Effects of Short- and Long-Term Dexamethasone Treatment on Growth and Growth Hormone (GH)-Releasing Hormone (GRH)-GH-Insulin-Like Growth Factor-I Axis in Conscious Rats

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Abstract. Although the inhibitory effects of a chronic excess of glucocorticoids (GC) on body growth and GH secretion are well established, the mechanisms involved remain unclear. In this study, we examined the chronic effects of a high dose of dexamethasone (DEX) on spontaneous GH secretion and insulin-like growth factor (IGF)-I in conscious rats. The animals were given daily ip injections of DEX (200 µg/day) for either one or four weeks. Body growth assessed by tibia length and serum IGF-I levels was significantly inhibited 1 week after treatment. By contrast, spontaneous GH secretion was not altered 1 week after the treatment. Neither hypothalamic GRH and somtatostain mRNA levels nor GH responses to GRH from single somatotropes were affected 1 week after the treatment. Four weeks after DEX treatment, body growth of the rats was noticeably suppressed. Interestingly, spontaneous GH secretion, hypothalamic GRH mRNA levels and GH responses to GRH were all inhibited 4 weeks after treatment. Pituitary GRH receptor mRNA levels were not altered 1 week after treatment, but increased after 4 weeks. These results indicate that a high dose of DEX initially impairs IGF-I production and subsequently inhibits spontaneous GH secretion in rats. Inhibition of spontaneous GH secretion resulting from chronic GC excess is due, at least in part, to the impairment of hypothalamic GRH synthesis and pituitary GH responsiveness. An increase in the pituitary GRH receptor may be caused by decreased GRH secretion.

Key words: Dexamethasone, GH, Insulin-like growth factor-I (IGF-I), GRH receptor, Rat

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CHRONIC excess of glucocorticoids (GC) impairs growth in humans and laboratory animals [1] and GH has been considered to be an important component in the GC-induced growth failure. In rats, many studies have shown that chronic treatments with GC inhibit GH responses to various stimuli including GRH [2–4]. Also in humans,

blunted GH responses to GRH have been demonstrated [5–7]. All these studies suggest that impaired GH secretion contributes to the growth failure associated with chronic GC excess. On the other hand, GC have been known to exert potent inhibitory effects on insulin-like growth factor (IGF)-I production by direct action in many types of cells [8–10], so that it is unclear which components most contribute to the growth failure. To date no reports have shown the time course of changes in spontaneous GH secretion and IGF-I levels under GC excess.

The mechanisms by which GH secretion is

impaired by chronic GC excess are not fully understood. Many lines of evidence suggest that hypothalamic somatostatin (SRIF) is chiefly responsible for GC-induced GH suppression [11–13]. It has previously been reported that hypothalamic SRIF content and mRNA levels are increased in dexamethasone (DEX)-treated rats [14–16]. Neutralization of SRIF secretion by SRIF anti-serum has been reported to restore the blunted GH responses to GRH in rats [17]. Hypothalamic GRH also plays an important role in regulating pulsatile GH secretion [18] so that, it is worth investigating whether hypothalamic GRH and its receptors are altered by chronic GC excess. In the present study, we administered a high dose of DEX to rats for either 1 or 4 weeks and examined the chronic effects of DEX on body growth and serum IGF-I/IGF-binding proteins (IGFBP). Spontaneous GH secretion, hypothalamic GRH mRNA levels, pituitary GRH receptor mRNA levels, and pituitary GH responses to GRH were also examined.

Materials and Methods

DEX treatment and measurement of spontaneous GH secretion

Eight-week-old male Wistar rats (Clea Japan Inc, Osaka, Japan) were kept in a room under a 12-h light/dark (between 0600–1800 h) cycle in a temperature- and humidity-controlled environment. The animals were given daily ip injections of DEX (200 μg/0.5 m*l*, Decadron^R, Merk USA) or saline, for 1 week and 4 weeks. The body weights and tibia lengths [17] were measured at each of these observation times. Spontaneous GH secretion was examined in conscious rats treated with DEX and saline under unrestrained conditions as previously described [19]. Blood samples were taken every 20 min from 1000 to 1600 h and GH concentrations in the plasma were measured with a rat GH RIA kit supplied by NIDDK. The results were expressed as ng per m*l* NIDDK rat GH RP-2. The pulsatile pattern of GH secretion during the 6-h period was analyzed with the Detect program [20] which calculates the number of hormone pulses, their peak height and their duration.

