Yamanashi Med. J. 1(2), 31~37, 1986

Original Article

Prostaglandin $F_{2\alpha}$ Metabolites in Plasma and Urine in Relation to Peripheral Plasma Levels of Progesterone and Estradiol-17β in the Rat During Pregnancy and Parturition

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Abstract: The major prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) metabolite in the plasma, 13.14-dihydro-15-keto-$PGF_{2\alpha}$ (dhk-$PGF_{2\alpha}$), and that in the urine, 5,7-dihydroxy-11-keto-tetranor-prosta-1,16-dioic acid ($PGF_{2\alpha}$-MUM), were measured by radioimmunoassay from day 13 of pregnancy to day 2 after parturition in the rat. Progesterone (P) and Estradiol-17β (E_2) in the rat plasma were also measured simultaneously. The levels of two $PGF_{2\alpha}$ metabolites increased remarkably from day 20 to parturition. The P concentration declined rapidly on day 21, while the E_2 concentration increased significantly from day 19 to parturition. These results indicate the increase of endogenous $PGF_{2\alpha}$ production during late pregnancy and parturition. The increased $PGF_{2\alpha}$, probably induced by the rise of plasma E_2, may be responsible for luteolysis and parturition in the rat.

Key words: Prostaglandin $F_{2\alpha}$ metabolite, Progesterone, Estradiol-17β, Rat pregnancy and parturition

Introduction

Administration of prostaglandin (PG)$F_{2\alpha}$ to pregnant rats is known to induce parturition[1)] and premature delivery[2)]. PG synthesis inhibitors given to pregnant rats during the final days of gestation resulted in delayed or prolonged parturition[3,4,5)]. Considering such evidence, $PGF_{2\alpha}$ seems to be an important mediator of the parturition process, and endogenous $PGF_{2\alpha}$ biosynthetic activity has been considered to increase at the end of pregnancy.

To evaluate the relationship between endogenous $PGF_{2\alpha}$ and rat parturition, the $PGF_{2\alpha}$ in the rat should be measured. However $PGF_{2\alpha}$ has been reported to be rapidly metabolized in the lung and kidney[11)], and its plasma half life has been estimated to be less than one minute[12)]. Thus, it has now been widely accepted that measurement of $PGF_{2\alpha}$ in the peripheral circulation or in urine does not always give a true picture of the total body synthesis of this compound[6)]. We measured the stable $PGF_{2\alpha}$ metabolite in the plasma (13, 14-dihydro-15-keto-$PGF_{2\alpha}$) and that in the urine (5,7-dihydroxy-11-keto-tetranor-prosta -1, 16-dioic acid) by a specific radioimmunoassay, which may be a useful method of monitoring $PGF_{2\alpha}$ production.

We also measured progesterone (P) and estradiol-17β (E_2) in the rat peripheral

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Received January 30th, 1986

plasma simultaneously to examine the periparturitional relationships between $PGF_{2\alpha}$ production and ovarian function in the rat.

Material and Methods

Female Wistar rats were cohabited with males overnight and were checked for the presence of sperm in the vagina the next morning. The day vaginal sperm was confirmed was regarded as day 1 of pregnancy.

Voided 24 hour urine was collected from 6 rats kept in a special cage on each day from day 13 of gestation to 2 days after parturition. Systemic blood samples were collected between 1000–1200 hs from 5–6 rats on days 13, 15, 17, 19, 20 and 21 of gtstations, intrapartum (day 22), and 24 and 48 hs after parturition. All blood samples were collected from the abdominal aorta with the aid of a heparinized syringe under ether anesthesia. They were immediately centrifuged and the plasma was separated. All samples were stored at −20°C until assay.

Parturition occurred in the late afternoon and evening of day 22 in all rats which were kept for the postpartal sampling.

Assays of $PGF_{2\alpha}$ metabolites and steroid hormones

13, 14-Dihydro-15-keto $PGF_{2\alpha}$ (dhk-$PGF_{2\alpha}$)

Plasma samples (1.0 m*l*) were acidified to pH3 with 1 N HCl and extracted twice with five volumes of ethyl acetate after addition of (5, 6, 8, 11, 12, 14 [n]-^{3}H) dhk-$PGF_{2\alpha}$ (approximately 3,000 dpm) for recovery.

