Pulsatile luteinizing hormone secretion during the first and the fourth cycle on two different oral contraceptives containing gestodene

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Oral contraceptives inhibit ovarian follicular growth by suppressing the release of gonadotropins from the pituitary. We studied basal and gonadotropin-releasing hormone-stimulated gonadotropin release, as well as pulsatile luteinizing hormone (LH) secretion, in ten healthy volunteers who had not used oral contraceptives before. Subjects received either a monophasic preparation containing 30 μg of ethinylestradiol and 75 μg of gestodene (group 1) or a triphasic formulation containing 30–40 μg of ethinylestradiol and 50, 70 and 100 μg of gestodene (group 2). Blood sampling at 10-min intervals during 6-h periods was performed on days 1, 8, 15 and 21 of both the first and fourth pill cycle. Thirteen healthy volunteers with regular ovulatory cycles served as normal controls. Both LH and follicle-stimulating hormone (FSH) were measured by a sensitive immunoradiometric assay. Pulsatile LH secretion was observed in all oral contraceptive users. Mean serum LH and FSH levels, number of pulses/6 h and the amplitude of LH pulses on day 1 in both the first and fourth pill cycle did not differ from early follicular phase controls in both groups. The FSH levels were suppressed rapidly in both groups, even in first cycles, while LH serum levels progressively declined in all cycles studied. In both groups, amplitudes of LH pulses decreased from day 8 onwards, with a substantial number of low-amplitude pulses (<0.75 U/l) interspersed between large-amplitude pulses. On day 1 of the fourth pill cycle a significant number of pulses were of low amplitude. These results confirm our earlier findings that pulsatile secretion of gonadotropins is maintained during oral contraceptive use but is profoundly modified by steroid feedback. There seems to be no major difference in the suppression of the hypothalamic–pituitary axis in the first cycle on an oral contraceptive as compared to subsequent cycles.

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Oral contraceptives (OCs) inhibit folliculogenesis and prevent ovulation by suppressing gonadotropin levels. Whether this effect is exerted primarily at the hypothalamic level by interfering with the pulsatile secretion of gonadotropin-releasing hormone (GnRH), or at the pituitary by reducing the synthesis and/or release of gonadotropins, is incompletely understood. Several investigators have tried to address this issue by performing GnRH challenge tests in OC users (1-7). These studies have yielded confounding results, because the response of the pituitary to a GnRH bolus not only depends on steroid feedback but also on preceding endogenous GnRH stimulation (8–11). Characterization of the pulsatile release of gonadotropins more accurately reflects hypothalamic–pituitary dynamics and is a more appropriate tool for investigating the feedback effects of contraceptive steroids. In a previous study in long-term users of sub-50 OCs containing either levonorgestrel or desogestrel, we showed that the pulsatile release of LH is influenced mainly by a hypothalamic effect of the gestagenic component of the OC, in a dose- and time-dependent way (12).

After the introduction of OCs in 1961, manufacturers have strived for a reduction in the amount of steroids contained in the OC in order to minimize side-effects. Initially the dose of estrogenic component was reduced successfully, while the synthesis of more potent gestagens, like levonorgestrel, resulted in further dose reduction. Gestodene, a potent gestagen with anti-estrogenic properties (13) and virtually no androgenic or glucocorticoid action, has been introduced recently in a monophasic and a triphasic contraceptive formulation. The biological potency of gestagens is generally determined by in vivo or in vitro bioassays, by measuring the effect on endometrium or on estrogen receptor depletion (14). By these standards, gestodene is a more potent gestagen as compared to desogestrel and levonorgestrel, which does not necessarily mean that the effect on the hypothalamic–pituitary axis is increased equally.

In this study we determined basal and GnRH-stimu-
lated gonadotropin release, as well as pulsatile LH secretion, in two gestodene-containing OCs. Women who had not been using OCs before were studied in their first and fourth cycle to ascertain possible differences in the endocrinology of the first cycle on an OC, compared to later cycles.

Materials and methods

Subjects
Ten healthy volunteers aged 19–28 years, with regular menstrual cycles (26–32 days) and who had not used OCs before, were recruited for the study. Subjects were randomized to receive either a monophasic preparation containing 30 µg of ethinylestradiol (EE) and 75 µg of gestodene (Minulet®, Wyeth Laboratories, Hoofddorp, The Netherlands; group 1, N = 5) or a triphasic formulation containing 30 µg of EE and 50 µg of gestodene from days 1 to 6, 40 µg of EE and 70 µg of gestodene from days 7 to 11 and 30 µg of EE and 100 µg of gestodene for the remaining 10 days (TriMinulet®, Wyeth Laboratories, Hoofddorp, The Netherlands; group 2, N = 5).

