The effect of danazol on gonadotropin secretion during the follicular phase of the menstrual cycle

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The effects of danazol on pulsatile luteinizing hormone (LH) release, basal LH and follicle-stimulating hormone serum levels, gonadotropin release induced by estradiol (E₂) and gonadotropin-releasing hormone were examined in five eugonadal women. Danazol administration resulted in a significant suppression of follicle-stimulating hormone serum levels. LH concentrations and LH pulse frequency appeared to be reduced, but these changes did not reach statistical significance. The pituitary response to exogenous gonadotropin-releasing hormone was not altered. The stimulatory effect of E₂ on LH secretion was completely abolished in one subject, severely diminished in three subjects, and unchanged in one subject. In addition, the time course of this response was altered. Serum prolactin concentrations were lowered, whereas basal E₂ and progesterone levels did not seem to be affected.

Danazol, a synthetic derivative of 17α-ethinyl testosterone, has been found effective in the treatment of endometriosis, benign breast disease, and angioneurotic edema. Although the compound has been used clinically for more than a decade, particularly in the treatment of endometriosis, its mechanism of action is still not well understood.

Based primarily on early experiments in rodents, the view had been advanced that the main action of danazol is antigonadotropic and that suppression of gonadotropin secretion causes reduction of steroidogenesis and thus endometrial atrophy. This view has been questioned recently, because basal gonadotropin levels were not found to be reduced by most investigators studying premenopausal subjects on danazol treatment. There is now considerable experimental evidence that suggests that danazol exerts major parts of its therapeutic action by direct inhibition of steroidogenesis at the ovarian level and by interference with steroid hormone action at the site of target tissues, particularly the endometrium. However, in most of the studies where basal gonadotropin levels were measured, blood samples were taken at daily or weekly intervals. As suggested by preliminary data, this sampling frequency may have been insufficient to detect subtle changes in circulating gonadotropin levels. In addition, very few of those studies included the appropriate controls.

The present study was designed to examine the effects of danazol on some key aspects of gonadotropin secretion. To this end, basal luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels, pulsatile gonadotropin release, as well as the response to gonadotropin-releasing

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hormone (GnRH) and estradiol benzoate (EB) administration were examined in healthy subjects during the follicular phase of a control cycle and during danazol treatment. Each subject served as her own control. The effects of the drug on prolactin (PRL), estradiol (E2), and progesterone (P) secretion were also determined.

MATERIALS AND METHODS

SUBJECTS

Five healthy women 24 to 32 years of age were studied after informed consent had been obtained. All had a history of regular menstrual cycles of 26 to 28 days' duration. None of them had received any hormone therapy for at least 6 months prior to the study. An intrauterine device was used by one subject for contraception.

Danazol (Winobanin, Winthrop GmbH, Neu- Isenburg, FRG) was administered at a dosage of 800 mg daily, divided in two doses of 400 mg, from day 1 through day 28 of the cycle. To assess the effects of danazol on hypothalamic-pituitary function, we performed the following tests:

Pulsatile Gonadotropin Secretion

To evaluate the effects of danazol on the pulsatile pattern of gonadotropin secretion, blood was drawn at 20-minute intervals over a period of 6 hours on days 3 and 4.

Response to Exogenous GnRH

On days 3, 4, and 8, 100 μg GnRH (LH-RH Ferring, Ferring GmbH, Kiel, FRG) was injected intravenously, and blood was drawn over a period of 3 hours at intervals ranging from 15 to 60 minutes.

Response to E2 Administration

On day 9, 5 mg EB (Progynon B oleosum, Schering AG, Berlin, West Germany) was injected intramuscularly immediately after blood collection at 8:00 A.M., and blood was drawn twice daily 8 to 12 hours apart from day 9 to day 16.

Basal Gonadotropin Secretion

Basal gonadotropin secretion was assessed from blood samples taken daily between 8:00 A.M. and 9:00 A.M. from day 1 to day 9 before the injection of EB. Additional daily blood samples were obtained from day 17 to day 28.

During a preceding control cycle, the women were subjected to the same procedures except for the administration of danazol. An interval of one normal menstrual cycle was allowed between the control cycle and that with danazol treatment. Basal body temperature was recorded throughout the whole study period.

LH, FSH, and PRL were determined by commercially available radioimmunoassays (IRE Diagnostics GmbH, Frechen, FRG). E2 and P were determined radioimmunologically after celite chromatography according to previously published procedures. Serum hormone levels were compared by means of Student’s t-test for paired observations.

