Differential effect of estrogen on pituitary responsiveness to GnRH in women with different forms of hypothalamic amenorrhea


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Abstract. The effect of treatment with estradiol valerate (6 days, 2-6 mg/day) on basal levels of LH and FSH and on response of LH and FSH levels to GnRH challenge (2 × 25 μg GnRH, iv) were investigated in women with "hypothalamic amenorrhea", but without other endocrine disorders. Three groups were studied: 11 women with primary amenorrhea, 10 women exhibiting secondary amenorrhea related with weight loss, and 7 women with normal weight and with amenorrhea persisting after a period of severe weight loss. Before treatment with estradiol valerate the estradiol concentrations in all women were at the lower limit of the follicular phase of a normal ovulatory cycle. In addition, there were no differences between the groups in basal LH and FSH levels and in responses to GnRH challenges. Treatment with estradiol valerate suppressed the basal levels of FSH but not of LH in all women. Estradiol did not affect the response to GnRH challenge in women with primary amenorrhea, weakly augmented the response in women with secondary amenorrhea associated with weight loss, and strongly increased the response in secondary amenorrheic women who had regained normal weight. The results are interpreted in the light of the well-established fact that estrogen augments the gonadotropin response only if the pituitary gland is not exposed to high concentrations of GnRH. It is hypothesized that the differential response to GnRH of the present patients after estrogen treatment reflects differences in GnRH exposure of the pituitary gland, with patients with primary amenorrhea having the highest level of GnRH exposure.

Disorders of the neuroendocrine system regulating ovarian function generally result in anovulation and amenorrhea. When no organic ovarian or pituitary lesion is demonstrable, yet the secretion of gonadotropins and gonadal steroids is clearly defective, the disorder is classified as "hypothalamic amenorrhea" (1,2).

Hypothalamic amenorrhea is a diverse clinical syndrome (3,4). The condition may result in either incomplete or even absent pubertal development (primary hypothalamic amenorrhea), but may also present itself post-pubertally (secondary hypothalamic amenorrhea). This latter type of regression of ovarian function is often seen in relation to mental stress (5), excessive physical exercise (6) or extreme weight loss (7). In these latter patients menses generally recur spontaneously once the body weight increases beyond a certain critical value (8-11).

Women with hypothalamic amenorrhea differ in their response to hormonal treatment depending on the clinical syndrome. Withdrawal bleeding following gestagen treatment (12) and induction of ovulation following clomiphene therapy are not observed in all patients (13,14). The way estrogens change the pituitary responsiveness to GnRH, too, is not always the same. In women with hypothalamic amenorrhea, estrogen may augment as well as inhibit the gonadotropin response (15,16). In cycling women, on the other hand, the gonadotropin response to GnRH is always augmented by estrogen (17-19).

In this study we investigated in women with hypothalamic amenorrhea the differential effects of estrogen on the pituitary gonadotropin response to GnRH. According to the type of amenorrhea, three clinically defined groups of patients with hypothalamic amenorrhea were compared (primary...
hypothalamic amenorrhea; secondary hypothalamic amenorrhea associated with weight loss, and secondary hypothalamic amenorrhea with normal weight after a period of weight loss). Basal levels of LH, FSH and estradiol as well as LH and FSH responses to GnRH challenge before and after a 6-day estrogen-treatment were measured.

Patients and Methods
This study includes 28 patients visiting our department. All patients had hypothalamic amenorrhea but no other endocrine disorder (adrenal and thyroid function were within normal limits; no patient had clinical evidence of galactorrhea and basal levels of prolactin were within the normal range).

Patients were divided into 3 clinically defined groups:
Group 1: Primary amenorrhea; age 27±1.4 (range 21-37) years; weight 65.6±3.2 kg; length 170.5±2.4 cm; Body Mass Index 22.5±1.0 (N=11). One of these patients had Kallmann's syndrome (age 37 years; weight 72.5 kg; length 165 cm; Body Mass Index 26.6).

Group 2: Secondary amenorrhea owing to weight loss (weight loss-related amenorrhea); age 29±1.5 (range 21-36) years; weight 48.3±1.0 kg; length 170.1±1.4 cm; Body Mass Index 16.7±0.4; duration of amenorrhea 5.3±0.9 (range 2-11) years, (N=10). These women did not respond to clomiphene therapy.

Group 3: Women who had returned to normal body weight, but who were still amenorrheic (normal weight with amenorrhea), age 27.3±1.1 (range 24-32) years; weight 59.2±1.5 kg; length 167.4±1.3 cm; Body Mass Index 21.2±0.6; duration of amenorrhea 6.1±1.0 (range 3-9) years; (N=7).

Data are means ± sem. Patients participated in the experiments at least 12 weeks after the last substitution treatment. Each woman gave written informed consent and the study was approved by the Groningen Medical Ethical Committee.