Chromatography and radioimmunoassay were carried out as reported previously[7].

5α, 7α-Dihydroxy-11-keto-tetranor-prosta-1, 16-dioic acid ($PGF_{2\alpha}$-MUM)

The $PGF_{2\alpha}$-MUM levels in urine were assayed according to the method of Ohki and associates[8].

Five m*l* of urine was adjusted with 0.1 N NaOH to pH 10. After the incubation at room temperature for 40 minutes, the pH was readjusted to 7.3 with 0.1 N HCl.

For the radioimmunoassay procedure which was described previously, 0.1 m*l* of 100–200 fold diluted urine was used[8].

Progesterone (P) and estradiol-17β (E_2)

For the measurement of P and E_2, 1.0 m*l* aliquots of plasma were transferred to 10 m*l* centrifuge tubes.

Approximately 1800–2300 dpm of ^{3}H-P and ^{3}H-E_2 were added to the plasma samples for recovery. The steroids were extracted twice with ether (4/1, v/v), separated by celite chromatography and quantitated by radioimmunoassay according to the methods of McNatty and Ryan[9,10].

^{3}H-dhk-$PGF_{2\alpha}$, ^{3}H-E_2 and ^{3}H-P were purchased from New England Nuclear Corp., Boston. The P-BSA and E_2-BSA antisera were supplied from Teikoku-zoki Co., Tokyo. The dhk-$PGF_{2\alpha}$ antiserum, $PGF_{\alpha 2}$-MUM antiserum and ^{125}I-$PGF_{2\alpha}$ MUM were supplied by Upjohn Co., Kalamazoo and Ono Phamaceutical Co., Osaka. Numerical data were analyzed by Student's test and the Welch Test.

Results

Dhk-$PGF_{2\alpha}$ in plasma

Up to day 19 of gestation, the dhk-$PGF_{2\alpha}$ concentrations in the plasma were almost in a range of 200–300 pg/m*l*. On day 20 the concentration increased significantly to 582 ± 172 pg/m*l* (mean ± S.D.) ($P < 0.025$), and then dropped to 470 pg/m*l* on day 21, which was still higher than the values on day 13–19. At parturition, the dhk-$PGF_{2\alpha}$ concentration was maximal

Table 1. The concentrations of dhk-$PGF_{2\alpha}$, Progesterone and Estradiol-17β in rat plasma (mean±SD)

Day of gestation	dhk-$PGF_{2\alpha}$ pg/ml	Progesterone ng/ml	Estradiol-17β pg/ml
13	276± 12(5) a	76.5±21.8(5) a	67.0±19.2(5) b
15	218± 76(5) a	87.4±25.1(5) a	64.8±13.0(5) b
17	250± 61(6) a	82.1±17.0(6) a	73.7± 8.7(6) b
19	316±103(5) a	78.4± 9.8(5) a	59.5± 6.7(5) b
20	587±172(6) bc	93.9±21.9(6) a	101.8±30.3(6) c
21	470±113(5) b	19.4± 7.6(5) b	221.2±74.4(5) cd
P	649±244(6) bc	10.5± 4.2(6) bc	176.3±52.5(6) cd
AP1	309±130(5) a	7.3± 1.9(5) bc	22.5±10.6(5) a
AP2	281± 85(5) a	4.2± 2.3(5) bcd	17.8± 8.9(5) a

Numbers in parenthesis=number of animals.
P=During parturition AP=After parturition
b: $P<0.05$ vs a, c: $P<0.05$ vs b, d: $P<0.05$ vs c, e: $P<0.05$ vs d