LH pulse frequency was determined by using a cycle detector program, kindly provided by Drs. Clifton and Steiner, University of Washington, Seattle, WA. Plausibility of the results provided by the computer program was assessed by visual analysis of the graphed data. For this purpose, a pulse was defined as an increase of LH concentrations of at least 30% (or 2 × standard deviation [SD] of duplicates) from a nadir occurring no more than 2 points before the peak, followed by a decline of over at least 2 consecutive points.

RESULTS

PULSATILE LH RELEASE AND BASAL GONADOTROPIN SECRETION

On days 3 and 4 of the follicular phase of the control cycle, a characteristic pulsatile pattern of LH secretion was observed. Pulses occurred every 180 ± 69 minutes and every 148 ± 62 minutes (mean ± SD), respectively. On day 3 of the treatment cycle, there was a reduction of LH pulse frequency in three of five women, and on day 4 in four of five women when compared with the control period. Those pulses occurred every 200 ± 100 minutes and every 257 ± 101 minutes (mean ± SD), respectively. This reduction, however, was not statistically significant.

Mean LH levels averaged over the 6-hour sampling period tended to be lower on day 3, and particularly on day 4, of danazol treatment when compared with the control levels, but this difference also failed to reach statistical significance. FSH pulses were observed concomitant with LH pulses in some subjects. Because they could

Braun et al. Danazol and gonadotropin secretion Fertility and Sterility
not be identified consistently, a statistical evaluation was not performed. Mean FSH levels over the 6-hour period were significantly suppressed on day 3 of danazol treatment when compared with the control cycle, and continued to decline on day 4. One typical example of the secretory pattern of LH and FSH over a 6-hour period during the control cycle and during danazol administration is shown in Figure 1. Figures 2 and 3 show the data averaged for all subjects. The hormone concentrations are given in Table 1.

When basal LH and FSH levels obtained daily from day 1 to day 9 of the control and treatment cycles were compared, LH levels were 9.0 ± 1.0 mIU/ml and 8.4 ± 1.1 mIU/ml (mean ± standard error of the mean [SEM]), respectively (P = 0.15), whereas FSH levels declined from 8.5 ± 1.4 mIU/ml to 7.3 ± 1.7 mIU/ml (mean ± SEM), respectively (P = 0.1).

PITUITARY RESPONSE TO GnRH

The pituitary response to 100 μg GnRH, administered as an intravenous bolus on days 3, 4, and 8 of the control cycle and during danazol treatment, is shown in Figure 4. The GnRH-induced gonadotropin release did not differ significantly between danazol and control cycles.

RESPONSE TO EB ADMINISTRATION

The injection of 5 mg EB on day 9 of the control cycle and during danazol administration produced peak serum E2 levels of 1941 ± 160 pg/ml and 1375 ± 425 pg/ml (mean ± SEM), respectively, which were not significantly different. The time courses of LH, FSH, and PRL concentrations following EB administration are shown in Figure 5. A composite is depicted in Figure 6. During the control cycle, injection of EB led to a decline of LH
and FSH serum levels lasting for 36 to 60 hours (negative feedback). This period of inhibition was followed by an abrupt increase of 48 to 76 hours' duration (positive feedback). Peak levels were reached 48 to 110 hours after EB administration. This pattern was dramatically altered when EB was administered during treatment with danazol. While the inhibitory effect of E2 on LH and FSH secretion was still apparent in all subjects, its stimulatory action on LH secretion was completely abolished in one subject, severely diminished in three subjects, and unchanged in one subject. Only in this last subject did an increment of FSH serum levels occur concomitant with those of LH; in the remaining four patients only a decline of FSH serum concentrations could be observed. In addition to its magnitude, the time course of the response to the stimulatory action of E2 was changed. Peak levels of LH were obtained earlier (24 to 36 hours after EB injection) as compared with the control cycle, and the duration of the peak was considerably shortened, lasting only 12 to 24 hours.

An increase in serum PRL concentrations occurred in all subjects when EB was administered during the control cycle, and this response was not altered in a characteristic way by danazol.

**EFFECTS ON BASAL PRL SECRETION**

Serum levels of PRL were averaged for the control and treatment periods from day 1 to day 9 (Fig. 6). During danazol treatment, there was a small but significant decrease of the mean values from $7.8 \pm 0.5$ ng/ml during the control cycle to $5.8 \pm 0.9$ ng/ml (mean ± SEM) during danazol treatment ($P < 0.05$).