Measurement of IGF-I, IGF-binding protein (IGFBP), nonesterified fatty acid (NEFA), glucose and insulin concentrations

The rats were killed by decapitation after DEX treatment and blood collected from their trunk. Serum IGF-I levels were determined with a commercial RIA kit (Somatomedin-C, Eiken II, Eiken Co., Tokyo, Japan) after acid-ethanol extraction [21]. Serum IGFBPs were analyzed by Western ligand blotting as described by Hossenlopp *et al.* [22]. The internal control was loaded onto each gel and the results were expressed as a percentage of the control value, which was set at 100%. The serum concentrations of NEFA and glucose were determined with an autoanalyzer [23]. The plasma insulin levels were determined with a commercial RIA kit (Rat insulin kit, INCSTAR, MN).

Pituitary GH response to GRH in vitro

Pituitary GH responses to GRH were examined by the reverse hemolytic plaque assay (RHPA) as previously described [24]. The day after the last injection of DEX, the anterior pituitary tissues were removed and dispersed into single pituitary cells. After 24-h incubation with Dulbecco's modified Eagle's medium (DMEM), monkey antirat GH serum (NIDDK) diluted 1:150 in DMEM was infused into the chambers alone or with rat GRH (Peninsula, CA) for 2 h. Plaque development was initiated by infusion of guinea pig complement (GIBCO, Grand Island, NY). The pituitary cells were stained with 0.5% toluidine blue and the plaque area was measured with a calibrated ocular reticule. An area of 50 plaques/slide was quantified.

Hypothalamic GRH and SRIF mRNA levels

Hypothalamic GRH and SRIF mRNA levels were examined by Northern blot hybridization as previously described [20, 25]. Total RNA was isolated from each hypothalamus after DEX treatments. Electrophoresis was performed with 40% of the RNA isolated from a single hypothalamus. RNA immobilized onto a nylon membrane was hybridized with [^{32}P]-labeled rat

GRH and SRIF complementary DNA probes [20, 25]. The hybridization signals were detected and quantified with an imaging analyzer (BAS 1000 system, Fuji Photo Film, Tokyo, Japan), and the results were expressed as a percentage of the saline-treated control value, which was set at 100%.

Pituitary GRH receptor mRNA levels

The competitive reverse transcribed-polymerase chain reaction (RT-PCR) method was used to quantify GRH receptor mRNA levels in the anterior pituitary tissue as previously described [26]. Briefly, total RNA was extracted from the anterior pituitary tissues in DEX-treated rats. The stock competitor was diluted from 10 pg to 10 fg by 10-fold serial dilutions. Five hundred nanograms of total RNA and each diluted competitor were simultaneously reverse transcribed into cDNA. Thirty cycles of PCR were carried out in a thermal cycler (Sanko Junyaku, Tokyo, Japan) and PCR products were electrophoresed in an agarose gel. Signal intensities of each product were quantified with an image scanner (JX-325M, Sharp Co, Tokyo, Japan) and NIH-Image. The concentration of GRH receptor mRNA was calculated by regression analysis between samples and competitors. The results were expressed as a percentage of the saline-treated control value, which was set at 100%.

Statistical analysis

Statistical comparisons were made by one-way analysis of variance and Student's *t*-test, with *P*<0.05 considered significant.

Results

Animals and body growth

Food intake was moderately lower in the DEX-treated rats than in the control rats (DEX-treated rats, 1 week; 19.6 g, 4 weeks; 18.9 g, control rats, 1 week; 24.3 g, 4 weeks; 24.0 g, mean per day, n=5). Although plasma insulin levels were high in the DEX-treated rats, plasma glucose and NEFA in the DEX-treated and control rats did not differ (data not shown). Changes in the body weight and tibial length are shown in Fig. 1. The body weight and tibial length were significantly reduced in the DEX-treated rats as compared with the control rats after 1 week of treatment. These inhibitory effects persisted throughout the 4 weeks of DEX treatment.