Thirteen healthy volunteers who had regular menstrual cycles and had not been using any hormonal medication for at least 3 months served as normal controls (group 3, N = 13).

The study protocol was approved by the subcommittee for the ethics on research involving human subjects of the hospital. Informed consent was obtained from all participants in the study.

Blood sampling

An indwelling catheter was placed in a forearm vein and blood was drawn into heparinized tubes every 10 min over 6 h (9.00–15.00 h). At 15.00 h an intravenous bolus of 100 µg of GnRH (HRF®, Wyeth Laboratories) was administered and blood was drawn 30, 60 and 90 min later. Blood was centrifuged and plasma was frozen at −20°C until assayed.

In groups 1 and 2, blood sampling was performed on day 1 before the first pill was taken and on days 8, 15 and 21, in both the first and fourth pill cycle (the day of the start of a fresh pill pack is designated as day 1). Subjects were instructed to take the pill after 16.00 h. The first pill cycle was started on the first or second day of menstruation. In group 3, blood sampling was performed in the early follicular phase (days 1–6) and in the midluteal phase (days 19–23) of the menstrual cycle. Ovulation was documented by a biphasic basal body temperature and elevated serum progesterone level (>20 nmol/l) during the midluteal phase.

Hormone measurements
In all samples LH was analyzed in duplicate with the use of an immunoradiometric coated tube assay (LH-sp, Medgenix, Fleurus, Belgium). The LH assay is calibrated against the first international reference preparation (IRP) 68/40 and has a sensitivity of 0.1 U/l (3 σ of the blank).

From each subject the LH concentrations in all samples during all pulse studies were measured in a single assay run. The same standard batch of kits was used for all the LH assays. The mean intra-assay coefficients of variation were 20-2% for values between 0.1 and 0.3 U/l, 7.4% for values between 0.3 and 0.5 U/l, 6.0% for values between 0.5 and 1.0 U/l, 2.2% for values between 1 and 3 U/l, 1.8% for values between 3 and 5 U/l, 1.1% for values between 5 and 10 U/l and 1.5% for values above 10 U/l. The inter assay coefficient of variation was 5.8% at the level of 3 U/l and 3.8% at the level of 17 U/l.

Policle-stimulating hormone, prolactin, estradiol and progesterone were measured in the first and last sample from each pulse study. The FSH and prolactin assays were performed with an immunoradiometric coated tube assay (Medgenix, Fleurus, Belgium). The FSH assay is calibrated against the 2nd IRP 78/549 and has a sensitivity of 0.45 U/l (3 σ of the blank). The interassay coefficients of variation for FSH were 12.8% at 4.4 U/l, 10.0% at 10.7 U/l and 7.6% at 33 U/l, respectively; for prolactin they were 12.8% at 2.9 U/l, 5.5% at 15.8 U/l and 2.2% at 43.8 U/l. The progesterone and estradiol measurements were performed with a radioimmunoassay (Coat-A-Count, Diagnostic Products, Los Angeles, CA, USA). The interassay coefficients of variation were 12.8% at 2.9 nmol/l, 7.8% at 22.1 nmol/l and 6.0% at 69.9 nmol/l for progesterone and 21% at 150 pmol/l, 9.7% at 450 pmol/l and 6.2% at 1650 pmol/l for estradiol.

Pulse analysis

The mean of the duplicate values for LH of each sample was taken as the hormone value. The analysis of pulsatile LH secretion was carried out by the method developed by Lambalk et al. (15). This method uses a threshold value for identification of hormone pulses. The sd calculated from the differences between duplicates was determined for the actual assay in which the pulse study was analyzed. This method takes into account the fact that the intra-assay variations among different runs of the same assay differ and that the precision of the assay varies at different hormone concentrations.

The sd of the differences between the nadir and peak of a hormone pulse (Sdiff) can be calculated from the sd of the range of the peak concentration (Sp) and the sd of the range of the nadir concentration (Sn), as follows

\[ S_{diff} = \sqrt{(S_{peak})^2 + (S_{nadir})^2} \]

When the difference between nadir and peak exceeds the threshold of 2 × Sdiff, the peak is considered to be a hormone pulse. Using this criterion, the probability of

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false-positive pulses is 0.02275 (one-tailed probability for normal distributions).

