Peripheral Blood and Ovarian Levels of Sex Steroids in the Lactating Rat

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Abstract

Changes in progesterone (P), 20 α -dihydroprogesterone (20 α -OHP), testosterone (T) and estradiol-17 β (E₂) in peripheral blood, in corpora lutea (CL) of pregnancy and lactation and non-luteal ovarian tissues (NLO), and serum levels of LH, FSH and prolactin (PRL) were determined by radioimmunoassay in lactating rats nursing 8 pups. CL of pregnancy and lactation and NLO were removed at various days after post-partum ovulation (Day 0 of lactation = day of parturition).

Serum E₂ remained low until Day 10 of lactation followed by a slight increase from Days 12 to 21. Serum T was unchanged throughout lactation. Low levels of T and E₂ in NLO were observed until Day 12, followed by a gradual increase on Day 15 and onward. These changes correlated with low levels of serum LH from Days 2 to 12 and a return to basal levels on Days 15 to 21. Throughout lactation serum FSH remained at basal levels corresponding to diestrous values during the estrous cycle. High levels of serum PRL were observed from Days 4 to 10. Serum levels of P reached maximal values between Days 8 and 10 and decreased after Day 12. Changes in serum 20 α -OHP were inversely related to serum P throughout lactation. The CL of pregnancy contained large amounts of 20 α -OHP and small amounts of P on Days 1 and 2 followed by an abrupt decline until Day 21. On the other hand, a marked increase in content and concentration of P in the CL of lactation occurred between Days 6 and 12 followed by a gradual decline by Day 21. Content and concentration of 20 α -OHP in the CL of lactation increased progressively from Day 1 to 21.

These findings indicate that the CL of pregnancy secrete a large amount of 20 α -OHP and a small amount of P during the early stages of lactation. Thereafter, the CL of lactation markedly increase their ability to secrete P. High levels of P and PRL during the first half of lactation along with strong suckling stimuli may be involved in the suppression of follicular development, presumably by lowering basal levels of serum LH.

During lactation in the rat, striking changes in ovarian histology occur, i.e. prolonged anovulation depending on the size of the litter and atrophy of the corpora lutea (CL) of pregnancy, while CL of lactation resulting

from post-partum ovulation increase their size and secretion of progesterone (P). However, CL of pregnancy are of considerable size during the early stages of lactation. Following post-partum ovulation, the number of healthy follicles in the ovary decreases and the number of atretic follicles markedly increases during the early stages of lactation in rats nursing 8 pups, whereas a few large healthy follicles are always present in the late stages of lactation (Taya and Sasamoto, 1981). Despite the

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great deal of work which has been done on the hypothalamic-hypophysial function during lactation in the rat, relatively little attention has been directed towards correlating serum levels of steroids with the steroidogenic capacity of the ovary *in toto* or the CL of pregnancy and lactation and the non-luteal ovarian tissues (NLO) to secrete steroids. The present study was therefore undertaken to provide a comprehensive account of changes in ovarian steroidogenesis throughout lactation in the rat by determining P, 20 α -dihydroprogesterone (20 α -OHP), testosterone (T) and estradiol-17 β (E₂) in serum and in isolated CL of pregnancy and lactation and NLO. To complete the picture, serum levels of gonadotropins were also measured.

Materials and Methods

Animals

Pregnant rats of the Holtzman strain with known dates of insemination were obtained from SASCO (Omaha, Nebraska) and were maintained in an environmentally controlled room illuminated daily from 0500 to 1900 h. They were placed in separate cages 5 days before parturition. The day of parturition was designated as Day 0 of lactation and the litter was adjusted to 8 pups. Any mother not having 8 pups was discarded. Between 0900 and 1000 h on the days of lactation indicated on the graphs, mothers were decapitated. Blood was collected and serum saved for radioimmunoassay (RIA) of P, 20 α -OHP, T, E₂, LH, FSH and prolactin (PRL). Immediately after blood collection, the ovaries were removed and placed in ice cold saline and all CL of pregnancy and lactation, as judged by their size and vascularity, were separated using jeweler's forceps with a dissecting microscope and cleaned of adhering ovarian tissue. From each animal 4-5 CL of pregnancy and lactation were selected at random. After weighing, the CL (2.4-23.4 mg) and the resultant NLO (7.4-27.3 mg) were placed in 0.4 ml 95% ethanol for steroid determinations. All tissues were homogenized in 95% ethanol and stored at -20°C until used for estimations of steroids.