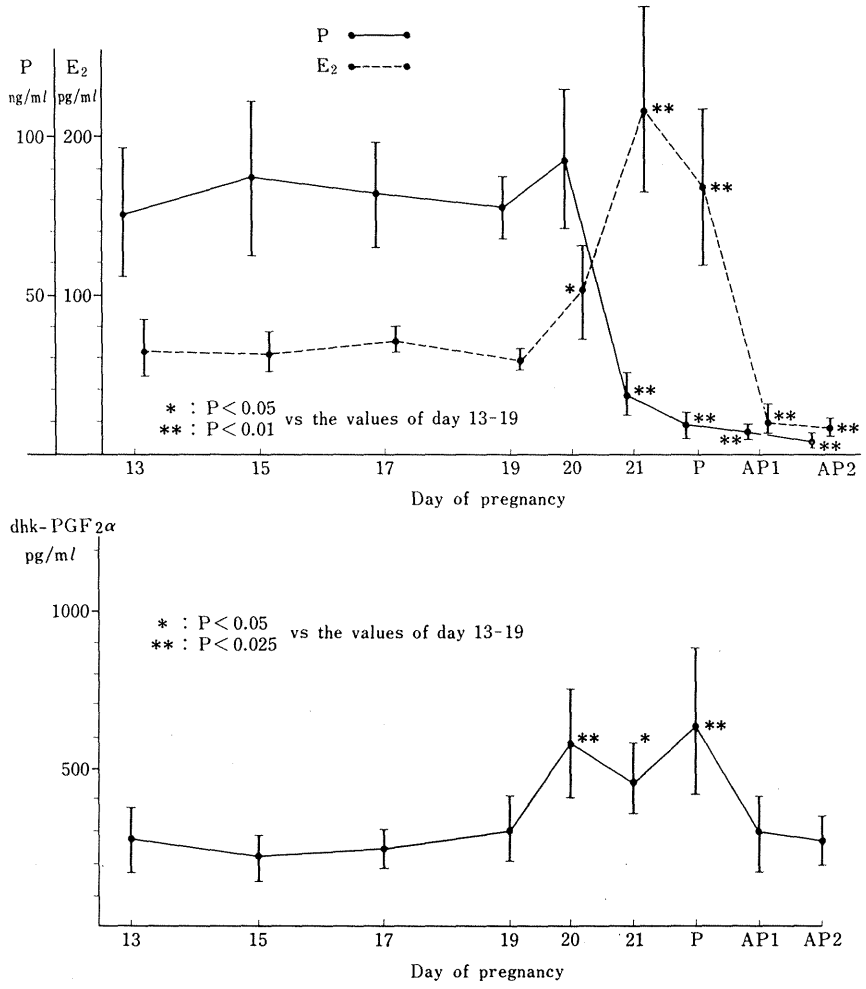

Fig. 1. The concentrations of progesterone (P), estradiol-17β (E_2) and dhk-$PGF_{2\alpha}$ in rat plasma during pregnancy and parturition.

Table 2. Urine volume, concentration, and daily excretion of $PGF_{2\alpha}$-MUM (mean±SD) of the rat during pregnancy and parturition

Day of gestation	Urine volume m*l*/day	Concentration μg/m*l*	Daily excretion μg/day	No. of Animals
13	19.5±2.1 a	1.13±0.21 a	20.1± 7.6 a	6
14	21.4±2.3 a	0.78±0.15 a	16.3± 4.0 a	6
15	20.3±1.9 a	0.94±0.13 a	19.2± 2.8 a	6
16	18.8±2.0 a	0.83±0.28 a	15.5± 5.1 a	6
17	17.6±1.6 a	1.12±0.32 a	20.2± 8.8 a	6
18	19.2±2.5 a	0.93±0.09 a	17.6± 4.3 a	6
19	18.4±1.3 a	1.25±0.26 a	23.4± 2.2 a	6
20	18.5±0.9 a	3.81±0.62 bc	69.4±10.3 bc	6
21	13.6±5.3 b	5.80±1.92 bcd	80.1±26.5 bc	6
22(P)	8.1±0.7 bc	12.60±3.16 bcde	91.6±22.1 bc	6
AP1	16.4±4.0 a	2.71±0.41 b	44.5±14.2 b	6
AP2	18.5±3.2 a	2.10±0.50 b	37.7±11.3 b	6

