BIOLOGY OF REPRODUCTION 10, 103–110(1974)

Temporal Relationship Between Secretory Patterns of Gonadotropins, Estrogens, Progestins, and Prostaglandin-F in Periparturient Rats

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Accepted July 20, 1973

To study changes in the secretory patterns of eight different hormones in relation to the day of parturition, blood samples were obtained sequentially from the ovarian vein, the uterine vein, and the posterior vena cava from each of 36 rats under chloral hydrate anesthesia. The blood sampling, which lasted for half an hour, was done during pregnancy from Day 18 to term and during the first 8 days after parturition. For each day, four rats were used. Plasma from the ovarian vein blood was analyzed for estradiol, estrone, progesterone, and 20α-dihydroprogesterone (20α-OH-P), that from the uterine vein for prostaglandin (PG) F, and that from the vena cava for LH, FSH, and prolactin by respective radioimmunoassays and the concentration of various hormones correlated with Day 22 when parturition occurred. The progesterone concentration dropped significantly on Day 19, but no significant rise in PGF was observed at this time. The 20α-OH-P secretion remained low until Day 20 and showed a striking rise on Day 21 which was maintained until Day 3 postpartum. The estradiol level rose significantly first on Day 21, i.e., some 48–72 h after a drop in progesterone, but estrone secretion showed no marked variation. The rise in estradiol secretion coincided with a significant increase in the PGF level and was followed by a surge in LH on Day 22. The FSH level showed a tendency for decline while prolactin concentration rose at approximately the same time as estradiol. It is suggested that the luteolytic action of PGF is unlikely to contribute to a reduction in the progesterone secretion on Day 19 and, since the withdrawal of "progesterone block" is believed to be one of the first steps in the initiation of parturition, it is unlikely that PGs provide a trigger for this process. It is likely that PGs participate in the later stages of parturition by expelling fetuses through their oxytocic property. The data suggest that, in some respects, hormonal interrelationship preceding parturition may be similar to that found on the day of proestrus in the cycle.

It is now well recognized that parturition is a complex process involving interplay of several endocrine factors. In the rat, it is known that secretion of progesterone drops and that of 20α-dihydroprogesterone (20α-OH-P) (Fajer and Barraclough, 1967; Hashimoto *et al.*, 1968; Wiest *et al.*, 1968), estrogens (Yoshinaga *et al.*, 1969; Shaikh, 1971), LH (Linkie and Niswender, 1972; Bast and Melampy, 1972), and prolactin (Amenomori *et al.*, 1970; Nagasawa and Yanai, 1972, Linkie and Niswender, 1972) rise prior to parturition. The secretion of FSH showed a tendency to decline (Linkie and Niswender, 1972). Since individual hormones were measured by different groups of workers in different studies, only a rough temporal relationship between gonadotropins, steroids, and parturition can be delineated from these studies. It was thought that by measuring all these hormones and prostaglandin (PG) F levels in the same rats both prior to parturition and during the immediate postpartum period, one might be able to examine more closely a temporal relationship between these hormones and PGF at the time of parturition. It is now

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widely believed that PGs are in some way involved in the process of parturition (for review see Labhsetwar, 1974). In the present study, we collected the ovarian venous blood for analysis of estradiol, estrone, progesterone, and 20α-OH-P, the uterine venous blood for determination of PGF levels, and the peripheral blood for assay of gonadotropins (LH, FSH, and prolactin) from each of 36 rats at various stages of pregnancy beginning on Day 18 to term and also during the immediate postpartum period. This permitted us to examine interrelationships between eight different hormones, some of which might be involved in the initiation of parturition. The necessity of collecting ovarian venous blood for estrogen determination was dictated largely by the presence of cross-reactive material of adrenal origin in the peripheral blood of rats (Labhsetwar, 1972). Since the ovarian venous blood contains estrogens in a nanogram range, this cross-reaction, which amounts to some 50 pg equivalents of estradiol, can be safely ignored.

