Intravenous Administration of Pulsatile Gonadotropin-Releasing Hormone in Hypothalamic Amenorrhea: Effects of Dosage*

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ABSTRACT. Eighteen women with well characterized hypothalamic amenorrhea underwent 30 cycles of pulsatile GnRH treatment in an effort to examine the role of GnRH dosage in pituitary and ovarian responses. GnRH was administered iv at 2 doses (25 and 100 ng/kg bolus) at a physiological range of frequencies (90 and 60 min) in the follicular phase of the induced cycles. After demonstration of ovulation by ultrasound and clinical parameters, the frequency of GnRH administration was progressively slowed from every 60 min to every 90 min and then to every 240 min to mimic the slowing of endogenous LH secretion that occurs during the luteal phase in normal women. The results of these induced cycles were compared to those of 62 ovulatory cycles from normal women.

Overall clinical and biochemical results revealed the following. Patients receiving doses of 25 ng/kg GnRH successfully ovulated only 80% of the time, with recruitment of a single dominant follicle. Two of 5 patients became pregnant. Peak estradiol levels were significantly lower than normal [261 ± 33 (±SE) vs. 342 ± 11 pg/ml, respectively; P < 0.02]. Integrated luteal phase progesterone production was also significantly reduced in the 25 ng/kg group compared to normal (78 ± 17 vs. 145 ± 8 ng/ml/entire luteal phase, respectively; P < 0.02). All women receiving bolus doses of 100 ng/kg GnRH ovulated; maturation of multiple follicles occurred in 5 of 20 cycles, and 6 of 7 women conceived. Peak estradiol values were significantly higher than those in either normal women or the 25 ng/kg group (478 ± 48 pg/ml; P < 0.02 for both), with integrated luteal phase progesterone levels significantly higher than those in patients receiving the 25 ng/kg dose (196 ± 28 ng/ml/luteal phase; P < 0.02).

This study demonstrates that 1) ovulation and fertility can be achieved with a physiological frequency regimen of pulsatile GnRH administration using bolus doses of both 25 and 100 ng/kg in women with hypothalamic amenorrhea; 2) the 25 ng/kg dose of GnRH may represent a threshold of stimulation of the pituitary-ovarian axis and recreates cycles with an inadequate luteal phase; and 3) a 100 ng/kg dose of GnRH may well cause a supraphysiological stimulation of the pituitary-gonadal axis. (J Clin Endocrinol Metab 62: 109, 1986)

HYPOTHALAMIC amenorrhea is the clinical syndrome that results from disordered secretion of endogenous GnRH (1–3). Due to this impairment of normal neuroendocrine function, these patients have arrested folliculogenesis and, thus, are ideal candidates for replacement with a GnRH regimen that simulates the normal program of hypothalamic secretion of this releasing factor. In addition, this disorder provides an opportunity to test various regimens of GnRH treatment and compare their abilities to reconstruct the normal ovulatory process in a physiological manner. Such an effort, however, assumes the prior acquisition of normative data which can be employed to derive a regimen of GnRH administration that mimics the pattern of GnRH secretion that occurs during the normal menstrual cycle.

We have previously accumulated data from 62 ovulatory menstrual cycles in normal women, in whom gonadotropins and sex steroids were measured daily for a complete cycle, and gonadotropins were measured at 10-min intervals at 1 of 6 key periods of follicular or corpus luteum function (4, 5). From these data, a program of frequency of GnRH administration was constructed to mirror that of normal endogenous GnRH secretion. Using this frequency program and the iv route of administration of GnRH (6, 7), we held these two critical variables constant and examined the impact of varying the dosage of GnRH on the resulting pituitary and ovarian responses.

Materials and Methods

Patient population

Normal subjects. The control series consisted of studies of 62
ovulatory menstrual cycles in 39 paid normal women, aged 18–39 (8). Each subject had 1) a history of regular 27- to 32-day cycles, 2) a body weight within 1 SD of normal (9), 3) no history of heavy exercise, 4) no evidence of hirsutism, galactorrhea, or genital abnormalities; 5) normal serum PRL and T levels, and 6) normal luteal function in the menstrual cycle preceding the study, as determined by a biphasic basal body temperature chart and a midluteal serum progesterone level greater than 6 ng/ml. None had received any medication for at least 6 months preceding the study.