To avoid false-positive pulses, owing to the poor quality of one of the duplicates (outlier), a second criterion was applied to the pulses detected by the threshold criterion: the value of either the nadir or peak with the greatest difference between duplicates ($\Delta_{\text{max}}$) is used in this method, which states that $D/\Delta_{\text{max}}$ should be $> 3$ (where $D$ is the amplitude of the pulse calculated from the threshold criterion) (16). Only hormone pulses detected by the threshold criterion that also fulfilled the criterion of the second method were identified as real pulses.

Because pulsatile LH secretion reflects hypothalamic GnRH secretion in portal blood and because these GnRH pulses immediately preceded an LH pulse, the nadirs are indicated as marker points in the hormone patterns, rather than the pulses themselves. Amplitudes were calculated as the difference between the peak value and the preceding nadir.

**Statistical analysis**

Differences between groups in the first and fourth pill cycle were analyzed by Wilcoxon’s signed rank test for paired observations (two-tailed test).

When there were no differences between the first and fourth cycles, data obtained in each group were pooled and differences within groups at different moments during the pill or control cycles were analyzed by the Kruskal–Wallis non-parametric analysis of variance, followed by a simultaneous test procedure based on the Mann–Whitney statistic (17). Differences between groups were determined by Wilcoxon’s rank sum test for unpaired observations (two-tailed test). The incidence of small-amplitude pulses was analyzed by linear regression, while differences between groups were analyzed by Fisher’s exact test. Probability values of $<0.05$ were considered to indicate significance.

Results are expressed as medians, with a range. The results of the GnRH-challenge tests are expressed as the area under the curve (AUC) for LH and FSH.

**Results**

All subjects completed the study protocol, resulting in 80 pulse studies in OC cycles. In the control group, 26 pulse studies were performed, equally distributed between the early follicular phase (EFP) and the midluteal phase (MLP) of the menstrual cycle.

Examples of LH pulse patterns that were observed in the OC users are shown in Fig. 1. For reference, pulse patterns typical for the EFP and MLP are included.

**Gonadotropins**

Serum FSH levels on day 1 of the OC cycles were not different from the EFP controls, either in the first or the fourth pill cycle (Fig. 2, left-hand panel). In both groups, serum FSH levels in first cycles were suppressed to the same extent as in the fourth cycles at all the moments studied. Therefore, the data were pooled. From day 8 onwards, FSH levels were significantly lower as compared to day 1 of the pill cycle and compared to EFP controls. The FSH levels were suppressed to values below those normally encountered in the MLP from day 8 onwards in group 1 and from day 15 onwards in group 2.

Basal serum LH levels on day 1 did not differ from EFP and MLP controls in either of the cycles studied (Fig. 2, right-hand panel). From day 1 onwards a gradual and progressive decrease of LH levels occurred in both groups, which was similar in both cycles, so again data from both cycles were analyzed simultaneously. The LH levels on day 8 were significantly lower when compared to levels on day 1, while maximally suppressed LH levels were reached by day 15.

**Pulse characteristics**

The number of LH pulses/6 h on day 1 of both the first and fourth pill cycles were no different from EFP controls in either of the groups (Fig. 3, left-hand panel). A significant reduction in pulse frequency, to values significantly lower than in EFP controls and comparable to MLP controls, occurred in both groups after day 1, without a significant difference between the first and fourth cycles. Analysis of the pooled data showed that in group 1 a significant reduction in the number of pulses was evident on day 8 without a further decrease in the remainder of the pill cycle; in contrast, in group 2 the number of pulses on day 8 was smaller than on day 1 but significantly higher as compared to group 1 ($p = 0.01$), while maximal suppression of the number of pulses/6 h to a level comparable to that in group 1 was only reached on day 15.

As there were no differences in pulse amplitudes in the first and fourth cycles, the analysis was performed on data pooled from both cycles. Amplitudes of LH pulses on day 1 in OC cycles did not differ from EFP controls (Fig. 3, right-hand panel). In group 1, amplitudes on day 8 were higher than on day 1 (although this difference just failed to reach significance; $p = 0.06$) and higher compared to EFP controls. From day 15 onwards, amplitudes were lower compared to day 1 and day 8, as well as compared to EFP and MLP controls. In group 2, however, amplitudes of LH pulses from day 8 onwards were significantly lower than on day 1 and lower when compared to EFP and MLP controls. The difference in pulse amplitudes on day 8 between groups 1 and 2 was significant ($p = 0.01$).