MATERIALS AND METHODS

A group of 36 mated rats was received in the laboratory on Day 16 of pregnancy (day of sperm = Day 1 of pregnancy) from the Charles River Co., Wilmington, Ma. Animals were housed under controlled environmental conditions of light (12 h light; 12 h dark) and temperature (71°–72°F) and provided free access to food and tap water.

Collection of blood samples. Rats were anesthetized with chloral hydrate (7.5% aqueous solution, 5 ml/kg, ip). After appropriate surgical manipulations (see Eto *et al.*, 1961), the uterine branch of the utero-ovarian vein was ligated on one side. A polyethylene cannula attached to a 23-gauge needle and filled with heparin solution was inserted into the utero-ovarian vein and ovarian vein blood collected for 15 min, as described by Eto *et al.* (1961). After ovarian vein and blood collection, the ovarian branch of the utero-ovarian vein on the other side was tied and the uterine vein blood collected for approximately 15 min. Finally, before killing the animal, peripheral blood sample was obtained from the posterior vena cava. Each animal remained anesthetized for approximately half an hour before three sets of samples could be collected. The blood samples were obtained on Days 18, 19, 20, 21, and 22 of pregnancy and on Days 1, 2, 3, and 8 postpartum. On each day, four rats were used. The group on Day 22 of pregnancy included two animals which showed vaginal bleeding while two animals were in the process of delivery, having partially expelled some of the fetuses. All the postpartum animals used in this experiment delivered on the night of Day 22, the next day being considered as Day 1 postpartum. During the postpartum period, animals were permitted to nurse their litters.

All blood samples were immediately centrifuged and plasma stored frozen until analyzed for hormones. The volume of plasma collected ranged from 0.5 ml (ovarian vein) to 1.5 ml (vena cava).

Steroid assays. Each ovarian vein plasma sample was analyzed for estradiol, estrone, progesterone, and 20α-OH-P by a method similar to that already described (Abraham *et al.*, 1971; Abraham, personal communication) using slight modifications (Labhsetwar *et al.*, 1973; Prasad *et al.*, 1973).

Chemicals. Radioactive steroids were obtained from New England Nuclear, Cambridge, with the following specific activites: [6,7-^{3}H]-estradiol-17β, 40–50 Ci/mmole; [6,7-^{3}H]-estrone, 30–50 Ci/mmole; [1,2-^{3}H]-progesterone 40–50 Ci/mmole, and [1,2-^{3}H]-20α-OH-P 40–50 Ci/mmole. All solvents were redistilled, and water was glass redistilled in the laboratory before use.

Extraction. The ovarian venous plasma samples were thawed and the volume brought to the nearest milliliter by the addition of distilled water. The plasma volume averaged about 0.5 ml. The following radioactive steroids were then added to each tube: progesterone-3000 cpm (5–10 pg/tube); 20α-OH-P 1500 cpm (5–10 pg/tube); 17β-estradiol 750 cpm (2.5–5 pg/tube); and estrone 750 cpm (2.5–5 pg/tube). This permitted calculation of losses incurred during the procedure. The samples were then extracted three times with 3 vol of ethyl acetate:cyclohexane (2:1, v/v) mixture. The organic phase was pooled and evaporated under nitrogen. The dried extract was applied to Celite microcolumns prepared according to the method of Abraham *et al.* (1971). The only difference was that Celite, which had been heated at 1000°F for 16–18 h a day earlier, was weighed in portions of 1.0 g before packing into the column to give more reproducible separation. The columns were washed with 15 ml of iso-octane prior to use. They were serially eluted under nitrogen pressure to obtain four fractions as follows:

Fraction A. Four and a half milliliters of iso-octane was passed through the columns. The nitrogen pressure was adjusted to obtain 6–8 drops/min.

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This fraction contains cholesterol, progesterone, Δ^4-androstenedione, etc.

Fraction B. This was followed by elution with 4.5 ml of 35% benzene in iso-octane which yielded fraction containing 20- or 20β-OH-P, testosterone, etc.

Fraction C. Four and a half milliliters of 15% ethyl acetate in iso-octane was used to elute estrone, androstanediols, etc.