Amenorrheic patients. Eighteen women, aged 17–39 yr (mean, 28.1 yr), who were otherwise healthy, had the following clinical and biochemical findings: 1) normo- or hypogonadotropic amenorrhea of at least 6 months duration, 2) body weight greater than or equal to 1 SD below average [mean weight 67.9 ± 20.0 (±SD) kg; mean height, 165.5 ± 8.5 (±SD) cm]; 9), 3) an absence of a history of intensive exercise, 4) a normal physical examination, with no hirsutism or ovarian enlargement, 5) normal serum TSH, T₄, T₃, and PRL levels, 6) no androgen excess (normal 24-h urinary 17-ketosteroid excretion and/or normal serum dehydroepiandrosterone sulfate and testosterone levels), and 7) no clinical or roentgenographic evidence of a hypothalamic or pituitary defect. None of these subjects had received any sex steroid therapy for at least 6 months before participation. Informed consent was obtained from each subject before participation. This study was approved by the Subcommittee on Human Studies of the Massachusetts General Hospital.

Nine of the study subjects had primary amenorrhea and, thus, were considered to have idiopathic hypogonadotropic hypogonadism (IHH); of these, three were anosmic (Kallmann’s syndrome). The diagnosis of IHH was confirmed in all women by normal cranial tomography of the hypothalamic-pituitary area and normal basal and stimulated levels of other anterior pituitary hormones in response to insulin (0.15 U/kg) and TRH (200 µg). The remaining nine patients had secondary amenorrhea, and all had abnormal baseline gonadotropin secretory profiles indicative of GnRH deficiency, as previously reported (3). In those patients desiring pregnancy, tubal patency was assured by either hysterosalpingography or laparoscopic dye studies, and postcoital tests were normal, as was seminal fluid analysis of the partner.

Protocol

Monitoring. Normal subjects: The normal women were monitored with a single daily blood sample for gonadotropin and sex steroid levels and basal body temperature charting.

Amenorrheic patients: All amenorrheic patients were monitored with daily blood sampling for LH, FSH, estradiol (E₂), and progesterone (P). Each blood sample was obtained 45 min after a GnRH dose. GnRH was administered iv via portable infusion pumps (Ferring), the reservoir of which contained a heparinized (100 IU/ml) solution of GnRH. Intravenous lines were inspected visually daily and were maintained for up to 14 days. The course of follicular maturation was followed by serial ultrasonography, cervical mucus examinations, and basal body temperature charting.

GnRH dosage and frequency. The frequency of GnRH admin-
Laboratory methods

All serum samples from each subject were processed in a single RIA for LH and FSH determinations, as previously described (4). Values were expressed as equivalents of the Second International Reference Preparation of human menopausal gonadotropin (milliinternational units per ml). The interassay and intraassay coefficients of variation for FSH were 12.3% and 7.8%, respectively; the corresponding values for LH were 5.4% and 5.8%. Serum E2 and P values were determined as previously described (4). The interassay coefficient of variation for E2 at 50% hormone binding was 5.8%; the corresponding value for the P assay was 5.8%.

Data analysis

Analysis of all results was standardized to the day of the midcycle surge. Only ovulatory cycles, defined as those in which luteal P rose to at least 3 ng/ml, were included in data analysis of all patients and normal women. The day of the midcycle surge, designated day 0, was required to contain three of the following four criteria: (1) the day of the LH peak, (2) the day of the FSH peak, (3) the day of or day after the peak E2 level, and (4) the day P levels doubled from baseline and/or reached 0.6 ng/ml (10). Using this system, data represented at the extremes of the figures consist of observations from progressively fewer patients. In addition, peak mean E2 levels (obtained by averaging individual peak values) may vary from the depicted mean on the day of the midcycle surge due to the criteria used for determination of the midcycle surge (see above). FSH levels were also analyzed according to the first day of menses in normal women or the first day of GnRH administration in the patient groups.