In pulse patterns obtained from OC users, a substantial number of LH pulses were of low amplitude, i.e. $<0.75$ U/l (see Fig. 1, where nadirs of small-amplitude pulses are marked with an asterisk). The cut-off point of 0.75 U/l was chosen arbitrarily because pulses of this amplitude are only seldom encountered in the follicular phase of
Fig. 1. Typical examples of luteinizing hormone (LH) pulse patterns in control and oral contraceptive (OC) cycles. Arrows indicate nadirs of significant LH pulses. Small-amplitude pulses (<0.75 U/l) are additionally marked with an asterisk. EFP: early follicular phase; MLP: midluteal phase.

the normal menstrual cycle. In first cycles, the number of LH pulses of <0.75 U/l on day 1 did not differ from EFP controls, but their occurrence increased in both groups with the duration of the pill cycle (linear regression analysis, p<0.05). In fourth cycles on day 1, significantly more pulses were of small amplitude as compared to day 1 of the first cycle and EFP controls (Fisher’s exact test, p<0.05), and their number remained fairly constant during the pill cycle (Fig. 4). The number of small-amplitude pulses did not differ between mono- and triphasic preparations at any moment during the pill cycle.

Gonadotropin-releasing hormone challenge tests
The response of FSH to a 100-μg GnRH bolus, expressed as the area under the curve (AUC-FSH), on day 1 of OC cycles was no different from EFP controls in either the
Fig. 2. Basal serum levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Data are presented in box plots: the box represents values contained in the second and third quartiles. The median value is represented by the horizontal bar in the box. The lines above and below the box depict the location of upper and lower extreme values (see Ref. 18). Open boxes: early follicular (F) and midluteal (L) phase controls. Hatched boxes: first cycle on the oral contraceptive. Dotted boxes: fourth cycle on the oral contraceptive. (*) Significantly different from values on day 1, \( p < 0.01 \); (***) significantly different from values on day 1, \( p < 0.001 \); (#) significantly different from values on day 8, \( p < 0.05 \); (■) significantly different from early follicular phase controls, \( p < 0.001 \); (●) significantly different from midluteal phase controls, \( p < 0.01 \).

Fig. 3. Number of pulses/6 h and amplitude of luteinizing hormone pulses in oral contraceptive and control cycles. Open boxes: early follicular (F) and midluteal (L) phase controls. Hatched boxes: first cycle on the oral contraceptive. Dotted boxes: fourth cycle on the oral contraceptive. (*) Significantly different from values on day 1, \( p < 0.05 \); (**) significantly different from values on day 1, \( p < 0.01 \); (#) significantly different from values on day 8, \( p < 0.05 \); (■) significantly different from early follicular phase controls, \( p < 0.01 \); (●) significantly different from midluteal phase controls, \( p < 0.01 \); (▲) monophasic (group 1) versus triphasic (group 2), \( p < 0.05 \).
first or the fourth cycle in both groups (Fig. 5, left-hand panel). From day 8 onwards, the AUC-FSH is decreased significantly in both cycles studied as compared to day 1 of the pill cycle, as well as compared to EFP and MLP controls.

The AUC-LH on day 1 of the first and fourth OC cycles did not differ from EFP controls in either of the groups (Fig. 5, right-hand panel). On day 8 the AUC-LH tended to be higher than on day 1 in both cycles, although this difference only reached statistical significance in group 2 (p<0.05, pooled data) and remained inferior to the response observed in MLP controls. From day 15 onwards the LH response is suppressed to levels below those in MLP controls.

**Ovarian steroids and prolactin**

Estradiol levels varied between 30 and 220 pmol/l at all stages of the OC cycles in all subjects. Progesterone levels were below 2 nmol/l and prolactin levels were within normal limits (<20 μg/l) in all subjects; these levels did not change during the OC cycle.