Fraction D. Four and a half milliliters of 40% ethyl acetate in iso-octane eluted 17β- or 17α-estradiol.

Progesterone assay. Fraction A was analyzed for progesterone. The eluate was dried and further purified by column partition chromatography on Sephadex LH-20. A slurry of Sephadex was made in 90% methanol in distilled water and 5-ml disposable pipets (5 mm diameter) were filled with the slurry (0.5 g/column). The column was flushed under gravity with 15 ml of iso-octane. The fraction A from the previous chromatogram was dried, concentrated in the tip of a conical tube, and transferred with 3 × 0.2 ml of iso-octane to the Sephadex column. The column was washed with 4.5 ml of iso-octane, and this eluate, which contains most of the fat, was discarded. Then 5 ml of cyclohexane was passed and this eluate, which contains progesterone, was saved. The columns can be reused up to three times when washed with 5 ml of 90% methanol between use.

The progesterone fraction was quantified using antibody developed by Drs. Stylos and Burstein in the rabbit by a procedure similar to that described before (Thornycroft *et al.*, 1970; Thornycroft and Stone, 1972). Rabbits were injected with progesterone conjugated at 11α-position to bovine serum albumin–hemisuccinate. The antibody was used in an initial dilution of 1:1000 and demonstrated no significant (<5%) cross reaction with 20α-OH P, testosterone, androstenedione, corticosterone, when each steroid was tested at a concentration of 0.5 ng/tube, but 17α-OH-progesterone showed 14% cross reaction. Since this steroid is eluted in Fraction B, this cross reaction can be ignored. To validate the method known, amounts of progesterone were added ranging from 1–50 ng/ml of human plasma (six levels, $n = 4$ at each level). For each nanogram of progesterone added, 0.988 ng (0.870–1.10 ng, 95% confidence limits) was recovered after adjusting for losses which ranged between 20 and 35%. The sensitivity of the assay was 20 pg with intra-assay coefficient of variation of 7.4% and interassay variation of 8.4%.

For quantitation of 20α-OH-P, Fraction B from the Celite column was dried, taken up in 0.5 ml of acetone, and treated with 0.1 ml of Jones reagent (CrO_3, 267 g, and H_2SO_4, 230 ml, made to 1 liter with water). The reagents were mixed and kept at room temperature for 10 min. The oxidation was stopped by the addition of 1.5 ml sodium carbonate (5%). Progesterone was then assayed as above. 17α-OH-Progesterone present in this fraction is converted to androstenedione after chromic acid oxidation. Since androstenedione showed no significant cross reaction with the antibody, the 14% cross reaction with 17α-OH-progesterone stated earlier does not affect the assay of 20α-OH-P. When known amounts of 20α-OH-P ranging from 0.5–10 ng/ml human plasma were added at five different levels ($n = 4$ for each level) for each ng of the steroid added, 1.01 ng (0.955–1.06, 95% confidence limits) of 20α-OH-P was recovered with intra-assay coefficient of variation of 9.1%. The sensitivity of the assay was 20 pg. The recovery of added steroid ranged between 50 and 80%.

Estradiol and estrone were assayed as described by Abraham *et al.*, (1971) using the antibody (SLC-6) already characterized (Thornycroft *et al.*, 1970). It was used in an initial dilution of 1:60,000 for estrone and 1:85,000 for estradiol. When known amounts of estradiol and estrone were added to human plasma in amounts ranging from 100–1000 pg/ml, 105% (99–111, 95% confidence limits) of added estradiol and 97% (94–101, 95% confidence limits) of added estrone were recovered, after adjustment for procedural losses, which ranged between 5 and 20% for estradiol and 15 and 30% for estrone. Both intra- and interassay coefficients of variation for estrone and estradiol were less than 10% and the least amount that could be distinguished from zero was 10 pg for each steroid. For an accurate measurement of estradiol, the sample must contain at least 50 pg of steroid.

