrations between baseline (saline study) and "physiological"/"reverse" cortisol infusion studies (0.43 ± 0.12 vs. 0.93 ± 0.22 and 0.98 ± 0.25 μg/L; $p = 0.02$). During "continuous" cortisol delivery, plasma GH was 0.8 ± 0.23 μg/L, with a trend toward

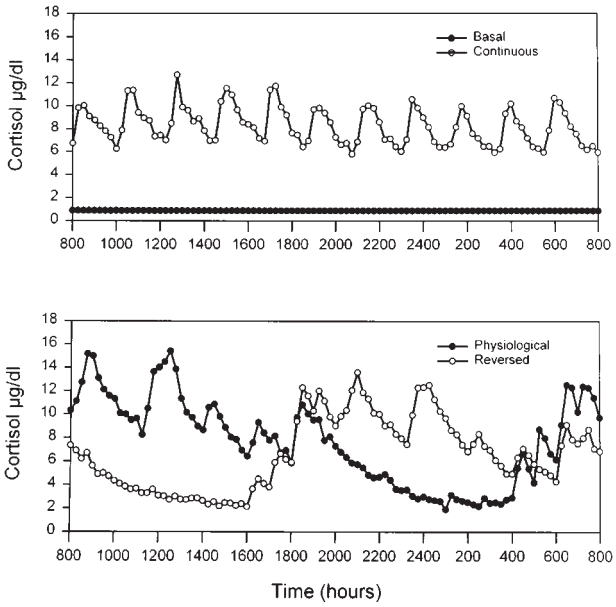

Fig. 1. *Mean plasma cortisol concentrations during various stages of the protocol. The data for the baseline and the "continuous" series are shown in the upper panel and for the "physiological" and the "reverse" series, in the lower panel. SE bars are omitted for clarity of presentation.*

increase vs. baseline (p = 0.08). There was no difference in pulse frequency between all steps of the protocol. At baseline, mean GH pulse amplitude was 1.14 ± 0.4 µg/L. It rose to 2.34 ± 0.57 and 2.15 ± 0.48 µg/L during "physiological" and "reverse" cortisol infusions (p = 0.02). Again, there was only a trend toward pulse amplitude increase during "continuous" cortisol delivery (p = 0.11). The interpulse GH levels during the

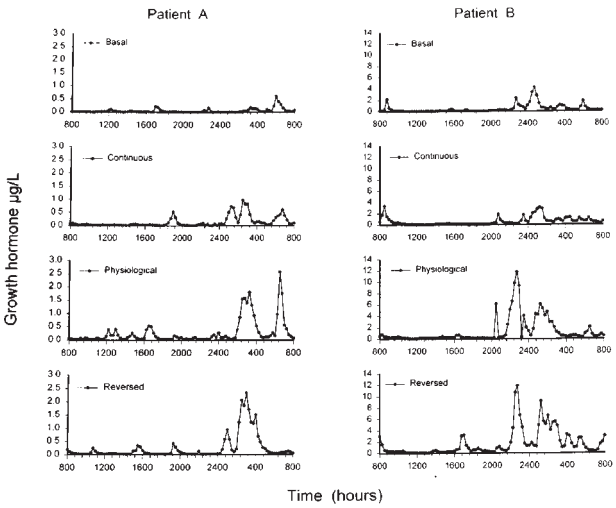

Fig. 2. *Plasma GH concentrations in 2 patients during various stages of the protocol*

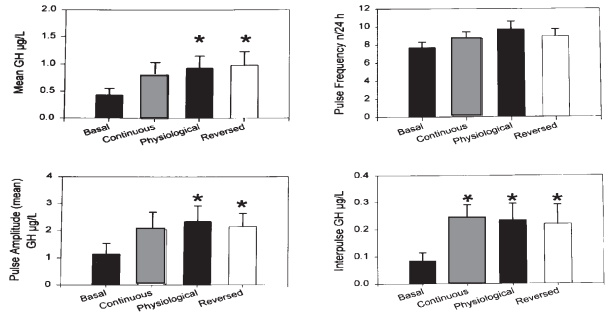

Fig. 3. *Discrete parameters of GH pulsatility during various stages of the protocol. *p < 0.02 vs. basal (saline infusion)*

baseline study (0.084 ± 0.03 µg/L) increased threefold during all cortisol infusions (0.24 ± 0.05 µg/L for the "continuous", 0.235 ± 0.06 µg/L for the "physiological" and 0.22 ± 0.07 µg/L during the "reverse" study; p = 0.01).

Diurnal rhythm of GH secretion was assessed using 1n transformed AUC data for 4h blocks (Fig. 4). Statistically significant increases in AUC were observed between midnight and 0400 hours in the "reverse" (p = 0.015) and "continuous" (p = 0.01) cortisol infusion series. GH AUC were at their highest at the same time during the "physiological" and baseline series, but only statistical trends were apparent (p = 0.12 and 0.06 respectively). The magnitude of these elevations were similar in all series, with the absolute increase in the 1n (AUC) between the lowest and the highest blocks being 1.3 for the baseline, 1.4 for the "continuous", 1.1 for the "physiological" and 0.8 for the "reverse" series. A change of 0.7 in 1n (AUC) is equivalent to a doubling of the AUC.

