Prostaglandin $F_{2\alpha}$ and E_1 in Plasma and Amniotic Fluid During Human Pregnancy and Labor

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Synopsis

In order to elucidate the significance of PGs in human labor, PGE_1 and $F_{2\alpha}$ in biological fluid during human pregnancy and labor were measured by RIA newly developed.

Analytical studies demonstrated that the levels of $PGF_{2\alpha}$ in maternal plasma were 3.7 ± 2.5 ng/ml a few days before parturition, 2.0 ± 0.9 ng/ml in the first stage of labor and 1.9 ± 1.4 ng/ml at delivery. Thus the concentrations of $PGF_{2\alpha}$ in maternal plasma showed no significant changes around parturition. On the other hand, the level of $PGF_{2\alpha}$ in amniotic fluid represented a significant increase up to 44.27 ± 32.81 ng/ml at delivery from 1.45 ± 0.76 ng/ml before labor at 38-40 weeks of pregnancy ($p < 0.05$), although it was uncertain whether this elevation was the cause or effect of uterine contraction.

The concentration of PGE_1 ranged from 2 to 14 ng/ml around parturition. This indicates that there was little remarkable difference between the levels of PGE_1 in plasma and amniotic fluid during the last month of pregnancy and labor.

Possible involvement of prostaglandins (PGs) in human labor has been discussed.

There are a lot of evidences supporting the suggestion that prostaglandins (PGs) may be one of the promising candidates to play a role in the initiation and maintenance of human labor. Much attention has been paid to the great ability of PGs, especially $PGF_{2\alpha}$, E_1 and E_2 to cause uterine contractions at various stages of pregnancy (Bygdeman *et al.*, 1968). This led obstetricians to use PGs clinically as abortients (Kinoshita *et al.*, 1971a; Wiquist *et al.*, 1972) and oxytocics (Kinoshita *et al.*, 1971b; Anderson *et al.*, 1972). It has, however, been difficult to document how PGs are concerned with pregnancy and labor, despite the accumulation of pharmacological and clinical data concerning the induction of

uterine contraction.

Karim and his associates, using bioassay (Karim and Devlin, 1967), found increased concentration of $PGF_{2\alpha}$ during labor in amniotic fluid, but not in plasma. However, bioassay systems used were not necessarily sensitive and specific enough to analyze PGs in biological samples. The development of more specific and relatively simple assay methods for PGs has, therefore, been urgently required and consequently several papers on radioimmunoassay systems (RIA) for PGE , F and A have been presented (Caldwell *et al.*, 1971; Jaffe *et al.*, 1973; Lindgren *et al.*, 1974).

Little is, however, known about the levels of PGE_1 and $F_{2\alpha}$ in maternal blood and amniotic fluid throughout pregnancy and labor. In the present paper, the ana-

lytical study of $\text{PGF}_{2\alpha}$ and E_1 during pregnancy and labor is reported, by our RIA newly developed.

Materials and Methods

Extraction of PGs:

Two ml of plasma or amniotic fluid was added with ^3H - $\text{PGF}_{2\alpha}$ (approx. 1,000 cpm, $50 \mu\text{Ci}/1.2 \mu\text{g}$) for recovery and extracted with 2 volumes of methylal-ethanol (5:1). The resultant precipitate was re-extracted with 5 ml of chloroform-methanol (2:1). The extracts were subsequently partitioned between 20 ml each of petroleum ether and 90% methanol to remove less polar lipids. The 90% methanol fraction was evaporated to dryness in vacuo.