PGF assay. The uterine vein plasma samples were thawed, labeled, $PGF_{2\alpha}$ (New England Nuclear) added (600–700 cpm, equivalent of 50 pg, 52% counting efficiency) to permit correction for losses and extracted with 3 vol of ethyl acetate as described by Skarnes and Harper (1972), fractionated on silicic acid columns (Caldwell *et al.*, 1971), and quantified by radioimmunoassay using conditions already described (Stylos *et al.*, 1972). Since the sensitivity of the method is 100 pg and the uterine venous plasma contained several nanograms of PGF/ml, only a small fraction of the original extract was necessary for final determination. Each uterine venous plasma sample was run in duplicate and values adjusted to 100% recovery. The mean recovery of added $PGF_{2\alpha}$ was 78% (range 61–93%). The coefficient of variation was 13%.

Gonadotropin assays. Peripheral plasma sample from each rat was assayed for LH, FSH, and prolactin, using respective NIAMD radioimmuno-

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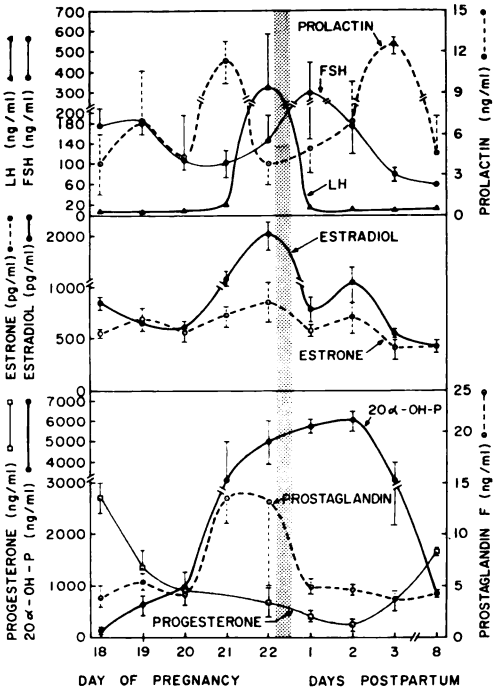

FIG. 1. The ovarian vein plasma levels of progesterone, 20α-dihydroprogesterone (20α-OH-P), estradiol, and estrone; the uterine vein plasma levels of PGF; and the peripheral plasma levels of LH, FSH, and prolactin in individual periparturient rats. All eight hormones were determined in the same rat. The shaded area denotes approximate time of the completion of parturition. The postpartum animals were permitted to nurse their litters. All values are expressed nanograms or picograms/per milliliters of plasma. Each value represents mean ±SEM ($n = 4$ per group). Standard errors of LH means except for Day 22 are not shown as they were less than 5% of the mean.

assay kits. Results were expressed using reference standards accompanying the kits (RP-1).

RESULTS

All results are illustrated in Fig. 1.

Progestins

Progesterone. Ovarian venous plasma levels of progesterone decreased significantly on Day 19 ($P < 0.05$) and remained low without any further changes until the day of parturition, i.e., Day 22. Even after parturition, the values on Days 1, 2, and 3 postpartum were comparable to those found on Day 19 of pregnancy. By Day 8 postpartum, however, progesterone concentration rose significantly as compared to that on Day 1 or 2 postpartum.

20α-OH-P. The concentration of this steroid was low on Day 18 and increased significantly by Day 20 when progesterone level had already reached a nadir. A further precipitous rise was observed on Day 21. The values on Day 22 of pregnancy and on Days 1, 2, and 3 postpartum remained comparable to those on Day 21 but dropped to the baseline by Day 8. Thus, the concentration of 20α-OH-P remained high for 3 days after parturition when progesterone level was lowest.

Estrogens

Estradiol. While the concentration of progesterone in the ovarian venous plasma dropped progressively from Days 18–20, the values for estradiol showed no significant fluctuations during this period. By Day 21 the estradiol level rose significantly reaching a peak on Day 22. Thus, there was a lag period of about 48–72 h between a significant drop in progesterone and a rise in estradiol. The estradiol concentration dropped significantly within 24 h after parturition and, except for a slight rise on Day 2 postpartum, decreased to a baseline value during the remainder of the postpartum period.