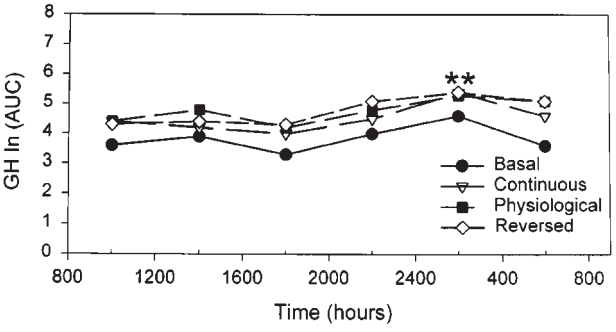

Fig. 4. *Circadian rhythmicity of GH concentrations during various stages of the protocol. Data are shown as mean of 1n (AUC) of 4h blocks. *p < 0.02 vs. other daily blocks. For details see Results.*

Discussion

This is the first study to evaluate spontaneous GH secretion in patients with Addison's disease with and without hydrocortisone replacement in a physiological daily dose. Since each subject was used as his/her own control, the potential influence of other variables, such as body composition, age, gender and use of other medications potentially affecting GH secretion (estrogen, thyroxine) was minimized. In the same patient population, significant changes in the magnitude and the pattern of TSH secretion were observed [12]. Thus, the duration of cortisol deprivation and the variability of plasma cortisol patterns were biologically meaningful. Therefore, this model is likely to shed light on glucocorticoid/GH interactions as well.

Virtually all studies addressing the issue of glucocorticoid effects upon GH secretion find that the states of endogenous [6] or exogenous [7] glucocorticoid excess are accompanied by the suppression of GH output. Thus, we expected that hydrocortisone infusions in patients with Addison's disease would also result in a lowering of mean 24 hour GH concentrations. Surprisingly, the reverse was true: GH ouput doubled during hydrocortisone administration compared to the baseline study. This was due to the doubling of the GH pulse amplitude and, to a lesser degree, to the tripling of the interpulse GH concentrations. This constellation of changes in the parameters of GH pulsatility is compatible with the decreased hypothalamic SRIF release [15]. Generally, it is assumed that glucocorticoids inhibit GHRH and stimulate SRIF secretion by the hypothalamus [1,2]. These conclusions, however, are based on experimental models utilizing supraphysiologic concentrations of glucocorticoids. In contrast, cortisol concentrations in our study were carefully maintained within a physiological range. In this regard, the study by Fernandez-Vazquez et al. [16] is very instructive and likely provides an explanation to the dichotomy between our and other studies. They have shown that the effects of corticosterone upon synthesis and secretion of GHRH and SRIF by cultured fetal rat hypothalamic cells are dose dependent. Corticosterone concentrations in the range of the glucocorticoid receptor Kd (3nM) increased while higher concentrations (30 or 300 nM) decreased, GHRH content and release. Similarly, corticosterone at 3nM did not affect SRIF release, but higher concentrations (30 and 300 nM) induced doubling to tripling of SRIF release. These data are fully compatible with our results, underscoring the important distinction between physiological and pharmacological glucocorticoid doses. We show that physiological concentrations of cortisol are stimulatory for GH secretion. Part of this effect might have also been expressed directly at the pituitary level, augmenting GH synthesis [4] and up-regulating GHRH receptors [3]. Indeed, glucocorticoids are indispensable for the full expression of the GH secretory potential of cultured human [17], monkey [18] and rat [5] pituitaries, as well as clonal cell lines of rat pituitary tumors [19]. Interestingly, acute administration of pharmacological doses of dexamethasone (4 mg i.v.) has been recently shown to elicit a marked GH release in humans [20,21]. On the other hand, physiological or only slightly supraphysiological doses of hydrocortisone inhibited (rather than stimulated) GH responses to GHRH in patients with Addison's disease [10]. Thus, the stimulatory effects of hydrocortisone upon GH pulse amplitude observed in our study are unlikely to be due to a direct pituitary effect of the steroid. By inference, the hypothalamic (SRIF suppression) effects of physiologic cortisol levels seem to provide a better and, likely, sufficient physiological basis for our results. Giustina et al. [22,23] have shown that hexarelin, a GH-secretagogue acting as a GHRH releaser and a functional SRIF antagonist is able to counteract the inhibitory effect of high glucocorticoid concentrations upon GH secretion in humans. These data are in full agreement with our interpretation of results.

We also tested the hypothesis that circadian GH rhythm is glucocorticoid-driven, similarly to the recently described effect of cortisol upon TSH secretion [12]. However, even after 48–72h of cortisol deprivation, the circadian rhythm of GH secretion still persisted and was not modified by the non-physiological "continuous" and "reverse" cortisol infusions. It is conceivable that a longer period of cortisol deprivation may be needed to abolish the diurnal pattern of GH secretion. However, prolongation of the glucocorticoid "wash-out" period in patients with adrenal insufficiency may precipitate Addisonian crisis; such a study would be unacceptable on ethical grounds. Importantly, the same duration of glucocorticoid deprivation was sufficient to abolish the nocturnal TSH rise [12]. Thus, we believe that the persistence of the circadian GH rhythm off glucocorticoids and the lack of effect of antiphysiologic cortisol rhythms upon GH periodicity disprove our original hypothesis and provide sufficient evidence against a potential role of cortisol rhythmicity upon circadian GH release.

In summary, we present the first evidence of the stimulatory role of physiologic levels of cortisol upon spontaneous GH secretion in patients with Addison's disease. This is likely to be mediated through inhibition of hypothalamic SRIF secretion. On the other hand, neither cortisol deprivation nor different modes of its replacement have any effect upon circadian GH rhythmicity. Thus the mechanism(s) subserving the nocturnal augmentation of GH release are glucocorticoid independent.

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