P=During parturition AP=After partrition
b: $P<0.05$ vs a, c: $P<0.05$ vs b, d: $P<0.05$ vs c, e: $P<0.05$ vs d

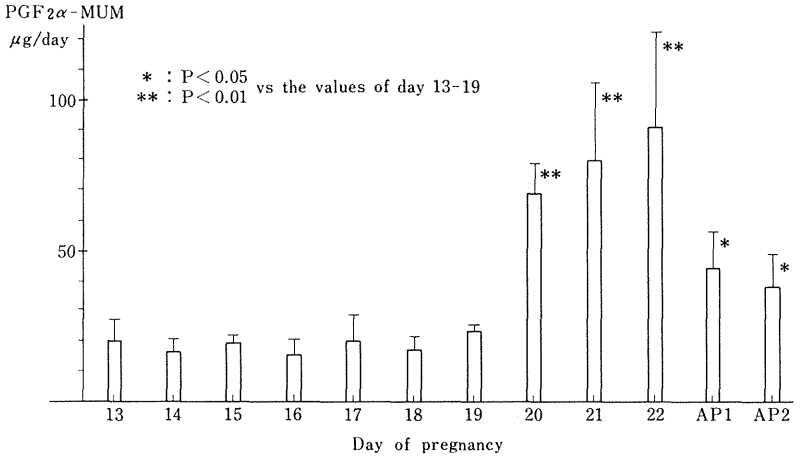

Fig. 2. The daily excretion of $PGF_{2\alpha}$-MUM of the rat during pregnancy and parturition.

at 649 ± 244 pg/m*l*. Twenty-four hs after parturition the dhk-$PGF_{2\alpha}$ concentration dropped to the level of day 13–19 (Table 1, Figure 1).

$PGF_{2\alpha}$-MUM

The concentration (ng/m*l*) and the daily excretion of $PGF_{2\alpha}$-MUM (μg/day) were estimated in 72 samples from 6 rats. Although the daily urine volume in the rats was almost constant in the range of 16–21 m*l* during pregnancy and after parturition, it decreased to less than 10 m*l* in 3 rats on day 21 and in all rats on the day of parturition (day 22) (Table 2).

Little fluctuation of $PGF_{2\alpha}$-MUM levels was observed until day 19. On day 20 excretion ($PGF_{2\alpha}$-MUM concentration: 3.81 ng/m*l*; daily excretion: 69.4 μg/day) increased significantly ($P<0.005$), followed by steady increase to the day of parturition. On 1–2 days after parturition $PGF_{2\alpha}$-MUM excretion remained relatively high (Table 2, Figure 2).

Progesterone (P) and estradiol-17β (E_2)

Changes in the peripheral plasma concentration of P and E_2 are shown in Table

l and Figure 1.

The P concentrations were at a high level from day 13 to day 20, but there was a rapid decline from 93.9 ± 21.9 ng/m*l* on day 19 to 19.4 ± 7.6 ng/m*l* on day 21 ($P < 0.005$), then gradually decreasing during parturition and postpartum. The E_2 concentration increased significantly from 53.5 ± 13.9 pg/m*l* on day 19 to 91.8 +39.3 pg/m*l* on day 20 ($P < 0.05$) and showed a maximal value of 221.1 ± 74.4 pg/m*l* on day 21, and decreased slightly at parturition.

Discussion

$PGF_{2\alpha}$ has been reported to be rapidly metabolized in the rat lung and kidney to a series of structually related prostanoic acid derivatives and excreted into urine in the form of a 16-carbon-membered metabolite[12]. 13, 14-Dihydro-15-keto-$PGF_{2\alpha}$ is the main metabolite in rat, rabbit and human plasma, and has a longer half life and is not generated during preparation of plasma or serum samples.

About 15 to 30% of the $PGF_{2\alpha}$ produced is excreted into urine in the form of a 16-carbon-membered metabolite, 5α, 7α-dihydroxy-11-keto-tetranor-prosta-1,16 dioic acid {the main urinary metabolite of $PGF_{2\alpha}$ ($PGF_{2\alpha}$-MUM)}[12].