Estrone. The concentration of estrone showed some fluctuations but they could not be established as statistically significant during the entire period of study.

PGF

The uterine vein plasma levels of PGF remained more or less constant from Days 18–20 of pregnancy when progesterone showed a significant drop and 20α-OH-P showed a significant rise. A significant rise was observed on Day 21, i.e., more than 24 h prior to the onset of parturition and coincided with a significant rise in estradiol in the ovarian venous plasma. The PGF

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values remained high on Day 22 but variation was very marked; one animal showed very high value. The PG level dropped sharply within 24 h after parturition and remained at the baseline level during the remainder of the postpartum period studied.

Gonadotropins

LH. The LH concentration in the peripheral plasma remained at the baseline level until Day 21 and rose significantly to a peak level on Day 22. The values dropped to the baseline with 24 h after parturition and remained low throughout the postpartum period. It is noteworthy that the peak in LH was preceded by a significant rise in estradiol secretion a day earlier, and LH rose 3 days after a drop in progesterone. It is also noteworthy that a significant drop in progesterone on Day 19 was not accompanied by a significant rise in the peripheral plasma LH.

FSH: Changes in FSH concentration were not synchronous with those in LH. The FSH level showed a tendency, although not statistically significant, to decline during 2–3 days preceding parturition when compared with values on days 18–19 of pregnancy. The peak FSH concentration occurred within 24 h after parturition, i.e., about a day out of phase with the LH peak. The FSH value on Day 8 is based on only one determination.

Prolactin. An increased secretion of prolactin was observed on Day 21 coinciding with rising levels of estradiol. The level dropped on Day 22 prior to parturition but was followed by a rise during the postpartum period.

DISCUSSION

The primary purpose of this study was to delineate the temporal relationship between eight different hormones in the periparturient rat. This study by no means provides a total picture as patterns of oxytocin and adrenal steroids were not monitored. Further, the stress imposed by surgical manipulations could alter or attenuate some of the hormonal patterns observed in this study. On the other hand, measurement of several hormones in the same rat is likely to provide a better understanding of the temporal relationship than the study of individual hormones in different rats.

The progesterone concentration decreased significantly several days before parturition between Days 18 and 19 and remained low during the remainder of gestation and up to 3 days postpartum. By contrast, the concentration of 20α-OH-P showed a negative relationship with progesterone as its concentration rose to peak levels on Days 21 and 22 when progesterone had already declined to the baseline level. These results confirm earlier studies of several investigators (Fajer and Barraclough, 1967; Hashimoto *et al.*, 1968; Wiest *et al.*, 1968). The nature of endocrine factor(s) responsible for inducing the drop in progesterone on Day 19 several days prior to parturition is not known. However, it is noteworthy that the decreased progesterone secretion was not associated with any significant rise in the uterine venous plasma levels of PGF. If the well-documented luteolytic action of PGF (see Pharriss *et al.*, 1972; Labhsetwar, 1974) is causally involved in progesterone decline on Day 19, then changes in the uterine secretion of PGF must be such that they cannot be detected by once-a-day sampling, and perhaps more frequent sampling may be required. The other possibility is that instead of PGs of uterine origin, those of ovarian origin may be involved. It is also possible that the decrease in progesterone secretion on Day 19 onward may not be due to an intervention of a luteolytic mechanism but may be due to a withdrawal of the luteotropic support of placental origin. The placental secretion of luteotropic hormone is well known in the rat (see Greenwald and Rothchild, 1968). The luteolytic action of LH (Rothchild, 1965) is unlikely to be involved in decreasing progesterone secretion as LH concen-

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tration remained at a baseline level during the period when progesterone secretion declined significantly.