**EFFECTS ON E2 AND P LEVELS**

Mean values measured from day 1 to day 9 during the control cycle were not different from those observed during danazol treatment. The E2 level during the control cycle was $123 \pm 22$ pg/ml; during danazol treatment it was $127 \pm 26$ pg/ml (mean ± SEM). The P level during the control cycle was $0.5 \pm 0.4$ ng/ml; during danazol treatment it was $0.9 \pm 0.3$ ng/ml (mean ± SEM).

**EFFECTS OF DANAZOL ON OVULATION AND BASAL BODY TEMPERATURE**

During the control cycle, all but one subject ovulated, as judged by the rise in serum P.

Table 1. *Effects of Danazol on LH and FSH Secretion*^a^  

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Control</th>
<th>Danazol</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (mIU/ml)</td>
<td>Mean Range</td>
<td>Mean Range</td>
</tr>
<tr>
<td></td>
<td>8.2</td>
<td>6.3-11.0</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>8.9</td>
<td>8.4-13.9</td>
</tr>
<tr>
<td></td>
<td>8.7</td>
<td>6.2-12.8</td>
</tr>
<tr>
<td></td>
<td>9.9</td>
<td>4.5-15.7</td>
</tr>
</tbody>
</table>

^aMean and range of LH and FSH serum levels during a 6-hour period on days 3 and 4 of a normal menstrual cycle (control) and during danazol administration. Blood samples were taken at 20-minute intervals.

^b$P < 0.025$.

^c$P < 0.0125$.  

40 Braun et al. *Danazol and gonadotropin secretion*  

*Fertility and Sterility*
Ovulation was delayed, however, probably as a consequence of the injection of EB. During danazol treatment, no ovulation occurred, as reflected by the consistently low levels of P.

The basal body temperature was biphasic in those subjects who ovulated during the control cycle and monophasic in all subjects during danazol administration.

DISCUSSION

The pharmacologic profile of danazol and its actions on the reproductive system are complex and include inhibitory effects on hypothalamic-pituitary function and ovarian steroidogenesis as well as interference with steroid hormone action in peripheral target tissues.\(^1\)\(^-\)\(^3\) This study was designed to evaluate the effects of danazol on hypothalamic-pituitary function.

Administration of danazol resulted in a significant decline of serum FSH levels within 3 days. This only became apparent, however, when blood samples were taken at 20-minute intervals; FSH levels in blood samples obtained daily were not significantly suppressed. This may explain the inability of earlier investigators to demonstrate a suppression of FSH serum concentrations by danazol, since most of these studies were based on blood samples taken at daily or weekly intervals.\(^6\)\(^-\)\(^10\)

Danazol has been reported to block the preovulatory rise in serum E\(_2\),\(^8\)\(^,\)\(^11\) which reflects final growth of the dominant follicle. The suppression of serum levels of FSH during danazol treatment observed in this study, although small in extent and only apparent in blood samples taken frequently, could account for this effect. It has been shown in rhesus monkeys that a slight suppression of FSH, occasioned by administration of minute amounts of E\(_2\), is sufficient to block the growth of the preovulatory follicle and thus to delay ovulation.\(^12\)

Basal serum levels of E\(_2\), however, did not seem to be influenced by the danazol-induced suppression of FSH, at least not for the first 9 days of treatment. Apparently, even diminished gonadotropin stimulation is sufficient to maintain basal estrogen levels, and this is in agreement with observations made in women with clomiphene-positive hypothalamic amenorrhea who have low FSH concentrations but estrogen levels typical for the early follicular phase of the cycle.\(^13\)

There was also a reduction in LH serum levels during danazol treatment which was, however, not statistically significant. The discrepant effects of the drug on LH and FSH serum levels may be related to the duration of treatment. They may, in addition, reflect higher sensitivity of FSH to negative feedback inhibition, as observed during the menstrual cycle,\(^14\) after estrogen administration in women with hypothalamic amenorrhea,\(^15\) or during menstrual cycles induced by the pulsatile administration of GnRH.\(^13\)\(^,\)\(^16\)

The preovulatory rise in serum E\(_2\) is the ovarian signal that induces, via positive feedback, the midcycle surge of LH and FSH and thus leads to

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**Figure 4**

Pituitary response to 100 \(\mu\)g GnRH injected on days 3, 4, and 8 of a normal menstrual cycle and during danazol administration. The data represent the mean \(\pm\) SEM of the responses of five subjects.

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**Braun et al. Danazol and gonadotropin secretion** 41
ovulation. Experimentally, LH and FSH discharges similar to those observed at midcycle can be induced by administration of E$_2$ during the early to midfollicular phase of the cycle, and this was again observed in this study. While it has been reported uniformly that danazol blocks the midcycle surge of gonadotropins, it was not clear whether this was due solely to the blockade of the preovulatory E$_2$ rise, or if, in addition, the compound interfered with the mechanisms resulting in the abrupt discharge of gonadotropins.