Steroids RIAs

The RIAs for steroids were the same as described previously (Terranova and Greenwald, 1978) using P antisera (Surve *et al.*, 1976), T antisera (Pang and Johnson, 1974) and E₂ antisera (Exley *et al.*, 1971). Since T antiserum cross reacts with 5 α -dihydrotesto-

sterone (58%) and androstendione (2%) the steroid levels are referred to as testosterone-equivalents. The antiserum to 20 α -OHP was supplied by Drs. C. N. Pang and J. Hilliard (University of California at Los Angeles) and the only steroid which cross reacted (> 1%) with the antiserum was 20 β -dihydroprogesterone (42%). The lower limits of sensitivity in the assay for P, 20 α -OHP, T and E₂ were 5 pg, 5 pg, 2 pg and 2 pg per tube, respectively.

Gonadotropin RIAs

LH, FSH and PRL were determined in sera by RIA, using kits provided by NIAMDD. The methods were similar to those of Bast and Greenwald (1974). Antisera used were anti-rat LH serum 3, anti-rat FSH serum 9 and anti-rat PRL serum 4. Reference preparations used for standards were rat LH-RP-1 (0.03 $\times$ NIH-LH-S1), FSH-RP-1 (2.1 $\times$ NIH-FSH-S1) and PRL-RP-1 (11 i.u./mg). Iodinated preparations were rat LH-I-1, FSH-I-1 and PRL-I-3. In the LH and FSH assay, 200 μ l sera were used whereas in PRL assays, 25 and 50 μ l were assayed, respectively. Duplicate serum samples were assayed. The lower limit of assay sensitivity for LH, FSH and PRL was 0.5 ng, 6.3 ng and 0.06 ng per tube, respectively.

Statistics

The significance of difference between two means was tested by Student's *t*-test, but when more than two means were compared an analysis of variance was carried out and the significance of the difference between means was determined by Duncan's multiple range test (Steel and Torrie, 1960). Differences were judged significant if $P < 0.05$.

Results

Changes in weight of CL of pregnancy and lactation and NLO (Fig. 1)

Post-partum ovulation occurred within 24 h after parturition with comparable number of oocytes shed (12.7 ± 0.6 , $n=6$) to cyclic animals (12.2 ± 0.6 , $n=6$). After parturition, the weight of the CL of pregnancy declined rapidly by Day 10, then continued to decrease gradually until Day 21. In contrast, the weight of CL of lactation increased steadily from the day of ovulation (Day 1) to the end of lactation (Day 21), although the maximum weight was only one half that of the CL of pregnancy. After parturition, the weight of NLO rapidly decreased by Day 4 and remained the same thereafter.

Fig. 1. Changes in weight of corpus luteum (CL) of pregnancy and lactation and non-luteal ovarian tissue (NLO) during lactation in the rat. In all figures, results are expressed as mean $\pm$ S.E.M. for 6 animals/group.

Fig. 2. Serum levels of P, 20α-OHP, T and E₂ during lactation in the rat.

Serum levels of steroids (Fig. 2)

Serum P gradually increased from Days 1 to 10 with the maximum value reached on Day 10 with the levels then decreasing slowly by Day 21. In contrast, serum levels of 20α-OHP were high on Days 1 and 2 followed by an abrupt decrease until Day 10. On Day 10, serum levels of 20α-OHP were at their nadir and thereafter gradually increased.