To evaluate corpus luteum function, P levels were integrated from day −2 (i.e., 2 days before the midcycle surge) to the day of subsequent menses using a computerized program which yielded a sum of the area under the P curve (Psum). Data from patients who conceived during GnRH therapy were not included in the luteal phase P data; however, gonadotropins and E2 values from these patients were combined with those of the other patients up to 7 days postmidcycle surge, i.e., before the onset of detectable serum hCG levels. Inadequate luteal phases were defined as those having a peak P value of 6 ng/ml or less and/or a duration of less than 10 days. Ovulatory cycles manifesting inadequate luteal phases were included in data analysis.

Statistics

Results of the GnRH-induced cycles were compared to those of normal women using two-tailed t testing. All gonadotropin and sex steroid values are given as the mean ± SEM.

Results

Normal women

The normal profiles of LH, FSH, E2, and P in the cycles from the normal women are portrayed as the shaded background in Figs. 1 and 2 and have been reported previously (8). By the above criteria, all of these cycles were ovulatory. Mean follicular phase length was 13.7 ± 0.3 days, and mean luteal phase length was 14.0 ± 0.3 days. Individual peak mean follicular phase E2 levels were 342 ± 11 pg/ml, and luteal phase P sums were 145 ± 8 ng/ml. Only one of these cycles had an inadequate luteal phase.

Amenorrheic patients: clinical results

The pretreatment baseline evaluation of the amenorrheic women revealed the following characteristics. Serum E2 levels were near assay detection limits (20 pg/ml) in these subjects, with a mean of 23 ± 2 pg/ml. PRL levels were normal (<15 ng/ml) in all subjects, with a mean of 5.2 ± 0.8 ng/ml. Serum total and free testosterone levels were 52.9 ± 11.2 and 0.64 ± 0.12 ng/dl, respectively, both well within normal limits (25–90 and 0.09–1.28 ng/dl). Twenty-four-hour 17-ketosteroid excretion, presumably due to the negligible contribution of ovarian androgens in these hypogonadal subjects, was 4.0 ± 1.0 mg/24 h, with a normal range of 4–16 mg/24 h.

The overall clinical results of the ovulation induction cycles are summarized in Table 1. Since not all patients who requested ovulation induction were attempting to conceive, the percentage of cycles in which conception occurred is adjusted for the number of women pursuing this outcome.

Amenorrheic patients: effects of GnRH dose

25 ng/kg cycles. The 25 ng/kg regimen resulted in folliculogenesis and ovulation in 8 of 10 cycles. In 1 cycle, folliculogenesis occurred, with growth of a single follicle to 2.9 cm and a rise in serum E2 to 224 pg/ml; however, P levels did not exceed 1.9 ng/ml after the initial E2 peak. The other anovulatory cycle was characterized by a minimal steroid response, no discernible follicular development, and termination of the cycle after 25 days. Two of 5 women attempting pregnancy conceived. Both conceptions occurred during the second cycle of GnRH. The mean durations of the follicular and luteal phases in the ovulatory cycles were 13.9 ± 2.0 and 12.7 ± 0.9 days, respectively, neither of which differed from normal. A single dominant follicle was seen by ultrasound examination in all of these ovulatory cycles, with circulating E2 and P levels consistent with a single ovulation in every case. Gonadotropin and sex steroid patterns in these 25 ng/kg cycles are contrasted with those in normal women in Fig. 1. Since the data shown in this figure are standardized to the day of the midcycle surge, as defined above, values at the extremes of this graph are representative of progressively fewer observations. As can be seen, E2 secretion followed a pattern similar to but
Fig. 1. Serum LH, FSH, E2, and P concentrations (mean ± SEM) during GnRH administration (25 ng/kg) to women with hypothalamic amenorrhea. Mean ± SEM values in normal women are represented by the shaded areas. Values beyond −6 or +9 days on the x-axis represent observations on progressively fewer subjects than the indicated number (n). Unconnected points represent observations on a single patient.