Hormonal treatment
On days 0 and 6 of the protocol, at t=0 and t=90 min (first injection given between 08.00 and 09.00 h), patients received iv 2 doses of 25 mg GnRH (Hoechst) according to Rozenman et al. (20). On days 0 through 6, the subjects received oral doses of estradiol valerate (days 0 and 1: 1×2 mg; days 2 and 3: 2×2 mg, days 4 and 5: 3×2 mg; day 6, 1 h before GnRH challenge: 2 mg). Blood samples for measurement of LH, FSH and estradiol were obtained via indwelling catheters in the arm at t=-15, 0, 60, 90, 110, 150 and 240 min. The blood was allowed to clot and the serum was then separated by centrifugation and frozen until assayed. Unstimulated levels of LH, FSH and estradiol were measured in the t=-15 and the t=0 sample, the latter of which was taken immediately before GnRH administration. All samples were measured simultaneously. LH, FSH and estradiol were measured at the Groningen Isotope Laboratory. Serum estradiol concentrations were measured by radioimmunoassay according to the method of Jurjens et al. (21) and expressed in nmol/l; serum concentrations of LH and FSH were measured by double-antibody solid-phase radioimmunoassay using LH-MRC 68/40 and FSH-MRC 69/104 as reference preparations and expressed in IU/l. Intra- and inter-assay

Table 1.
Basal LH and FSH (IU/l serum) and estradiol (nmol/l serum) levels in 3 groups of amenorrheic women before and after treatment with estradiol valerate. Group 1: primary amenorrhea (N=11); group 2: weight loss-related amenorrhea (N=10); group 3: normal weight after a period of severe weight loss and amenorrhea (n=7). Values are means ± sem. For the effect of estradiol valerate: x-xx: p<0.05 (Wilcoxon's signed rank test).

<table>
<thead>
<tr>
<th>Type of amenorrhea</th>
<th>Basal LH Before estradiol valerate</th>
<th>After estradiol valerate</th>
<th>Basal FSH Before estradiol valerate</th>
<th>After estradiol valerate</th>
<th>Serum estradiol Before estradiol valerate</th>
<th>After estradiol valerate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary hypothalamic amenorrhea</td>
<td>3.2±0.6</td>
<td>3.2±0.7</td>
<td>5.1±0.5*</td>
<td>2.7±0.5 **</td>
<td>0.06±0.01</td>
<td>0.51±0.07</td>
</tr>
<tr>
<td>Weight loss-related amenorrhea</td>
<td>3.3±0.8</td>
<td>1.8±0.4</td>
<td>4.9±0.7*</td>
<td>1.6±0.7**</td>
<td>0.07±0.01</td>
<td>0.44±0.09</td>
</tr>
<tr>
<td>Normal weight and amenorrhea</td>
<td>3.8±0.7</td>
<td>4.1±1.3</td>
<td>6.2±1.2*</td>
<td>3.5±1.0**</td>
<td>0.11±0.03</td>
<td>0.49±0.10</td>
</tr>
</tbody>
</table>

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coefficients of variation were for both assays 2-4% and 3-7%, respectively; the sensitivity of the LH assay was 1 IU/l and that of FSH 0.5 IU/l.

**Parameters and statistics**

Data are expressed as mean ± SEM. LH and FSH responses were judged according to the mean maximal increment of the LH/FSH concentrations in the serum. Incremental values were calculated by subtracting the basal concentrations (i.e. the mean of the t=−15 and the t=0 samples) from the maximal LH and FSH concentrations induced by the second GnRH injection. Maximal LH and FSH concentrations were reached at t=110 min and t=150 min, respectively. Pairs of data were compared statistically by Wilcoxon’s signed rank test. Groups of data were compared by Duncan’s multiple comparison test (22).

**Results**

There was no significant difference between basal LH, FSH and estradiol levels of the pre-estradiol treatment samples between the various groups studied (Table 1). The pretreatment estradiol levels were at the lower limit of the normal range during the early follicular phase of the ovulatory cycle (days 1-5: 0.07-0.30 nmol/l; N=10). After treatment with estradiol valerate, the mean serum estradiol levels were increased to about 0.50 nmol/l in all groups at the time of the second GnRH challenge. Estradiol valerate significantly suppressed the serum FSH levels in patients with primary hypothalamic amenorrhea, weight loss-related amenorrhea, and patients with normal weight and amenorrhea. Estradiol valerate did not change basal levels of LH.

