Dichotomic action of glucocorticoids on growth hormone secretion

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Abstract. The mechanism of apparently discrepant actions of glucocorticoids (GC) on GH secretion, in vivo suppression and in vitro potentiation, was studied in rats. Dexamethasone (Dex), at the concentration of 50 nmol/l, potentiated basal and GHRH-stimulated GH release from monolayer culture of normal rat pituitary cells in 48 h. On the other hand, in vivo administration of Dex, 165 μg daily for 3 days, consistently suppressed serum GH levels in female rats. In these rats, the hypothalamic content of immunoreactive (IR) SRIH was significantly increased, whereas that of IR-GHRH was significantly decreased in comparison with the untreated rats. Bioassayable GH-releasing activity was also lower in Dex-treated rats. These findings indicate that the suppressing effect of GC on GH release in vivo is, at least partially, due to the increase in hypothalamic SRIH release and probably also to the decrease in GHRH release, and these effects surpass the potentiating effect of GC on GH release at the pituitary level, resulting in a net inhibitory effect in vivo.

In vivo administration of glucocorticoids (GC) is known to inhibit growth (Loeb 1976). Although the peripheral actions of GC on somatotrope-generating (Mosier et al. 1976) or -effecting organs (Loeb 1976) are responsible to this growth inhibition, the secretion of GH per se is considered to be suppressed by supraphysiological doses of GC in vivo (Franz & Rabkin 1964; Hartog et al. 1964; Nakagawa et al. 1969; Thompson et al. 1972). Also in the majority of patients with acromegaly, plasma GH levels are decreased by pharmacological doses of GC (Nakagawa & Mashimo 1973).

In contrast, these steroids have repeatedly been shown to enhance GH release from pituitary somatotropes in vitro; studies on normal monkey pituitary cells (Kohler et al. 1968), rat GH secreting pituitary tumour GH3 cells (Bancroft et al. 1969) or human normal pituitary (Bridson & Kohler 1970), and somatotrope adenoma cells (Bridson & Kohler 1970) demonstrated potentiating action of GC on GH release by day-unit incubations, although the growth of these cells was inhibited by GC as reflected by population doubling time and total protein synthesis (Bancroft et al. 1969). Vale et al. (1983) also reported increased amount of GH released in monolayer culture of rat pituitary cells in response to GHRH when they were pre-treated with dexamethasone (Dex) for 24 h. We confirmed this effect in human somatotropinoma cells by 48 h-pre-treatment with Dex (Nakagawa et al. 1985). These observations were further supported by the finding that GC increase GH gene transcription (Tushinski et al. 1977), and, more recently, that the activated GC receptor protein binds to the first intron of the hGH gene (Moore et al. 1985).

To clarify the mechanism of this apparently discrepant actions of GC on GH secretion in vivo and in vitro, studies on the effects of Dex treatment on the pituitary and extra-pituitary GH regulating system were performed.

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Materials and Methods

In vitro effects of Dex

Monolayer cultures of rat pituitary cells were prepared from the anterior pituitary glands excised from Wistar-strain female rats as described previously (Nakagawa et al. 1980), except that trypsin and collagenase were used to disperse the cells (Nakagawa et al. 1985). The number of cells was approximately $2 \times 10^6$ per dish. After 4 days, the medium was discarded and the dishes were incubated with 3.5 ml of filtered Dulbecco's modified Eagle medium (DMEM) containing 0.5% bovine serum albumin (DMEM-BSA). Randomly selected one half of dishes contained Dex at the concentration of 50 nmol/l. When tested with GHRH, hGHRH (1–44 amide; Peptide Institute, Minoh, Japan) was included in all dishes at the concentration of 1 nmol/l. Forty-eight h after the beginning of the incubation at 37°C in humidified 95% air/5% CO2, the medium was removed and stored at -20°C for subsequent GH determination. The dishes were washed twice, each time 1.5 ml of DMEM-BSA and frozen. At the time of assay, the cells were removed with a rubber policeman and homogenized in 2 ml of 0.01 mol/l phosphate buffer, pH 7.6, containing 0.15 mol/l NaCl and 0.5% BSA.