Fig. 2. Comparison of serum FSH levels (mean ± SEM) during the early follicular phase of the menstrual cycle in normal women (shaded background; ±1 SE) and in women receiving 25 and 100 ng/kg GnRH. Data are standardized to the onset of menses in normal women or the first day of GnRH administration. Mean FSH levels in the 25 ng/kg group increased at the beginning of the cycles, but integrated FSH levels over the first 6 days of the 25 ng/kg cycles were slightly but not significantly lower than those in normal women.

<table>
<thead>
<tr>
<th>Dose</th>
<th>No. of patients</th>
<th>No. of cycles</th>
<th>% Ovulation</th>
<th>% Conception</th>
<th>E2 peak (pg/ml)</th>
<th>Psum (ng/ml)</th>
<th>% P &gt; 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 ng/kg</td>
<td>7</td>
<td>10</td>
<td>80</td>
<td>40</td>
<td>261 ± 33*</td>
<td>78 ± 17*</td>
<td>87.5</td>
</tr>
<tr>
<td>100 ng/kg</td>
<td>12</td>
<td>20</td>
<td>100</td>
<td>86</td>
<td>478 ± 48*</td>
<td>196 ± 25*</td>
<td>100</td>
</tr>
<tr>
<td>Normal</td>
<td>39</td>
<td>62</td>
<td>100</td>
<td></td>
<td>342 ± 11*</td>
<td>145 ± 8*</td>
<td>98.4</td>
</tr>
</tbody>
</table>

The percentage of conceptions is adjusted for the number of women attempting pregnancy in each group.
* P < 0.02 vs. 25 ng/kg.
* P < 0.02 vs. 100 ng/kg.

slightly lower than normal. The individual mean peak E2 level was 261 ± 33 pg/ml, which was significantly lower than that in normal women (P = 0.012). In addition, P production by the subsequent corpus luteum was well below that in the normal women, as demonstrated by the luteal phase sums of P (78 ± 17 ng/ml/luteal phase; P = 0.01). The early follicular phase FSH levels in the 25 ng/kg cycles also were below the lower limits of the normal range. In Fig. 2, FSH levels are shown standardized to day 1 of the normal menstrual cycle or the first day of pulsatile GnRH administration. Mean FSH levels in the 25 ng/kg group increased at the beginning of the cycles, but integrated FSH levels over the first 6 days of the 25 ng/kg cycles were slightly but not significantly lower than those in normal women.
100 ng/kg cycles. The 100 ng/kg cycles are compared to those in the normal women in Fig. 3. All patients who received this dose of GnRH ovulated, and 6 of the 7 women attempting pregnancy successfully conceived (all within 3 cycles of therapy). There was 1 twin pregnancy. Once again, the durations of the follicular and luteal phases (12.8 ± 0.8 and 14.1 ± 0.6 days, respectively) did not differ from those in normal women or the 25 ng/kg group. In contrast to the situation in the 25 ng/kg group, ultrasonic evidence of recruitment of multiple follicles was common in the 100 ng/kg group (seen in 5 of 20 cycles), with E₂ and P values in these cycles approximately twice normal, consistent with multiple ovulation. As can be seen in Fig. 3, E₂ secretion was greater than normal, with an individual mean peak value of 478 ± 48 pg/ml (P = 0.011 vs. normal). P secretion by the corpus luteum of the 100 ng/kg cycles was slightly but not significantly greater than normal (196 ± 25 ng/ml/luteal phase; P = 0.073), due to the scatter of values engendered by the presence of single and multiple corpora lutea. Integrated luteal P sums in the 100 ng/kg cycles were significantly increased compared to those in the 25 ng/kg cycles (P < 0.01). The pattern of augmented FSH secretion in the early follicular phase of the 100 ng/kg cycles (Fig. 2) was not statistically different from that in normal women or women receiving 25 ng/kg.