Spontaneous GH secretion

Representative profiles of spontaneous GH secretion are shown in Fig. 2. The profiles of

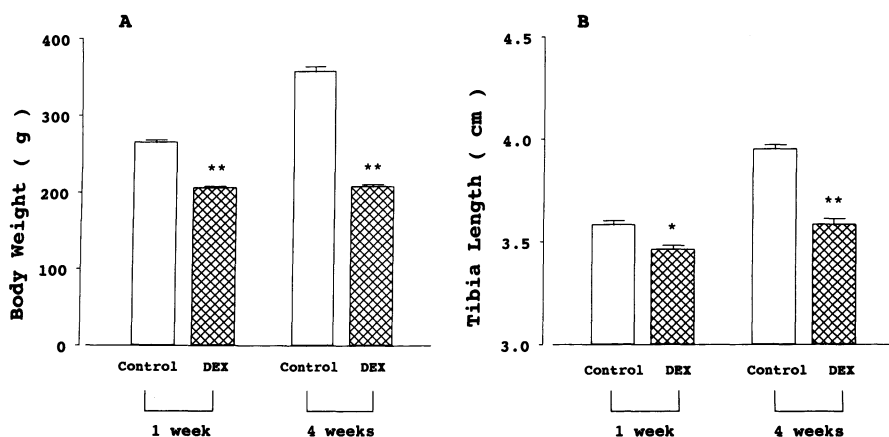Fig. 1.   Effects of DEX on body weight (A) and tibia length (B) in rats. Each group was treated with DEX for 1 and 4 weeks, respectively. Each column represents the mean ± SEM (n=5). *, *P*<0.05 *vs*. control. **, *P*<0.01 *vs*. control.
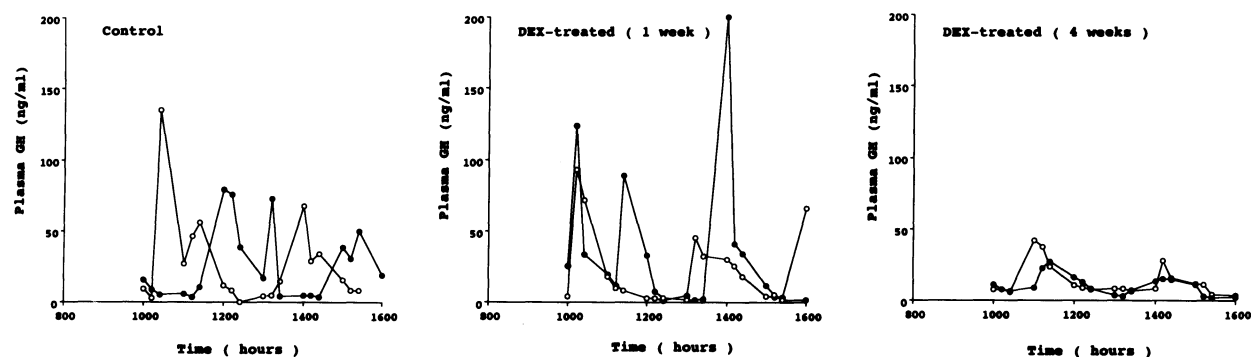
Fig. 2. Individual representative 6-h plasma GH secretory profiles in control and DEX-treated rats. Each group was treated with DEX for 1 and 4 weeks, respectively. Two representative cases are shown.

Table 1. Analysis of GH surges in DEX-treated rats assessed by computer program (Detect)

Group	(n)	Peak number (Peaks/6 h)	Peak height (ng/ml)	Peak area (ng/ml/20 min)	Trough level (ng/ml)
Control	(7)	1.86 ± 0.26	101.36 ± 14.03	165.44 ± 19.83	8.97 ± 1.45
DEX-treated, 1 week	(6)	1.33 ± 0.21	117.86 ± 38.21	168.02 ± 49.59	4.93 ± 0.78*
DEX-treated, 4 week	(7)	2.29 ± 0.42	33.91 ± 6.93*	55.96 ± 11.26*	7.71 ± 0.49

