Influence of estrogen administration on growth hormone response to GHRH and L-Dopa in patients with Turner's syndrome

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Abstract. The modulating effect of estrogen on GH secretion was studied in 22 patients with Turner's syndrome. Estrogen administration (0.5 μg/kg ethinylestradiol) for a period of 4 weeks resulted in a significant increase in basal GH concentrations (2.6 vs 4.8 μg/L, P< 0.01). The L-Dopa-stimulated GH concentrations were also significantly increased (P< 0.01), whereas no effect of estrogen substitution on GH responses to GHRH (1−44) and Sm-C levels was seen. Our findings demonstrate a priming effect of estrogen on GH secretion in patients with Turner's syndrome. These patients generally lack the puberty-associated rise in GH secretion, which might be due to ovarian failure and the concomitant estrogen deficiency.

In a recent study, reduced spontaneous GH secretion (basal levels and GH pulse amplitudes) was reported in girls of pubertal age with Turner's syndrome whereas prepubertal patients had a normal GH secretion when compared with age-matched controls, and a possible GH secretory disorder was suggested (Ross et al. 1985). In this study, 14 out of 23 patients had a diminished GH response to L-Dopa but not to insulin or arginin (Ross et al. 1985).

As a consequence of primary ovarian insufficiency, patients with Turner's syndrome lack endogenous estrogens and the well-known pubertal increase of sex steroids. It has been shown that estrogens have a modulating effect on GH secretion (Dawson-Hughes et al. 1986; Lang et al. 1987; Shulman et al. 1987) and on Sm-C levels in humans and animal models (Caufriez et al. 1986; Wilson 1986). A possible explanation for the reduced GH secretion in patients with Turner's syndrome at pubertal age could be the deficit of estrogens.

The aim of this investigation was to analyse the influence of a low-dose estrogen administration on GH response to GHRH and L-Dopa as well as on serum Sm-C in girls of pubertal and adolescent age with gonadal dysgenesis.

Patients and Methods

Twenty-two patients with gonadal dysgenesis participated in the study. The diagnosis was established by lymphocyte karyotype analysis. Nineteen patients had the karyotype 45 XO. Three had mosaics (45 XO/46 XY, 45 XO/46 XX, 46 XIX). Chronological age was 14.2 ± 0.7 years, bone age was 12.3 ± 0.6 years (mean ± SEM) assessed...
according to the method of Greulich & Pyle (1959). Patients had short stature (height $-3.2 \pm 0.67$ standard deviation scores (SDS), mean $\pm$ SEM) and were overweight (+2.0 $\pm$ 1.47 SDS, mean $\pm$ SEM). Ten patients (mean chronological age 16.6 $\pm$ 0.9 years) had been treated with E before the study period; this therapy was interrupted at least 4 weeks prior to the investigation. The other 12 girls (mean chronological age 12.2 $\pm$ 0.7 years) were prepubertal and had never received any androgen or estrogen treatment.

None of the patients had clinical or biochemical symptoms of hypothyroidism; two patients had increased titres of microsomal and thyroglobulin autoantibodies.

Patients were hospitalized at the endocrine ward of the university children’s hospital for the investigation. Informed consent from the patients and their parents was obtained.

Study protocol
GH stimulation tests with GHRH and L-Dopa were performed on two consecutive days before and after a 4-week treatment period with ethinylestradiol (E$_2$) (0.5 µg/kg body weight po). None of the patients had any other medication during the whole study period.

Basal GH levels were determined by drawing blood samples at $-30$ min (L-Dopa) or $-10$ min (GHRH) and immediately before the stimulation test. GH stimulation was performed with GHRH 1–44 (Bissendorf-Peptide, FRG), 1 µg/kg body weight iv. The second stimulation test was performed with L-Dopa, 300 mg/m$^2$ body surface in combination with propranolol 0.75 mg/kg po. A maximal GH level of $>10$ µg/l was considered as normal, $>4$ to $<10$ µg/l as subnormal and $<4$ µg/l as insufficient. In addition, basal serum concentrations of Sm-C, LH, FSH, T$_4$, and T$_3$ were determined before and after the treatment period.