In vivo effects of Dex

Wistar-strain female rats were used for 4 series of the experiments of the in vivo effects of Dex. In each experiment, 6–7 rats received 200 µg of Dex sodium phosphate (4 mg/ml; Decadron®, Nippon Merck Banyu Co), equivalent to 165 µg of Dex and diluted 1:10 with physiological saline, for 3 days (once daily in Exp. I and in two divided doses in Exp. II to IV). Same numbers of rat received same volume of physiological saline at the same time schedule. The body weights of the rats were 347.1 ± 13.9 g (mean ± SEM; control group) vs 355.7 ± 10.8 (Dex-treated group) in Exp. I, 260.0 ± 5.2 vs 254.2 ± 3.7 in Exp. II, 344.2 ± 4.2 vs 344.2 ± 4.3 in Exp. III, and 147.0 ± 1.9 vs 145.7 ± 1.9 in Exp IV. Three to 4 h after the last injection, they were sacrificed by decapitation with shortest intervals and blood was collected. In Exp. II and IV, the anterior pituitary and the hypothalamus including the pituitary stalk were removed as described previously (Nakagawa et al. 1980). The anterior pituitary was immediately frozen and, when assayed, homogenized in 5 ml of 0.01 mol/l phosphate buffer, pH 7.6, with 0.5% BSA. The hypothalamus was homogenized immediately in 0.5 ml of 2 mol/l acetic acid and centrifuged. The residue was extracted again with the same volume of acetic acid and the combined extracts were frozen until assayed. For the fractionation of SRIH and GHRH, 1/10 (SRIH) or 3/10 (GHRH) of an extract from each group was subjected to Sephadex G-25 (SRIH) or G-75 (GHRH) column (1 x 50 cm) chromatography, eluted with 0.5 mol/l acetic acid with 0.1% BSA.

Assays

Rat GH was measured by RIA with NIADDK-rGH-I-5 for radioiodination with 125I, NIAMDD-anti-rGH-S-3 and NIADDK-rGH-RP-2, each generously supplied by the National Pituitary Agency or National Hormone and Pituitary Program of USA.

Hypothalamic immunoreactive (IR) SRIH was determined by an enzyme-linked immunoassay. The method originally reported (Nakagawa et al. 1978) was partially modified; SRIH-14 (Peptide Institute) was conjugated with alkaline phosphatase (AP; from bovine intestine, Type VII, Sigma Chemical Co, St. Louis) with glutaraldehyde (50–100 µg of SRIH vs 330 µg of AP). The conjugate was purified with Sephadex G-200 column (1 x 50 cm) and then with anti-SRIH-antibody-coupled Sepharose 4B column (0.7 x 10 cm), the latter eluted with 3.0 mol/l potassium thiocyanate. Anti-SRIH-IR serum was raised in a rabbit immunized with SRIH-human α-globulin conjugate. Anti-rabbit γ-globulin-antibody precipitated AP-SRIH was determined with the phenyl phosphate method using Alkaline Phospha K-Wako kit (Wako Pure Chemical Co, Osaka, Japan). The minimal detectable dose was 5 pg per tube and the intra- and inter-assay variations were less than 5.9% and 11.2%, respectively. A standard displacement curve is depicted in Fig. 1. SRIH-14 (Peninsula Laboratories, San Carlos, USA) showed an equimolar displacement in this assay.

Hypothalamic IR rat GHRH (rGHRH) was determined by a radioimmunoassay, rGHRH was purchased from Peninsula Laboratories. Anti-rGHRH serum was raised in a rabbit immunized with rGHRH-BSA conjugate. This anti-rGHRH antisera cross-reacted with hGHRH (1–44 amide; Peptide Institute) and hGHRH (1–29 amide; generously supplied by Sumitomo Pharmaceutical Co, Osaka, Japan) by less than 0.1%. The minimal detectable dose was 10 pg per tube and the
intra- and inter-assay variations were less than 8.5% and 12.0%, respectively. A standard displacement curve is shown in Fig. 2. In the assay of rGHRH in the hypothalamus, the acid extract was neutralized and 20 mg of silicic acid was added. After 2 washes, each with 1 ml of distilled water, the absorbed materials were extracted with 1 ml of 0.2 mol/l HCl/acetone (1:4). The acid-acetone was washed with an equal volume of petroleum ether and evaporated. The residue was dissolved in the buffer used for RIA.