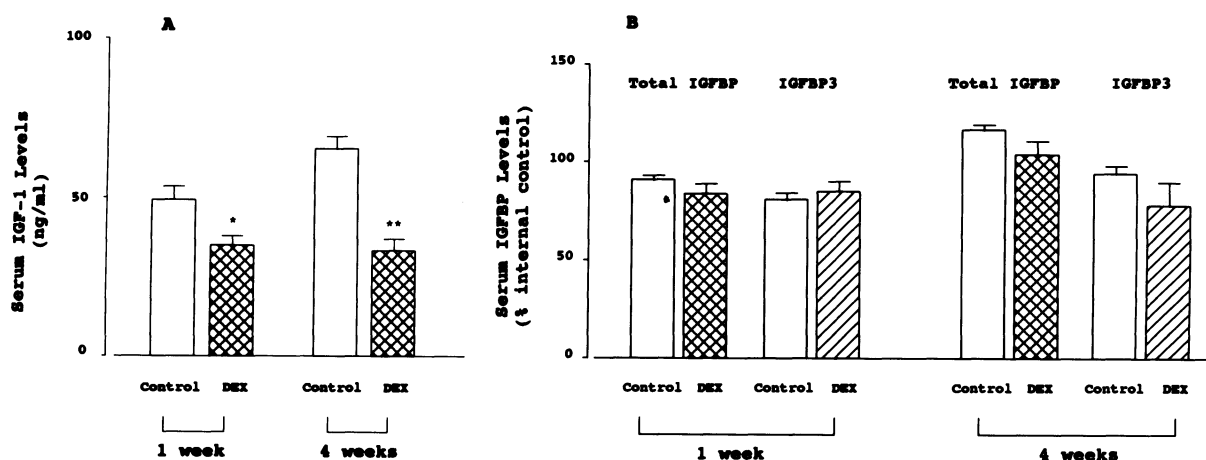Values are the mean ± SEM. *$P<0.05$ (vs. control).

Fig. 3. Effects of DEX on serum IGF-1 levels in rats (A). Effects of DEX on serum levels of total IGFBPs and IGFBP3 in rats (B). Each group was treated with DEX for 1 and 4 weeks, respectively. Each column represents the mean ± SEM (n=5). *, $P<0.05$ vs. control. **, $P<0.01$ vs. control.

pulsatile GH secretion were not altered 1 week after DEX treatment, but spontaneous GH secretion was partially suppressed in DEX-treated rats after 4 weeks. Table 1 shows the computer program (Detect) analysis of spontaneous GH secretion in the control and Dex-treated rats. The peak height and peak area were significantly reduced in the rats treated with DEX for 4 weeks compared with the control rats, but the number of peaks was not significantly altered by DEX treatment. The trough level of GH secretion was significantly lower in the rats treated with DEX for only 1 week.
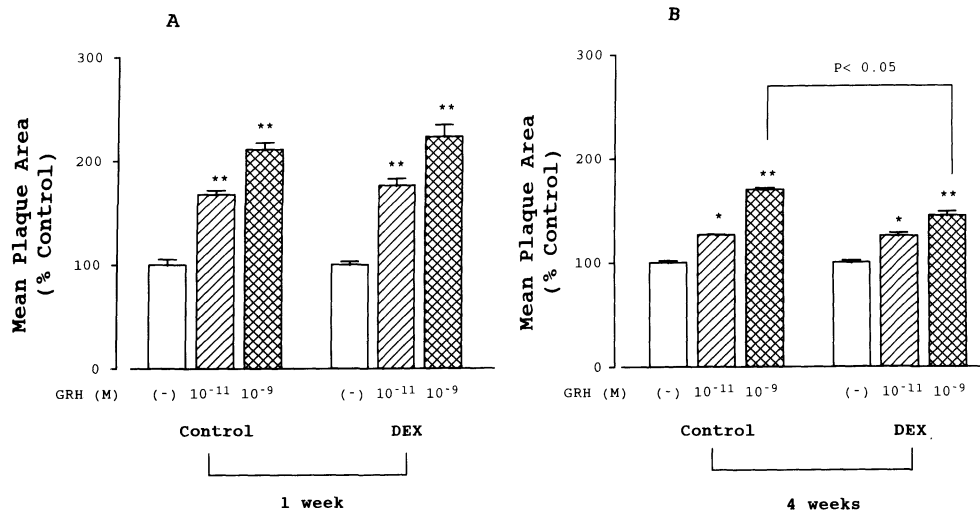
Fig. 4. Effects of administration of DEX for 1 week (A) and 4 weeks (B) on mean plaque area of single pituitary cells assessed by RHPA. Cultured pituitary cells were incubated with GRH at a concentration of 10^{-11} or 10^{-9} M. Each column represents the mean ± SEM (n=5). *, $P<0.05$ vs. control. **, $P<0.01$ vs. control.

Serum IGF-I and IGFBPs

Serum IGF-I levels were much lower after 1 week of DEX treatment than in the control, and remained low for 4 weeks (Fig. 3A). The serum levels of total IGFBPs and IGFBP3 were not significantly changed during the DEX treatment (Fig. 3B).