Methods
GH was measured with a RIA test kit (Behring, FRG). The detection limit of this assay was 0.9 µg/l. For comparison the WHO standard 66/217 was used. The intra-assay CV was 3.6% and the inter-assay CV was 4.9%. Sm-C determination was done by RIA (Nichols Institute, USA) with an intra-assay CV of 5.4% and an inter-assay CV of 9.1%.

LH, FSH and T$_4$ levels were measured by commercial RIA-test kits (Behring, FRG; Serono, FRG) using the 1st IRP 68/40 and the 2nd IRP 78/549 standard for LH and FSH, respectively. Intra-assay CV was 5.1 and 5.3%, inter-assay CV 7.3 and 7.6%, respectively, for LH and FSH. E$_2$ serum levels were measured by RIA described by Hümpe1 et al. (1979), with a detection limit of $<50.6$ pmol/l and an intra-assay variance of 3.9%. Samples from individual patients were measured in the same assay.

For statistical analysis we used the Wilcoxon two-sample test.

Results
Basal hormone levels
Basal GH and Sm-C levels before E$_2$ treatment were in the lower normal range when compared with an age-related control group (N = 20, basal GH 4.1 $\pm$ 0.5 µg/l; basal Sm-C 65 $\pm$ 22.5 nmol/l). LH and FSH levels were increased in these patients with gonadal dysgenesis and T$_4$ values were in the normal range (Table 1).

After E$_2$ therapy, basal GH levels increased significantly, whereas there was no significant change in Sm-C in the whole patient group. As expected, gonadotropin levels were significantly lower after E$_2$ therapy, but were not completely suppressed. The decrease in FSH was obviously more pronounced than in LH. T$_4$ levels increased in all patients after E$_2$ without any change in basal TSH values (data not shown).

Serum E$_2$ concentrations were low or undetectable before therapy and could be measured in all but 3 patients at the time of the second investigation ($<50.6$ pmol/l vs 107.3 $\pm$ 42.9 pmol/l).

L-Dopa test (Fig. 1)
Before E$_2$ treatment, the mean GH peak after L-Dopa was 13.6 $\pm$ 1.4 µg/l, which is in the lower normal range of our laboratory (controls N = 21, peak 20.9 $\pm$ 1.2 µg/l). Nine girls had a subnormal

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Basal hormone concentrations before and after E$_2$ therapy in 22 patients in Turner's syndrome (median and range).</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>GH (µg/l)</td>
<td>2.6 (0.9–5.6)</td>
</tr>
<tr>
<td>Sm-C (nmol/l)</td>
<td>32.5 (17.3–92.5)</td>
</tr>
<tr>
<td>LH (U/l)</td>
<td>23.1 (3.8–88.5)</td>
</tr>
<tr>
<td>FSH (U/l)</td>
<td>108.0 (14.1–129)</td>
</tr>
<tr>
<td>T$_4$ (nmol/l)</td>
<td>105.5 (86–141)</td>
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</tbody>
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Growth hormone response to L-Dopa and 2 of them had GH peaks below 4 µg/l. Four of these 9 patients with subnormal GH response to L-Dopa were severely overweight (> +2 sds). After E2 treatment, a significant increase was found in stimulated GH levels and in the area under the curve (942.9 ± 143.1 vs 1764.0 ± 155.4 µg · l⁻¹ · min, P < 0.01).

**GHRH test** (Fig. 2)
The mean maximal level after GHRH was in the normal range of our laboratory (controls N = 12, peak 26.9 ± 3.2 µg/l). Five girls had peak GH levels below 10 µg/l before E2 treatment; 3 of them had also a low response to the L-Dopa test. After E2 therapy, the mean maximal GH concentration increased insignificantly.