The concentration of 20α-OH-P rose significantly on Day 20 with a further striking rise on Days 21 and 22. The high levels were maintained during the first 3 days postpartum. Wiest *et al.* (1968) have reported that LH stimulates 20α-hydroxysteroid dehydrogenase in the ovary under experimental conditions and increased LH secretion may enhance secretion of 20α-OH-P from the ovary prior to parturition. However, the plasma level of LH did not rise when ovarian secretion of 20α-OH-P rose significantly. It is clear that, in addition to LH, other factors are probably involved in stimulating 20α-OH-P production. One of the factors Wiest *et al.* (1968) suggested is the withdrawal of placental support as mere dislocation of placentas of about midgestation markedly stimulated 20α-hydroxysteroid dehydrogenase activity in the ovary. It is thus probable that the withdrawal of placental support leads to not only a drop in the progesterone output but also to a rise in the 20α-OH-P secretion.

The concentration of estradiol rose significantly 3 days after a drop in progesterone secretion, attaining a peak on Day 22, i.e., on the day of parturition. Coincidentally, PGF concentration in the uterine venous blood also rose at this time. Since estrogen can stimulate uterine synthesis of PGs (Blatchley *et al.*, 1971), it is possible that the rise in PGF secretion was due to a concomitant increase in the estrogen output from the ovary. That these raised levels of PGF may be involved in parturition is suggested by the fact that inhibitors of PG synthesis delay parturition (Chester *et al.*, 1972) and administration of exogenous PGs can cause a premature littering of pups in rats (unpublished observations). The time when we found increased secretion of PGF in the uterine vein blood coincides with the ability of uterine strips to synthesize increased amounts of PGF *in vitro*, the rate of synthesis being higher on Days 21 and 22 than on Day 19 of pregnancy (Aiken, 1972). It is interesting that no further drop in progesterone concentration was observed in our study during the period of an increased uterine secretion of PGF. Thus, our results suggest that the withdrawal of "progesterone block" does not occur as a result of the luteolytic action of PGF. It appears unlikely that PGs provide a trigger for the initiation of parturition but their oxytocic property may be involved in the later stages of parturition. Thus, an increased secretion of estrogen preceding parturition may serve a triple purpose—stimulate synthesis of PG in the uterus, sensitize the uterus to oxytocic property of PGs as the estrogen-dominated muscle is more responsive to PGs than the progesterone-dominated muscle (see Speroff and Ramwell, 1970), and finally trigger LH release from the pituitary for postpartum ovulation.

The secretion of LH was low on Days 19, 20, and 21 of pregnancy despite the fact that progesterone values had already dropped significantly on Day 19. LH showed a surge on Day 22, i.e., 1 day after a significant rise in the estradiol secretion and several days after a drop in the progesterone secretion. This result suggests that the rise in LH preceding parturition is not secondary to a withdrawal of progesterone with a consequent interruption of the negative feedback mechanism. Rather, it appears to be due to an intervention of a positive feedback of estrogen similar to that known to occur in the estrous cycle (Ferin *et al.*, 1969; Labhsetwar, 1970). This LH rise is almost certainly involved in the postpartum ovulation (Hoffman and Schwartz, 1965).

The changes observed in FSH were not synchronous with those in LH. FSH showed a tendency to decrease toward the end of pregnancy as was recently reported by Linkie and Niswender (1972). On the day when LH rose to peak values, i.e., Day 22, the FSH concentration did not show

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a surge. This finding contrasts with a pattern during the estrous cycle when LH and FSH rise to peak values on the afternoon of proestrus, athough probably not synchronously (Gay *et al.*, 1970; Daane and Parlow, 1971). In this respect, the pattern of LH and FSH secretion preceding postpartum ovulation seems to differ from that preceding spontaneous ovulation during the cycle.

Interpretation of prolactin data is difficult in view of the susceptibility of its secretion to a variety of stimuli including anesthesia (see Meites *et al.*, 1972, for review). The effect of such stress on the secretion of LH and FSH is much less marked (Ajika *et al.*, 1972), one study finding no effect of ether anesthesia on LH release (Neill, 1970).

ACKNOWLEDGMENTS

We thank C. McDonald, P. Dukes, L. Levy, and C. Roberson for assistance during various phases of this work, Dr. B. Stylos for providing antiserum to PGF and progesterone, and NIAMD for supplying radioimmunoassay kits of gonadotropins. The study was partially supported by Contract CSD/2837 from A.I.D. and a grant from the Ford Foundation.

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