Pituitary GH responses to GRH in vitro

Figure 4 shows GH responses to GRH in single somatotropes assessed by RHPA. The mean plaque area in single somatotropes obtained from control rats for 1- and 4-week treatment was 3308.8 ± 377.0 and 2605.0 ± 125.8 μm^2 (mean ± SEM, n=5), respectively. The mean plaque area in single somatotropes obtained from the rats treated with DEX for 1 week was similar to that in control rats (Fig. 4A). In contrast, the mean plaque area in single somatotropes obtained from rats treated with DEX for 4 weeks was much lower in the presence of 10^{-9} M GRH than in the control rats (Fig. 4B). The number of somatotropes was not significantly altered by DEX in any of the experiments.

Hypothalamic GRH/SRIF mRNA levels, and pituitary GRH receptor mRNA levels

Figure 5 shows the changes in hypothalamic GRH and SRIF mRNA levels, and pituitary GRH receptor mRNA levels in the DEX-treated rats. Neither hypothalamic GRH nor SRIF mRNA levels were altered by DEX 1 week after the treatment. When a lower dose (40 μg/rat) of DEX was administered to rats for 1 week, the results were quite similar (data not shown). Only hypothalamic GRH mRNA levels were significantly decreased after 4 weeks (Fig. 5A). Although pituitary GRH receptor mRNA levels were not altered by DEX 1 week after DEX treatment, they were significantly increased by DEX 4 weeks after the treatment (Fig. 5B).

Discussion

GC exhibit a number of catabolic changes including increased proteolysis, insulin resistance, collagen degradation, and bone loss [27]. It has also been well documented that GC cause growth failure in humans and laboratory animals [1]. In the present study, high doses of DEX significantly
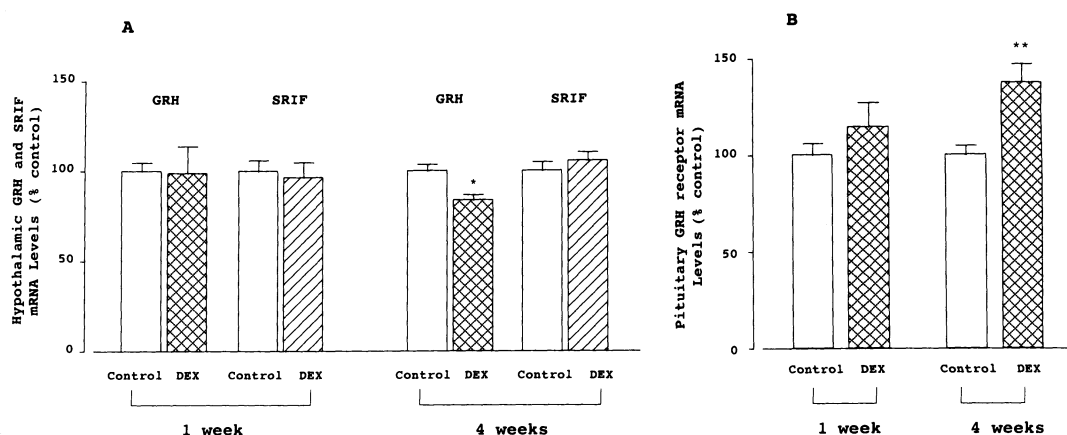
Fig. 5.   Effects of DEX on hypothalamic GRH and SRIF mRNA levels in rats (A). Effects of DEX on pituitary GRH receptor mRNA levels in rats (B). Each column represents the mean ± SEM (n=5). *, $P<0.05$ *vs.* control. **, $P<0.01$ *vs.* control.

inhibited the tibia length in rats 1 week after the treatment and these effects continued with longer (4 weeks) treatment. Similarly, serum levels of IGF-I were lowered 1 week after the DEX treatment and the effects persisted throughout the 4 weeks. Since serum levels of total IGFBPs and IGFBP3 were not affected by DEX, interference in the measurement of IGF-I can be excluded. The inhibitory effects of DEX on serum IGF-I levels are in agreement with previous studies demonstrating that GC directly inhibit the production of IGF-I in many types of cells [8, 9]. By contrast, spontaneous GH secretion was not altered in DEX-treated rats 1 week after the treatment. This observation strongly suggests that DEX-induced suppression of growth and IGF-I is independent of spontaneous GH secretion at the early stage of GC excess. Direct effects of GC on the production of IGF-1 [8, 9] and a reduction in GH receptors [28] appear to be more important.