GH-releasing activity of the hypothalamus was measured with the monolayer culture of anterior pituitary cells from the untreated female rats which was detailed previously for prolactin releasing activity (Nakagawa et al. 1980). A 1/8 and 1/4 of each hypothalamic extract was added for each sample.

Statistical analysis
The significance of difference was tested by unpaired t-test.

Results
In vitro effects of Dex
The effects of 48-h treatment with 50 nmol/l of Dex on the release and synthesis of GH with or without 1 nmol/l of GHRH in monolayer cultures of normal rat pituitary cells are presented in Table 1.

The basal GH release without GHRH was significantly greater in Dex-treated than in control rats. The GH content in the cells adhered on dishes was also significantly higher in Dex-treated than in the controls.

With 1 nmol/l of GHRH, the release of GH in Dex-treated rats was more than twice that in control rats. The content in the cells, on the other hand, was significantly lower in Dex-treated than in control rats.

In vivo effects of Dex
In all 4 experiments of in vivo Dex administration, the serum GH levels were significantly lower in Dex-treated than in control rats as shown in Table 2.

In 2 series examined, the pituitary GH content was slightly increased in Dex-treated rats as compared with the controls, but the difference was not statistically significant (Table 2).

In these 2 series, the hypothalamic content of IR-SRIH was determined. IR-SRIH in Dex-treated groups was significantly higher than in control groups in both series (Table 2). The dilution curves of the hypothalamic extracts were parallel to the standard curve (Fig. 1) and the elution patterns from Sephadex G-25 column were similar in both groups, showing two peaks corresponding to standard SRIH-28 and SRIH-14 (Fig. 3).

Hypothalamic rGHRH content was also determined in these 2 series. The IR-rGHRH content in Dex-treated rats was significantly decreased in
Table 2.
In vivo effect of Dex.

<table>
<thead>
<tr>
<th></th>
<th>Serum GH (µg/l)</th>
<th>Pituitary GH (µg)</th>
<th>Hypothalamic SRIH (ng)</th>
<th>Hypothalamic GHRH (ng)</th>
</tr>
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<tbody>
<tr>
<td>Experiment I</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>30.6 ± 5.4</td>
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<tr>
<td>Dex-treated</td>
<td>14.3 ± 1.1</td>
<td></td>
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<td></td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt; 0.01</td>
<td></td>
<td></td>
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<tr>
<td>Experiment II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>24.1 ± 2.4</td>
<td>166.3 ± 7.0</td>
<td>41.3 ± 3.4</td>
<td>8.72 ± 0.85</td>
</tr>
<tr>
<td>Dex-treated</td>
<td>16.2 ± 1.2</td>
<td>179.9 ± 5.1</td>
<td>63.7 ± 2.5</td>
<td>6.28 ± 0.32</td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt; 0.02</td>
<td>n. s.</td>
<td>&lt; 0.01</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Experiment III</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>28.2 ± 6.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dex-treated</td>
<td>9.5 ± 1.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt; 0.02</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Experiment IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>35.5 ± 6.0</td>
<td>180.7 ± 11.9</td>
<td>35.3 ± 2.3</td>
<td>7.31 ± 0.23</td>
</tr>
<tr>
<td>Dex-treated</td>
<td>13.3 ± 5.1</td>
<td>197.4 ± 15.5</td>
<td>45.5 ± 2.0</td>
<td>6.19 ± 0.22</td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt; 0.05</td>
<td>n. s.</td>
<td>&lt; 0.02</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Mean ± SEM.

Fig. 3.
Elution patterns of IR-SRIH of hypothalamic extracts from the control (*) and Dex-treated rats (○) on Sephadex G-25 column chromatography (lower panel). In the upper panel, elution patterns of authentic SRIH-14 (■) and SRIH-28 (□) are shown.

Fig. 4.
Elution patterns of IR-rGHRH of hypothalamic extracts from the control (*) and Dex-treated rats (○) on Sephadex G-75 column chromatography (lower panel). In the upper panel, elution patterns of authentic rGHRH is shown.
comparison with the control rats in both series (Table 2). The elution patterns from Sephadex G-75 column were similar in both groups, although some IR fractions were found around the main IR peaks corresponding to authentic rGHRH (Fig. 4).