**Discussion**

The main objective of oral contraception is to prevent folliculogenesis. Because FSH promotes follicular growth and maturation, inhibition of pituitary FSH output should be accomplished effectively by an OC. Both preparations used in this study showed a prompt and profound suppression of serum FSH levels, as well as a reduced pituitary sensitivity to release FSH, as judged by the impaired FSH response to a GnRH challenge. This suppression of FSH is achieved as early as day 8 of the cycle, and occurs in first cycles as reliably as in later cycles. As synthetic gestagens seem to exert no effect on FSH release (19), the decrease of FSH levels is probably caused by the negative feedback of EE.

Although serum LH levels were decreased uniformly during OC use, the effects of contraceptive steroids on the pulsatile release characteristics of LH are more differentiated. A uniform property of both of the contraceptive formulations studied is a reduced number of LH pulses from day 8 onwards. As the pituitary sensitivity to GnRH is not impaired, at least initially, this phenomenon can be attributed to a slowing of the hypothalamic GnRH generator by the gestagenic component of the OC, a process that is thought to be mediated by endogenous opiates (20–25) and is dependent on the duration of exposure to gestagens (26). The finding that the number of LH pulses/6 h on day 8 in the triphasic formulation was higher than in its monophasic counterpart is difficult to interpret, because the doses of EE and gestodene differ between both preparations. Furthermore, a sampling period of only 6 h is a relatively short time in which to draw firm conclusions regarding the differences in pulse frequency.

Although the median pulse amplitude declines during the OC cycle, this is a poor parameter with which to characterize the nature of pulsatile LH release because pulse patterns in OC users are characterized by two different classes of pulses: pulses of relatively high amplitude interspersed with pulses of very low amplitude that are also seen in the luteal phase of the normal menstrual cycle (27–29). In both preparations in this study, the incidence of small-amplitude pulses increased with the duration of pill ingestion in the first cycle. This is in keeping with the observation that the occurrence of these small pulses is related to the duration of exposure to gestagens (28). The fact that in fourth cycles the number of small-amplitude pulses was fairly constant from the first day of the cycle onwards suggests that this gestagenic effect does not wear off during the 7-day pill-free interval. There were no differences in the occurrence of small pulses in the monophasic and triphasic OCs with gestodene, which is in contrast with our previous findings in long-term users of two levonorgestrel-
containing formulations, where the increase in small-amplitude pulses was related to the dose of the gestagenic component (12). The difference in dose regimens of the levonorgestrel- and gestodene-containing OCs may be responsible for this difference, because in the triphasic preparation of the former a 40% reduction is achieved in the total amount of gestagens ingested over the 21-day pill cycle as compared to its monophasic counterpart, while in the latter no reduction occurs (in fact there is a 5% increase).

The origin of small-amplitude pulses is still obscure: pituitary desensitization to endogenous GnRH would be one explanation, but because small-amplitude pulses sometimes immediately precede normal amplitude pulses and because they occur at a moment when pituitary sensitivity, as measured by a GnRH-challenge test, is not reduced, this would seem unlikely. The occurrence of GnRH pulses of small amplitude, demonstrated in sheep (30) and possibly important in stimulating the synthesis of gonadotropins by the pituitary (31), seems a more attractive explanation. This could imply that progesterone modulates GnRH pulse amplitude (32, 33) or, alternatively, that the varying level of desensitization of the pituitary due to the low GnRH pulse frequency (34, 35) allows small GnRH pulses to be translated occasionally into an LH signal.

After a 7-day pill-free interval all endocrine parameters, including FSH levels and pulsatile LH release, have normalized, as evidenced by a pulse pattern on day 1 that cannot be distinguished from EFP controls, as has been demonstrated earlier (36). This means that the feedback effects of contraceptive steroids wear off rapidly. The only exception is the increased amount of small-amplitude pulses on day 1 in OC users, which probably reflects a carry-over effect of the gestagenic component of the OC.

From these results we conclude that:

(i) suppression of FSH levels and of pituitary GnRH-stimulated FSH release occurs in both gestodene dose regimens, equally early and equally effective, even in first cycles;
(ii) pulsatile LH secretion is maintained during the use of OCs, although the pulse pattern is strongly modified by feedback effects of the gestagenic component in the OC;
(iii) modulation of the amplitude of LH pulses and the occurrence of small-amplitude pulses is related to the duration of exposure to gestagens;
(iv) steroidogenic feedback effects of OCs seem to wear off rapidly, because on day 1 of the pill cycle an almost normal EFP pulse pattern is found, except for the presence of small-amplitude pulses in long-term OC users;
(v) there seems to be no difference in the endocrinology of first cycles on an OC, as opposed to later cycles.
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