Measurement of these two metabolites in blood and urine may be a practical way of monitoring $PGF_{2\alpha}$ production. The levels of dhk-$PGF_{2\alpha}$, progesterone and estradiol-17β have been presented as concentrations in plasma on samples collected once a day from the same rat on each day of gestation. Therefore no consideration of possible diurnal or individual variations was made.

The P and E_2 concentrations in rat plasma during pregnancy and labor were essentially the same as those reported earlier by several investigators[14,15,16]

Measurement of $PGF_{2\alpha}$ metabolites revealed that there was a remarkable increase in the level of the two $PGF_{2\alpha}$ metabolites, dhk-$PGF_{2\alpha}$ in plasma and $PGF_{2\alpha}$-MUM in urine from day 20 to parturition. The increase in excretion rate of $PGF_{2\alpha}$-MUM was much greater than the increase in the plasma dhk-$PGF_{2\alpha}$ levels.

These results clearly demonstrated an increase in the production of $PGF_{2\alpha}$ associated with rat parturition.

Although the daily excretion of $PGF_{2\alpha}$-MUM increased steadily from day 20 until parturition and declined gradually in the postparturitional period, the dhk-$PGF_{2\alpha}$ concentration in plasma showed a slight drop on day 21 and low values in the postpartum. High levels of $PGF_{2\alpha}$-MUM on day 21 and after parturition might be due to excretion of the $PGF_{2\alpha}$ on day 20 and at parturition.

Shaikh et al.[16] reported that PGF concentration in rat uterine venous plasma increased on day 20 of gestation, dropped on day 21 and remained relatively high during and after parturition. The P concentration in plasma reflects the luteal function in the pregnancy. $PGF_{2\alpha}$ causes not only uterine contraction but also luteolysis in many species[17,18]. The pregnant uterus has been considered the main source of $PGF_{2\alpha}$[20] In response to $PGF_{2\alpha}$, the corpora lutea in pregnant rats shows a remarkable increase in 20α-hydroxysteroid dehydrogenase activity and a significant drop in peripheral plasma P concentration[21]. The E_2 concentration was reported to control the $PGF_{2\alpha}$ synthetase activity in the rat uterus[22].

In light of the above findings and our results, we assume that the $PGF_{2\alpha}$ production, induced by the rise of plasma E_2

concentration, increases on day 20 and causes luteolysis in the rat by increasing 20α-hydroxysteroid dehydrogenase activity in the corpora lutea. This could lead to a fall in the P concentrations in the plasma, withdrawal of the progesterone 'block'[23,24], and stimulation of uterine muscle activity.

In conclusion, the increased endogenous $PGF_{2\alpha}$ plays an important role in luteolysis and parturition in rat.

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ラット分娩周辺期の血中，尿中の $PGF_{2\alpha}$ 代謝物の動態および血中プロゲステロン，エストロゲン濃度との相関

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内因性 Prostaglandin $F_{2\alpha}$ のラット分娩への関与を明らかにするため，妊娠13日より分娩後２日までのラットから血液　尿を採取し，血中および尿中の $PGF_{2\alpha}$ の主要代謝物 {13, 14-dihydro-15-keto $PGF_{2\alpha}$(dhk-$PGF_{2\alpha}$), 5, 7-dihydroxy-11-keto-tetranor-prosta-1, 16-dioic acid ($PGF_{2\alpha}$,-MUM) を，血中 progesterone (P), estradiol-17β (E_2) とともに RIA にて測定した。Dhk-$PGF_{2\alpha}$, $PGF_{2\alpha}$-MUM とも妊娠19日までは比較的一定であるが，妊娠20日に有意の上昇をみせ，分娩時に最高値となり分娩後は低下した。血中Ｐは妊娠21日より急速に低下し，一方 E_2 は妊娠20日より有意の上昇を示す。以上の結果からラットでは妊娠20日より内因性 $PGF_{2\alpha}$ の産生増加が起こることは明白である。またこの $PGF_{2\alpha}$ がラットの黄体退縮および分娩進行に関与していること，および $PGF_{2\alpha}$ 産生がE_2 により誘導される可能性が示唆された。