In Exp. IV, the GH-releasing activity in the hypothalamus was assayed in monolayer culture of normal rat pituitary cells. The hypothalamic extracts from Dex-treated rats released less GH into the medium (Table 3), whereas the GH content in the cell layers was greater in Dex-treated rats (with 0.25 hypothalamus; 825 ± 25 in controls vs 1025 ± 50 ng in Dex-treated; \( P < 0.05 \)).

**Table 3.**

<table>
<thead>
<tr>
<th>Hypothalamus</th>
<th>rGH released (ng/dish)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>20.0 ± 4.5</td>
</tr>
<tr>
<td>0.125</td>
<td>102.0 ± 5.0</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Dex-treated</td>
<td>88.0 ± 6.2</td>
</tr>
<tr>
<td>( P )</td>
<td></td>
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<tr>
<td>0.25</td>
<td>184.0 ± 10.6</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Dex-treated</td>
<td>132.0 ± 12.2</td>
</tr>
<tr>
<td>( P )</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Mean ± SEM.

control rats. However, female rats, as used in the present studies, have been reported to show much less remarkable pulses of GH secretion than male rats (Edén 1979), and the changes by Dex seems to have exceeded the variation owing to pulsatile secretion, if it exists. Therefore, the secretion of GH can be considered to be suppressed by in vivo Dex administration.

In these rats with suppressed serum GH levels, IR-SRIH was found to increase significantly in hypothalami from the rats treated with Dex in vivo. Conversely, IR-rGHRH was decreased in hypothalami of Dex-treated rats. Decreased bio-assayable GH-releasing activity in hypothalami of Dex-treated rats confirmed these changes of SRIH and GHRH.

An increase in SRIH content in the hypothalamus would not necessarily indicate an increase in SRIH release from the hypothalamus. However, several previous reports concerning the changes of the hypothalamic content of SRIH, decrease by hypophysectomy (Berelowitz et al. 1981; Fernández-Durango et al. 1978; Wakabayashi et al. 1976) or thyroidectomy (Berelowitz et al. 1980) and increase by GH injection (Berelowitz et al. 1981; Hoffman & Baker 1977), seem to indicate the parallel change of hypothalamic content and release, at least in such subchronic or chronic states. Therefore, it can be presumed that the release of SRIH from the hypothalamus is increased in Dex-treated rats.

The hypothalamic content of GHRH was found to have decreased in the Dex-treated rats in the present study. Recently, Katakami et al. (1986) reported decreased GHRH content in hypothyroid rats. They also observed a parallel change of content and in vitro release of GHRH in the hypothalamus of hypophysectomized rats, though both parameters were increased (Katakami et al. 1987). It is likely, therefore, that the release of GHRH from the hypothalamus was decreased in Dex-treated rats.

Wehrenberg et al. (1983) reported an enhanced GH response to GHRH by Dex administration in vivo in rats. In view of the present study, this seems to be due to the pentobarbital anaesthesia, which is considered to be an inhibitor of SRIH release (Fernández-Durango et al. 1978), and, in their study, only the potentiating effect of Dex at the pituitary level would have been manifest.

GC have diverse pleiotropic actions and the suppression of GH secretion by pharmacological
dose of GC might be induced through a sequence/sequences of more unspecific changes in vivo. The Dex-treated rats in the present experiments lost their weight by 7.2 to 8.9% more than the controls. However, weight loss per se is known to be accompanied by increased GH secretion. Serum TSH levels generally also decreased in the Dex-treated rats, but with rather increased hypothalamic TRH contents (data not shown). Although several other points remain to be examined, the present results explain, at least partially, the discrepancy between in vivo and in vitro studies on the effect of GC on GH release; GC induce dichotomous effects on pituitary GH secretion, i.e. a suppressing effect indirectly by increasing SRIH release, and probably also by decreasing GHRH release, from the hypothalamus, and a potentiating effect directly at the pituitary level. In normal conditions, the former surpasses the later, resulting in an inhibitory effect in vivo.

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