**Influence of body weight**
All 22 patients had a height-related body weight above the 50th percentile (Tanner et al. 1966) (mean +2.04, range +0.8 to +6.8 sds); 5 of these girls presented with severe overweight (range: +2.1 to +6.8 sds). Compared with the moderately obese patients, these patients had basal GH levels (1.8 ± 0.29 vs 3.2 ± 0.39 µg/l, P < 0.01) as well as GHRH-stimulated GH levels (9.3 ± 3.3 vs 21.0 ± 1.7 µg/l, P < 0.01) that were significantly lower before treatment with E2 was started. After E2 treatment there was no significant difference in basal (5.8 ± 0.2 vs 6.3 ± 0.9 µg/l), GHRH-stimulated (23.7 ± 2.1 vs 18.1 ± 4.1 µg/l), and L-Dopa-stimulated (24.03 ± 1.9 vs 23.7 ± 3.9 µg/l) GH concentrations between the moderate and the severely obese patients.
Discussion

Several recent investigations have demonstrated a dose-dependent effect of estrogens on basal GH secretion (Ross et al. 1983; Moll et al. 1986). Very low doses during short-term application (Copeland et al. 1984; Moll et al. 1986; Ross et al. 1983; Wilson 1986) did not influence basal GH levels, whereas after pharmacological doses of estrogen, a significant increase in basal GH secretion was the most consistent finding in animal models (Copeland et al. 1984) and in men independent of age (Dawson-Hughes et al. 1986; Moll et al. 1986). In agreement with these observations we found a significant increase in basal GH levels after E_2 in patients with Turner’s syndrome.

The influence of sex steroids on GH response to various forms of stimulation is still a matter of discussion. Short-term application of sex steroids has been shown to increase the GH response in patients with constitutional delay of growth and development (Moll et al. 1986) and is used for ‘priming’ in diagnostic tests. Liu et al. (1987) have shown an increase in spontaneous GH secretion in adult patients with idiopathic hypogonadotropic hypogonadism by substitution therapy with sex steroids, but also with gonadotropins or with gonadotropin-releasing hormone. GH response to exercise (Greene et al. 1987) and to stimulation with arginin (Sperling et al. 1970) as well as spontaneous GH secretion (Mauras et al. 1987) increase significantly with the progress of pubertal development.

On the other hand, GHRH-stimulated GH responses are rather constant during childhood and puberty (Gelato et al. 1986). Low-dose estrogen had no influence on GHRH-stimulated GH in castrated female rats, whereas high doses decreased the GH response (Shulman et al. 1987). Similarly, there was no increase in GHRH-stimulated GH levels after E_2 treatment for 28 days in postmenopausal women, although basal and exercise-stimulated GH concentrations increased (Dawson-Hughes et al. 1986). Our results are in agreement with these observations as we could demonstrate a significant increase in basal and L-Dopa-stimulated GH concentrations in patients with Turner’s syndrome when treated with E_2, whereas the response to GHRH was hardly influenced.

In contrast to our results and the study of Dawson-Hughes et al. (1986) is the observation of Lang et al. (1987) who reported a significant positive influence of endogenous estradiol on GHRH response in male and female adults. It is not clear whether age dependency or different effects by exogenous or endogenous estrogens or the known variability of the GHRH test response (Gelato et al. 1986) may account for this discrepancy.

The effect of estrogen on Sm-C has been found to be dose- and age-dependent and dissociated from the effect on GH (Wilson 1986; Ross et al. 1983; Dawson-Hughes et al. 1986).

In our patients, Sm-C was in the lower normal range. We failed to detect an increasing influence of E_2 on Sm-C, which might be due to the relatively high E dosage (Ross et al. 1983). Overweight is a common finding in Turner’s syndrome. In obese children (Rosskamp et al. 1987) and adults (Williams et al. 1985), the responsiveness of GH to various stimuli was blunted, which could be reversed by weight reduction (William et al. 1985). It was not surprising that the most obese of our patients had the lowest basal and stimulated GH levels. Therefore, body weight should be considered when GH values are evaluated in patients with Turner’s syndrome.

Our data indicate that the absence of ovarian steroids might be the cause of the impairment of GH and Sm-C secretion in patients of pubertal age with Turner’s syndrome. It remains to be clarified whether low-dose estrogens and their modulating effect on GH and Sm-C secretion may be of advantage in growth-promoting therapeutic regimens for patients with Turner’s syndrome.

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References


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