The RHPA study indicates that GH responses to GRH from single somatotropes are unaffected by DEX 1 week after the treatment. These seem to be incompatible with previous *in vitro* observations in that GC augment GH responses to GRH by their acute effects [29–31]. In addition, pituitary GRH receptor mRNA levels were not altered by DEX 1 week after the treatment in the present study, although we have previously reported that GRH receptor mRNA levels were stimulated by 6-h and 24-h incubation with DEX in cultured rat anterior

pituitary cells [26]. These discrepancies could be explained by the differences in the doses and durations of DEX treatments between *in vivo* and *in vitro*. It is possible that stimulatory effects of DEX on GH release might be masked with longer exposure to a high dose of DEX *in vivo*. Many lines of evidence suggest that hypothalamic SRIF tone is increased by DEX treatment *in vivo* [14–15]. Although we did not find an increase in hypothalamic SRIF mRNA levels in DEX-treated rats in the present study, the trough levels of GH secretion were decreased in these rats. This suggests that SRIF tone is enhanced in the DEX-treated rats, because the trough levels are mediated chiefly by hypothalamic SRIF secretion [32]. The trough levels returned to the normal levels 4 weeks after the DEX treatment. The long-term treatment with DEX might alter SRIF tone or cause a desensitization to SRIF.

In the present study, DEX significantly inhibited spontaneous GH secretion in the rats 4 weeks after the treatment. Furthermore, hypothalamic GRH mRNA levels were significantly decreased in the DEX-treated rats. The inhibitory effects of DEX on GRH mRNA levels seem unlikely due to direct action, since the short-term (1 week) exposure to DEX had no effect on hypothalamic GRH mRNA levels. Long-term treatment with DEX may result in many metabolic changes including protein catabolism, insulin resistance, and lipolysis in the peripheral tissue [27]. It is therefore possible that

reduced GRH mRNA levels are due to metabolic changes in DEX-treated rats. Hypothalamic GRH mRNA levels are influenced by blood glucose levels [25, 33]. We have shown that the diabetic state noticeably inhibits hypothalamic GRH mRNA levels in mice [33], but hyperglycemia did not occur even in rats undergoing long-term (4 weeks) treatment with DEX in the present study. Although hyperinsulinemia was observed in DEX-treated rats, insulin itself is not thought to alter GH secretion or hypothalamic GRH mRNA levels [26]. FFA inhibit GH secretion [34], but this was not altered in DEX-treated rats, so that other metabolic factors including amino acids might be involved. A dietary amino acid, histidine, has been reported to regulate GRH synthesis [35]. We do not preclude the possibility that chronic GC excess affects the metabolism of this amino acid. In addition, since DEX treatment moderately reduced the food intake (21% reduction per day) in rats, a protein deficiency might contribute to the decrease in hypothalamic GRH mRNA levels [35].

Although hypothalamic GRH mRNA levels were decreased by DEX 4 weeks after the treatment, pituitary GRH receptor mRNA levels were inversely increased by the DEX treatment. Miki *et al.* have reported that pituitary GRH receptor mRNA levels were increased by immunoneutralization of GRH in rats [36]. This suggests that decreased GRH secretion leads to an increase in GRH receptors in DEX-treated rats. Similarly, in the DEX-treated rats, enhanced expression of the pituitary GRH receptor mRNA

levels might be due to the decreased GRH secretion. On the other hand, our RHPA study showed that GH responses to GRH from single somatotropes were moderately diminished in DEX-treated rats. Post GRH receptor signaling might be impaired in somatotropes exposed to a high dose of DEX for long periods.

In man, acute administration of GC simulates GH secretion [37]. By contrast, chronic GC excess causes an inhibition of spontaneous GH secretion in man [38] as well as in rats as shown in the present study. In children, prolonged exposure to GC leads to considerable impairment in linear growth [39]. The clinical application of GH for such growth retardation is attractive. Our study suggests that GH resistance might occur in chronic GC excess, since growth failure appeared regardless of spontaneous GH secretion in DEX-treated rats. Further studies are required to elucidate whether GH could be used to prevent the growth retardation in GC excess.

In conclusion, our results indicate that a high dose of DEX exhibits differential effects on IGF-I production and the GH-regulatory system. Chronic exposure to DEX initially inhibits IGF-I production and subsequently inhibits spontaneous GH secretion. Inhibition of GH secretion resulting from a chronic excess of GC is due, at least in part, to the impairment of hypothalamic GRH synthesis and pituitary GH responsiveness. An increase in the pituitary GRH receptor may be caused by decreased GRH secretion.

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