Five women in the 100 ng/kg group underwent their initial and second cycles of GnRH replacement in immediate succession. The follicular phase lengths, luteal phase durations, peak E₂ levels attained, and P sums were not significantly different between the first and second treatment cycles.

Side-effects of iv therapy

Maintenance of iv lines for periods up to 14 days in ambulatory, otherwise healthy young women was relatively free of complications. One case of mild phlebitis necessitating catheter removal and local therapy only occurred. Blood and catheter tips were routinely cultured whenever iv lines were changed. Aside from the occasional growth of skin contaminants (e.g. Staphylococcus epidermidis) on the catheter tip (8 positive reports in the 30 cycles) or the rare blood culture (1 positive report in the 30 cycles), all cultures were sterile. Patients were instructed to record their oral temperatures twice a day, and no subject had a febrile episode attributable to the presence of the iv catheter.

Discussion

The advantages and disadvantages of various GnRH dosage regimens and routes of administration have been explored by several investigators (6, 7, 11–19). Recent observations in women with hypothalamic amenorrhea (11) revealed that iv GnRH doses as high as 20 μg induced supranormal E₂ and P responses, occasional mild ovarian
hyperstimulation, and multiple pregnancies compared to a smaller group of normal women. We have employed a considerably lower dose of 100 ng/kg (5–6 μg) of GnRH and demonstrated a similar, though milder, supraphysiological response. Although most investigators agree that the iv route is considerably more effective in inducing ovulation in women with hypothalamic amenorrhea (6, 11), others have studied both iv and sc administration (7, 11, 13) or used the sc route alone for reasons of convenience (14–19). Studies employing the sc route for GnRH therapy have generally indicated that higher doses are required for longer periods of time, lower rates of ovulation are achieved, and unphysiological patterns of gonadotropin secretion can result, since prolonged and variable GnRH absorption may occur (6, 7, 11, 15). In the present work, we chose to employ the iv method exclusively due to its superior ability to deliver a discrete dose of GnRH to the pituitary and to elicit a pattern of LH release that most closely resembles that in normal women (6, 7). Utilization of this route then allowed us to compare the pituitary and gonadal responses of these subjects with those of normal women, and thereby modify our GnRH replacement regimen appropriately.

The optimal concentration of exogenous GnRH required for restoration of cyclic function in women with hypothalamic amenorrhea is controversial. Since considerable interspecies differences in GnRH sensitivity exist, previous animal experience is of limited value in establishing a dose of GnRH that mimics the normal amplitude of gonadotropin release during the menstrual cycle (20–25). Measurements of GnRH in the peripheral circulation are not helpful, since they do not correlate with the portal levels of GnRH (26), probably due to its short half-life and relatively confined space of distribution within the hypothalamic-pituitary blood supply. Furthermore, GnRH secretion may change in quantity during the course of the normal menstrual cycle, rendering direct hypophyseal-portal blood measurements insufficient to provide the necessary data (27).

The 25 ng/kg dose of GnRH used in this study was derived from previous information concerning the physiological range of GnRH doses used to restore normal gonadotropin secretory patterns, sex steroid production, and gametogenesis in GnRH-deficient men (28, 29). However, in contrast to the experience in GnRH-deficient men, the 25 ng/kg GnRH dose in women appears to represent a threshold dose for folliculogenesis, since ovulation occurred in only 8 of 10 cycles. The overall deficient P secretion by the subsequent corpus luteum in those cycles that proved ovulatory suggests that the 25 ng/kg dose is marginal in its ability to restore the normal amplitude of pituitary-gonadal responses in GnRH-deficient women. This observation taken together with our previous experience in GnRH-deficient men implies that women in the follicular phase secrete a larger quantity of GnRH per secretory episode than adult men.