Levels of serum T remained unchanged at about 400 pg/ml throughout lactation. Serum E₂ was constantly low from Day 1 through Day 10 and then increased slightly from Day 12 to Day 21.

Concentration of T and E₂ in NLO (Fig. 3)

In general, throughout lactation the change

in non-luteal concentration of T was similar to that of E₂. The NLO concentrations of T and E₂ were low until Day 12, followed by an abrupt increase by Day 15 and a further increase by Day 21.

Levels of P and 20α-OHP in the CL of pregnancy and lactation (Figs. 4 and 5)

Since the growth of the CL in the rat represents hypertrophy rather than hyperplasia, luteal levels of P and 20α-OHP were calculated at the basis of content (per CL) or concentration (per mg CL).

On day 1, the CL of pregnancy contained small amounts of P and the content dropped progressively until Day 21, whereas the con-

Fig. 3. Concentration of T and E₂ in NLO during lactation in the rat.

Fig. 5. Content and concentration of 20 α -OHP in the CL of pregnancy or lactation during lactation in the rat.

Fig. 4. Content and concentration of P in CL of pregnancy or lactation during lactation in the rat.

centration of the hormone was relatively constant throughout lactation (Fig. 4). On the other hand, on Days 1 and 2, the CL of pregnancy contained high amounts of 20 α -OHP, but the content and concentration declined abruptly by Day 3. Thereafter the content of the hormone decreased gradually until Day

21, whereas there was no significant decline when they were expressed as the concentration of the hormone (Fig. 5).

In contrast, luteal content and concentration of P in the CL of lactation significantly increased by Day 3 and continued to increase markedly until Day 12 (Fig. 4). Thereafter, luteal levels of P in the CL of lactation decreased gradually until Day 21 regardless of which way they were expressed. Although luteal content of P on Days 1 and 2 was significantly higher in the CL of pregnancy than the CL of lactation, the luteal concentration of the hormone was not different between the CL of pregnancy and lactation. After Day 4, luteal content and concentration of P in the CL of lactation was significantly higher than in the CL of pregnancy. Luteal levels of 20 α -OHP, in the CL of lactation increased gradually throughout lactation (Fig. 5). The maximal content and concentration of 20 α -OHP in the CL of lactation was noted on Day 21. On Days 1 through 4, luteal levels of 20 α -OHP were significantly higher in the CL of pregnancy than in the CL of lactation, whereas

after Day 12, the values were much higher in the CL of lactation compared to the CL of pregnancy.

Serum levels of LH, FSH and PRL (Fig. 6)

Serum levels of LH were relatively high on Day 1 and abruptly decreased by Day 2; the values were low from Days 2 to 12 compared to the diestrous levels of cyclic rats. A gradual rise in serum LH began after Day 15.

Relatively high levels of serum FSH were observed on Day 1. Thereafter, serum FSH was maintained at basal levels throughout the period of lactation comparable to diestrous values in the estrous cycle.

There was a marked increase in serum PRL on Day 4 and high levels were maintained

until Day 10, followed by an abrupt decline by Day 12. Serum PRL was relatively constant from Day 12 to Day 21.

Discussion

In the present study, during the first half of lactation the low level of serum E_2 correlates with the absence of large antral follicles (Taya and Sasamoto, 1981). There were increased concentrations of E_2 and T in NLO after Day 15 of lactation (Fig. 3), indicating that follicular maturation was then initiated. These results agree with previous findings that no ovulation in response to HCG was observed and no follicles of 401–500 μ m in diameter were present during the early stages of lactation, whereas ovulation is readily produced by HCG during the second half of lactation correlated with follicles larger than 401 μ m in diameter (Taya and Sasamoto, 1977; 1981). The serum levels of E_2 agree in general with previous findings that there are low levels of estrogen during the early stages of lactation (Smith and Neill, 1977). In the present study, serum T was unchanged throughout lactation. Serum levels of T during lactation have not been reported previously.