Separation of PGs:

One g of silicic acid was immersed in benzene-ethyl acetate-methanol (60:40:0.5) (solvent 1) to make slurry. After adding the slurry to each glass column (i.d. $0.7 \times 20 \text{ cm}$), the silicic acid was washed with 30 ml of solvent 1. Sample residues were dissolved in 1 ml of solvent 1, and were applied to the columns which were allowed to run dry. Prostaglandin fractions were obtained by developing the columns serially with 14 ml of solvent 1 (PGA and PGB), 14 ml of solvent 2 (benzene-ethyl acetate-methanol 60:40:3.5) (PGE), and 10 ml of solvent 3 (benzene-ethyl acetate-methanol 60:40:10) (PGF) (Fig. 1). The fractions were taken to dryness by evaporation in air at 40°C and dissolved in 1 ml of methanol. Half of the sample was transferred to 10 ml of toluene scintillation solution for counting to permit calculation of recovery. Another 0.5 ml of the sample was evaporated to dryness and dissolved in 0.1 ml of 0.1 M phosphate buffer (pH 7.4) containing 0.15 M NaCl and 0.1% gelatin (assay buffer).

Antisera:

$\text{PGF}_{2\alpha}$ - and PGE_1 -bovine serum albumin conjugates, as antigen, were prepared by carbodiimide reagent and supplied by Dr. Hirata of Ono Central Research Institute. Adult rabbits were immunized subcutaneously or intramuscularly once a week for four weeks, with an emulsified mixture of 1 ml each of complete Freund's adjuvant and physiological saline containing 1 mg of the conjugate.

Radioimmunoassay system:

A mixture of 0.1 ml each of tritiated prostaglandin ($6,000 \text{ cpm}$, ^3H - $\text{PGF}_{2\alpha}$ $50 \mu\text{Ci}/1.2 \mu\text{g}$ or ^3H - PGE_1 $50 \mu\text{Ci}/1.2 \mu\text{g}$) and of unknown sample or unlabeled prostaglandin (0.5–10 ng) in assay buffer was incu-

bated for 1 hour at 37°C . Separation of antibody-bound from unbound ^3H -PG was accomplished by adding to the reaction mixture 0.1 ml of dextran coated charcoal (Norit A, 5 g; dextran T 40, 0.5 g in 100 ml of assay buffer). After 10-min centrifugation at 4°C , 3,000 rpm, the supernatant (containing antibody bound ^3H -PG) was immediately decanted into 10 ml of Bray's scintillation solution. Radioactivity was counted in an Aloka liquid scintillation counter Model 653.

Plasma and amniotic fluid samples:

One hundred and six plasma samples for PG analysis were collected from pregnant women during late pregnancy and labor. In addition, specimens of amniotic fluid, maternal venous plasma and cord plasma were obtained from patients at normal delivery. Amniotic fluid was obtained by amniocentesis from patients before labor.

Fig. 1. Elution pattern for PGs separated by chromatography on silicic acid column.

Results

Specificity of antibody:

Cross reactions of various PGs with an antiserum to $\text{PGF}_{2\alpha}$ and PGE_1 were shown in Figs. 2 and 3, respectively, and the specificity of antiserum was demonstrated in Tables 1 and 2. An antiserum to $\text{PGF}_{2\alpha}$ had relative cross-reactivities of 19.2%, 14.2%, 0.39%, 0.03% and 0% to PGE_1 ,

Fig. 2. Cross reactions with antiserum to $\text{PGF}_{2\alpha}$ at a dilution of 1/600. Fig. 3. Cross reactions with antiserum to PGE_1 at a dilution of 1/800.Table 1. Specificity of Antiserum
against $\text{PGF}_{2\alpha}$ -BSA

PGs etc	Quantity on 50% Inhibition (ng)	Relative Cross Reaction (%)
$\text{F}_{2\alpha}$	1	100
E_1	5.2	19.2
$\text{F}_{1\alpha}$	8.6	14.7
A_2	260	0.39
A_1	3000	0.03
Metabolite	>>	<<

Table 2. Specificity of antiserum
against PGE_1 -BSA

PGs	Quantity on 50% Inhibition (ng)	Relative Cross Reaction (%)
E_1	0.5	100
E_2	8.0	6.2
$\text{F}_{1\alpha}$	13.0	3.8
$\text{F}_{2\alpha}$	>>	0
A_1	>>	0
A_2	>>	0
B_1	>>	0

PGF_{1α}, PGA₂, PGA₁ and urinary metabolite of PGF_{2α}, respectively and bound 50% of ³H-PGF_{2α} added at 600-fold dilution. On the other hand, an antiserum to PGE₁ had relative cross-reactivities of 6.2%, 3.8%, and 0% to PGE₂, PGF_{1α}, and other PGs, respectively and bound 50% of ³H-PGE₁ added at 800-fold dilution. Both antisera were, therefore, satisfactory enough for RIA in terms of specificity.