LH and FSH responses induced by GnRH before and after estradiol treatment are shown in Fig. 1. There was no significant difference between the pre-estradiol treatment responses of the 3 groups studied, but after estradiol treatment there was a significant differential response to GnRH. In patients with primary hypothalamic amenorrhea, the pre- and post-estradiol treatment responses were the same; in patients with weight-loss related amenorrhea there was a small yet significant augmentation of the LH and FSH response, and in women with normal weight and amenorrhea the response was strongly augmented and was significantly higher than that of patients with primary hypothalamic amenorrhea. The height of the response in patients with weight loss-related amenorrhea was intermediate to that of subjects with primary hypothalamic amenorrhea and subjects with normal weight and amenorrhea.

**Discussion**

This study shows that in women with different forms of hypothalamic amenorrhea the effect of estrogen on GnRH-induced gonadotropin responses is also different: estradiol had no effect on

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**Fig. 1**

LH (left panel) and FSH (right panel) increments of the serum LH and FSH concentrations following GnRH challenge (25 μg iv) in 3 groups of amenorrheic women before (open bars) and after (closed bars) treatment with estradiol valerate. Values are means ± SEM.


For the effect of estradiol valerate, x-xx p<0.005; 0-00 p<0.025 (Wilcoxon’s signed rank test); a,b vs c: p<0.05 (Duncan’s multiple comparison test).
the responses of patients with primary amenorrhea, had a weak augmenting effect in patients with weight loss-related amenorrhea, and a strong augmenting effect in amenorrheic patients who had returned to normal body weight after a period of severe weight loss. In fact, in these latter subjects the effect of estrogen was essentially normal again (cf. 17-19).

Our data show that the effect of estrogen on the gonadotropin response varies with the type of the defect of the system controlling gonadal function. They also show that the effect of estrogen on the pituitary gland varies with the body mass. Data like these raise the question of how the effect of estrogen on the pituitary gland is controlled.

Rozenman et al. (20) observed in women whose LH and FSH levels did not respond to gestagen and clomiphene therapy that estrogen increased the response to GnRH challenge only before a 3-day treatment with GnRH (5 pulses of 20 µg/day). After such an GnRH-treatment, the augmenting effect of estrogen did not appear any more. Similar data have recently been obtained in rats. In these animals estradiol augmented the gonadotropin response of the pituitary gland only if the gland was exposed to "low" concentrations of GnRH (less than about 70 pmol/l). With higher concentrations the effect of estradiol was inhibitory (23,24). Apparently, the effect of estrogen on the pituitary gland (augmenting or inhibitory) depends on the concentration of GnRH to which the pituitary gland is exposed at the beginning of estrogen administration.

In fertile women low GnRH exposure of the pituitary gland is ensured by the negative feedback of gonadal hormones, notably estrogens, on the secretion of GnRH by the hypothalamus (25). In this condition an increase in estrogen augments the gonadotropin response to GnRH. In patients with hypothalamic amenorrhea GnRH exposure of the pituitary gland is also believed to be low, because the disorder is generally held to be caused by insufficient secretion of GnRH by the hypothalamus (1,2). Consequently, one would expect estrogen to exert a strong augmenting effect in patients with hypothalamic amenorrhea of all types. This, however, is not the case. Moreover, patients with hypothalamic amenorrhea still maintain significant serum levels of LH and FSH, and the fact that the latter is suppressible by estrogen suggests that the hypothalamus secretes significant amounts of GnRH. Taken together with the absence of any effect of estradiol in patients with primary hypothalamic amenorrhea and only a weak augmenting effect in patients with weight loss-related amenorrhea, this observation may suggest that the pituitary gland of such individuals is exposed to elevated rather than to too low concentrations of GnRH.

Exposure to elevated concentrations of GnRH may not only explain the absence of the augmenting effect of estradiol in patients with primary hypothalamic amenorrhea but also the very condition of hypothalamic amenorrhea: continuous exposure of the pituitary gland to elevated concentrations of GnRH or GnRH analogue desensitizes the gland and suppresses the blood levels of LH, FSH and gonadal steroids (26). This may lead to hypogonadotropic hypogonadism, referred to as "chemical castration".

We present an alternative hypothesis concerning the nature of hypothalamic amenorrhea. We suggest that hypothalamic amenorrhea may be due to some yet undefined dysregulation of the secretion of GnRH by the hypothalamus resulting in either a permanent and irreversible (primary hypothalamic amenorrhea) or a temporal and reversible (secondary hypothalamic amenorrhea) "hypersecretion" of the neurohormone. The good correlation between the magnitude of the estrogen effect and the seriousness of hypothalamic amenorrhea may suggest that the putative hypersecretion of GnRH may vary between a minimal dysfunction (patients with normal weight and amenorrhea) and a major defect (patients with primary hypothalamic amenorrhea). According to this hypothesis hypothalamic amenorrhea resembles the state of "chemical castration". Obviously, this hypothesis needs further study.

Acknowledgments

Estradiol, LH and FSH in human serum were measured by Dr J. J. Pratt at the Groningen Isotope Laboratory. The comments of Dr S. Jager are gratefully acknowledged.

References