Conversely, 100 ng/kg, while uniformly effective in inducing ovulation in our small series of patients, is probably a slightly supraphysiological dose of GnRH for the follicular phase of the cycle. This observation is based on the higher than normal early follicular phase FSH levels, the significantly elevated midcycle E2 peaks, the occurrence of multiple follicular development by ultrasonic detection, the high early luteal phase P levels, and twinning in one of seven pregnancies. Clearly, in patients with multiple folliculogenesis, E2 levels rose well above the normal range, to individual levels as high as 1046 pg/ml, and luteal P values during these cycles were twice those in normal women. In concert, these findings suggest that endogenous negative feedback mechanisms of E2 regulation of FSH secretion are intact during pulsatile GnRH therapy, but overriding of this negative feedback can occur with the use of supraphysiological levels of GnRH. Of interest was the finding that the LH peak in patients receiving 100 ng/kg bolus doses of GnRH was significantly blunted compared to normal women (mean, 65 ± 8 vs. 111 ± 7 mIU/ml; P = 0.001), suggesting that suppression of positive feedback on the pituitary gland by these increased levels of E2 may have occurred. Alternatively, nonsteroidal substances produced by the overstimulated ovary may be responsible for suppressing the LH surge, as has been suggested by others (30). Luteal phase ultrasonography was also performed routinely 7 days postovulation. Although ascites was never detected in any patient who received 100 ng/kg, mild ovarian enlargement with multiple cysts compatible with corpora lutea was observed in five women receiving the 100 ng/kg dose. Thus, subtle adjustments of the GnRH dose during the follicular phase might be important in avoiding multiple pregnancy while using the minimum effective amount that will induce ovulation. Conversely, the use of higher doses of GnRH in the follicular phase of the cycle may well have a role in ovulation induction attempts associated with in vitro fertilization programs where multiple folliculogenesis is a goal (8, 31).

In humans, GnRH exerts a self-priming effect by sensitizing the pituitary gland to subsequent GnRH exposure for up to 1 month (29, 32). From these findings, one might anticipate that an initial cycle of exposure to exogenous GnRH would be less than satisfactory, but could lead to improved responses in following cycles. In the limited context of these studies, however, it did not appear that a previous cycle exerted either a positive or a negative influence on succeeding cycles. The pituitary response was neither increased nor decreased in the five women who underwent initial and second cycles of GnRH replacement without interruption. Furthermore, gonadal responses did not differ between these two
groups, implying that the ovaries of women with long-standing hypothalamic amenorrhea are ready to respond as soon as a physiological program of GnRH is instituted.

Cycles of conception were not included in evaluation of luteal phase P secretion. Nonetheless, P secretion during the 100 ng/kg cycles compared favorably with normal. Since no difference occurred in initial and second cycles, it is unlikely that priming cycles skewed comparisons of luteal phase P production. Furthermore, since all conceptions occurred within three cycles of GnRH administration and were approximately evenly distributed among the first, second, and third cycles, the non-conception cycles were unlikely to have been due to unfavorable patterns of P secretion or other hormonal factors. Indeed, this distribution of conceptions suggests the pregnancy pattern that occurs in normal women.

The difference in frequency of GnRH administered in the early follicular phase (90 vs. 60 min) to women who received 100 ng/kg GnRH failed to cause different patterns of gonadotropin secretion, folliculogenesis, or luteal function. The small numbers of cycles studied might be insufficient to elucidate a subtle difference. On the other hand, it is possible that the hypothalamic-pituitary-ovarian axis may respond identically to frequencies that fall within a tolerable range (8, 33).

Intravenous pulsatile GnRH administration appears to be an effective and feasible technique for restoring fertility in women with IHH. The adoption of a physiological frequency of GnRH stimulation resulted in uniform ovulation in properly selected patients suffering from a presumed deficiency of endogenous GnRH secretion. Inadequate quantities of GnRH stimulation sometimes caused attenuated folliculogenesis, with subsequent inadequate corpus luteum function. Slightly excessive doses of GnRH resulted in more exuberant follicular growth and corpus luteum function, but with concomitant problems of overstimulation, multiple folliculogenesis, and multiple gestation. Further studies of different dosages of GnRH will be required to determine that which simulates the physiology of the normal menstrual cycle more closely.

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