The results of this study clearly demonstrate that danazol interferes with both the inhibitory and stimulatory effects of E$_2$ on pituitary LH and FSH release and either blocks or alters the time course and extent of the stimulatory action of the steroid on gonadotropin secretion. Our findings are at variance with those reported by Chimbira et al. and Luciano et al., who stated that the E$_2$-induced positive feedback on pituitary LH secretion was not abolished after 8 to 10 weeks of danazol treatment. Whether these discrepant results are related to the different duration of treatment, different dosage, or other unknown factors cannot be decided at present.

The sites at which danazol acts to decrease serum FSH levels and interferes with the effects of E$_2$ are unknown. The compound may act on the central nervous system, altering GnRH secretion from the hypothalamus, at the level of the pituitary gland, or at both of these sites. The data obtained in this study are consonant with each of these views.

The apparent decrease in LH pulse frequency, although not statistically significant, points at a hypothalamic site of action. The frequent sampling period of 6 hours and the duration of danazol administration may have been too short to demonstrate significant changes of this phenomenon. In addition, LH pulse frequency is only an indirect indicator of hypothalamic activity. Its assessment is critically dependent upon the amplitude of LH pulses, which in turn may be modulated and obscured by effects at the pituitary level. From experiments in rhesus monkeys, an increase in FSH levels would be expected as a consequence of a reduced frequency of GnRH pulses. However, FSH levels declined during danazol treatment, which suggests a primary pituitary site of action of the drug. The view of a pituitary site of action of danazol in inhibiting FSH secretion is not contradicted by the unimpaired response of the gland to GnRH, because a
large bolus of the decapeptide was administered. This may override inhibition at the pituitary level and may not reflect the response to endogenous GnRH pulses in a physiologic setting. A normal or exaggerated pituitary response to exogenous GnRH administration has also been observed by Goebel and Rjosk, Asch et al., Rannevik, and Luciano et al. after long-term danazol administration, and this may be related to the finding that pituitary gonadotropin content is not affected by the compound.

The site and the mechanisms by which danazol interferes with the effects of E2 on gonadotropin secretion are equally unknown. It has been shown in primates that E2 exerts its negative and positive feedback effects on gonadotropin secretion at the level of the pituitary gland, and this would suggest that interference with these actions by danazol occurs also at the pituitary level. There is, however, a striking similarity between the effects of danazol observed in this study and those described for P, which has also been shown, depending on the experimental circumstances, to block or to advance and shorten the E2-induced gonadotropin discharge in women and in rhesus monkeys. It has been shown in female rhesus monkeys with lesions in the arcuate region of the hypothalamus that P acts at the central nervous system to block the E2-induced gonadotropin surge, while its modulatory effects are exerted at the pituitary level. Whether those findings can be extrapolated to explain the action of danazol has to be further investigated.

While the progestational action of danazol itself is controversial, the view of a similar action of P and danazol on gonadotropin secretion is indirectly supported by the observation that a major metabolite of danazol, ethisterone, is a progestational agent.

There is good experimental evidence that danazol binds to the androgen receptor, translocates to the nucleus, and is able to evoke androgenic responses in target tissues. While it is suggested by elegant experiments of Krey and associates, using androgen-receptor-deficient (Tfm) rats, that the androgenic actions of danazol could account for the reduction in basal gonadotropin levels, an androgenic effect is an unlikely explanation for the blockade of the E2-induced gonadotropin release, because androgen administration was unable to block this phenomenon in castrated male and female primates.

The administration of E2 induced a significant increase in circulating levels of PRL. In contrast to its effects on gonadotropin secretion, danazol did not interfere with this action of estrogen, although it lowered basal PRL concentrations. This observation suggests that the molecular mechanisms underlying the stimulatory effects of E2 on gonadotropin secretion and those that lead to an increase in PRL are affected differently by danazol administration.

In summary, the data obtained in this study show that administration of danazol inhibits basal FSH and probably also LH secretion, appears to reduce the number of LH pulses, interferes with the feedback effects of E2 on gonadotropin secretion in a way similar to that of P, but does not interfere with the pituitary response to a single bolus of GnRH.

Effects of the compound on gonadotropin secretion may thus be sufficient to account for the anovulation produced by the drug. This does not exclude, however, that additional effects at the endometrial target cells are responsible for the
Effectiveness of this compound in the treatment of endometriosis.

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