Validation of the assay method:

Accuracy was estimated by recovery experiments performed by measuring the levels of PGF_{2α} present in plasma to which known amounts had been added. The value measured did not differ by more than 20% from the expected value (Table 3). The same experiment demonstrated the validity of RIA for PGE₁ as well.

Table 3. Radioimmunoassay of known amounts of PGF_{2α} added to plasma

Added PGF _{2α} (ng)	n	Plasma Volume	Found, mean ± SD (ng/ml)
0.0	4	2.0	2.63 ± 0.64
1.0	3	2.0	3.73 ± 0.26
5.0	3	2.0	6.67 ± 0.58
10.0	4	2.0	11.01 ± 2.22
20.0	3	2.0	21.03 ± 3.49

Measurement of PGF_{2α} and PGE₁:

The standard curve for PGF_{2α} was shown in Fig. 4 using an antiserum at 600-fold dilution.

The levels of PGF_{2α} in plasma obtained before and after parturition were demonstrated in Fig. 5. The concentration of PGF_{2α} ranged from 1 to 6 ng/ml and no significant changes in the level of PGF_{2α} were found in plasma around parturition. The levels of PGF_{2α} in plasma were 3.7 ± 2.5 ng/ml (n=10) a few days before the initiation of labor and 2.0 ± 0.9 ng/ml (n=10) (mean ± SD) in the first stage of labor.

PGF_{2α} plasma levels followed serially from the near term to delivery in 10 patients

are shown in Fig. 6. Half of the patients showed higher concentrations of PGF_{2α} a few days before parturition than those in the first stage of labor, whereas the other half did not represent such a trend. Thus the levels of PGF_{2α} in each patient plasma during the last month of pregnancy and labor did not show a uniform pattern.

The levels of PGF_{2α} in maternal peripheral blood, umbilical venous and arterial blood, retro-placental blood and amniotic fluid obtained at normal delivery (n=10) were 1.92 ± 1.42, 3.41 ± 2.40, 2.74 ± 1.27, 12.5 ± 9.79, and 44.27 ± 32.80 ng/ml (mean

Fig. 4. Standard curve for PGF_{2α} using antiserum diluted 600-fold.

Fig. 5. Plasma PGF levels in different physiological states.

Fig. 6. PGF levels in peripheral plasma of ten patients throughout their course of normal pregnancy and delivery.

$\pm$ SD), respectively (Fig. 7). The concentration of $\text{PGF}_{2\alpha}$ in amniotic fluid obtained before labor at 38–40 weeks of pregnancy was $1.45 \pm 0.76 \text{ ng/ml}$ ($n=6$) (Fig 8). The level of $\text{PGF}_{2\alpha}$ in amniotic fluid at delivery was significantly higher than those in amniotic fluid obtained without any labor pain and in maternal plasma ($p < 0.05$).

The concentrations of PGE_1 in maternal plasma in the last month of pregnancy and labor were demonstrated in Fig. 9. There was no statistically significant difference among the mean values in each week of pregnancy and labor. The levels of PGE_1 were around 10 ng/ml in the last month of pregnancy.

Fig. 10 showed the concentration of PGE_1 in plasma of 8 cases before and after the initiation of labor. The results showed that there was a considerable individual variation in the concentration of PGE_1 around parturition and that the levels of PGE_1 had no correlation with the progress

Fig. 7. Plasma and amniotic fluid PGF levels at delivery.

of cervical dilatation.