The marked reduction in E_2 and T in NLO during the first half of lactation coincided with decreasing secretion of LH and increasing secretion of PRL whereas the secretion of FSH was unchanged (Figs. 3 and 6). The serum levels of FSH, LH and PRL (Fig. 6) agree with those of previous papers (Ford and Melampy, 1973; Smith and Neill, 1977; Taya and Sasamoto, 1981). These results suggest that decreasing serum levels of LH during the first half of lactation are insufficient to optimally support thecal cell synthesis of androgen and thus follicular E_2 secretion, since thecal cells have been shown to be the source of androgen precursors which are converted to estrogen by an FSH-dependent aromatizing enzyme system present in the granulosa cell (Erickson and Ryan, 1976; Fortune and

Fig. 6. Serum levels of LH, FSH and PRL during lactation in the rat. The dotted line of the graph represents the mean of the serum LH (6.8 ± 0.4 , $n=6$) and FSH (101.0 ± 4.0 , $n=6$) levels at 0900 h on diestrus II of cyclic rats.

Armstrong, 1977; Makris and Ryan, 1977; Tsang *et al.*, 1979).

It has been well established that the active secretion of P by the CL of lactation during lactation can be ascribed to the luteotropic action of prolactin (Yoshinaga, 1974; Ford and Yoshinaga, 1975b; Tomogane *et al.*, 1975). In the present study, the levels of P in serum and CL of lactation reached a peak on Days 10 and 12, respectively, and decreased gradually until the end of lactation. On the other hand, serum levels of PRL decreased abruptly on Day 12 and relatively low levels were maintained thereafter. The reason for the delayed response of luteal P secretion to an abrupt decline in serum PRL is not clear. In the present experiments, although the level of serum PRL was measured only at 0900 hr on each day, van der Schoot *et al.* (1978) have reported that there were at least two diurnal peaks of serum PRL in post-parturient rats during the first 4 days after removal of a 10-pup litter on Day 13 of lactation. Therefore, it is possible that nocturnal or diurnal increases in serum PRL may occur during the second half of lactation in lactating rats nursing 8 pups. It has also been reported that LH plays an important role in the secretion of P during lactation in the rat, since the administration of antisera against LH to lactating rats causes a decline in the concentration of P in ovarian venous blood (Yoshinaga *et al.*, 1971; Ford and Yoshinaga, 1975a). Previous reports have demonstrated that the ability of LH to stimulate P is augmented by the presence of PRL (Armstrong *et al.*, 1969; Wu and Wiest, 1976), most probably in increasing the number of LH receptors in the CL (Grinwich *et al.*, 1976; Holt *et al.*, 1976; Behrman *et al.*, 1978). Serum levels of 20 α -OHP show an inverse relationship throughout lactation to serum P (Fig. 2). Our data also indicate that during the early stages of lactation the CL of pregnancy contain considerable amounts of 20 α -OHP and small amounts of P. On the other hand, in the CL of lactation luteal levels of P and 20 α -OHP increased gradually. These

results clearly indicate that during the early stages of lactation the high levels of serum 20 α -OHP represent a proportionally larger contribution from the CL of pregnancy than the CL of lactation. Furthermore, the maximal content and concentration of P in CL of lactation are much greater than those of CL of pregnancy during gestation (Taya and Greenwald, 1981), though the CL of lactation are much smaller than the CL of pregnancy. Ford *et al.* (1975) have reported that the corpora lutea of pregnancy are the major contributor to ovarian 20 α -OHP secretion during midlactation using post-partum ovulation blocked rats by antisera against LH releasing hormone. The present results on changes in peripheral P and 20 α -OHP levels agree in general with previous findings in the ovarian venous blood (Tomogane *et al.*, 1969; Yoshinaga *et al.*, 1971). During the first half of lactation, high levels of serum P secreted from the functional CL associated with the suckling stimulus and high levels of PRL may suppress serum LH, since exogenous P injected into diestrous rats lowers serum LH within 1 h (Taya *et al.*, 1981).

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