The samples collected at delivery were also measured by RIA for PGE_1 . The measurement of PGE_1 in amniotic fluid obtained during labor showed no increase in the concentrations, differing from those of $\text{PGF}_{2\alpha}$. Namely, $4.4 \pm 1.8 \text{ ng/ml}$ ($n=3$) in amniotic fluid during labor and $5.0 \pm 1.2 \text{ ng/ml}$ ($n=7$) without uterine contractions were presented. The concentrations of PGE_1 in umbilical artery, umbilical vein, maternal plasma, uterine vein and retroplacental blood obtained at delivery were 1.9 ± 0.4 , 3.8 ± 2.3 , 6.8 ± 5.7 , 1.8 ± 0.4 and $3.0 \pm 0.8 \text{ ng/ml}$, respectively ($n=7$).

Thus, as far as the levels of PGE_1 in plasma and amniotic fluid are concerned, there were no significant changes observed in relation to uterine contractions.

Fig. 9. PGE_1 levels in maternal plasma during pregnancy and labor.

Fig. 8. Amniotic fluid PGF levels in different physiological states at 38-40 weeks of gestation.

Fig. 10. PGE_1 levels in maternal plasma during pregnancy and labor.

Discussion

There have recently been several reports that antibodies to several kinds of PGs were prepared and their usefulness was evaluated. Since specificity and simplicity of RIA are thought to depend ultimately on the serological specificity of the antigen-antibody reaction, the property of antisera decides the reliability of the assay.

Antisera to $\text{PGF}_{2\alpha}$ reported in this paper had 19.2% of cross-reaction with PGE_1 . The extracts from samples, therefore, were necessary to be subjected to column chromatography using silicic acid to separate $\text{PGF}_{2\alpha}$ from E. In addition, our antisera had 14.2% of cross-reaction with $\text{PGF}_{1\alpha}$ and, moreover, chromatographical separation of $\text{PGF}_{2\alpha}$ from $\text{F}_{1\alpha}$ on a silicic acid column was impossible. The data presented here concerning $\text{PGF}_{2\alpha}$ levels, therefore, should be evaluated as total PGF values.

On the other hand, it has generally been impressed that it is very difficult to prepare the specific antisera to PGE_1 , since dehydration of PGE to A may easily occur during conjugation procedure using carbodiimide (Levine *et al.*, 1971) and further conversion of PGA to B may often take place after the injection into animals, of which plasma contains the isomerase catalyzing the reaction (Piper *et al.*, 1970). However, the antisera to PGE_1 we prepared had the high degree of specificity to allow reliable determination of PGE_1 in biological fluid.

The study for validation of the assay system showed that our RIA was considered to be quite satisfactory with respect to precision, recovery and reproducibility.

It was evidenced from the data presented that there were no remarkable variations in the levels of $\text{PGF}_{2\alpha}$ and E_1 in maternal plasma in relation to parturition. On the contrary, the significant elevation

of $\text{PGF}_{2\alpha}$ concentrations in amniotic fluid was clearly demonstrated only during labor. But it was uncertain whether this elevation was the cause or effect of uterine contractions.

Conflicting data have been reported by several investigators that PGF levels in peripheral plasma and serum during pregnancy were unchanged, increased slightly (Patrono, 1973), reached to a peak during the second trimester (Jutierrez-Cernosek *et al.*, 1972), or showed the lowest values during the same period (Hennam *et al.*, 1974). Moreover, there was a report that high levels of $\text{PGF}_{2\alpha}$ in plasma during labor were observed (Brunner and Craft, 1973), whereas in another paper no correlation between plasma $\text{PGF}_{2\alpha}$ levels and the stage of labor was reported (Johnson *et al.*, 1975).

On the contrary, as to the levels of $\text{PGF}_{2\alpha}$ in amniotic fluid, Salmon and Amy (1973), and Keirse *et al.* (1974) reported the similar results to ours. These data may suggest that $\text{PGF}_{2\alpha}$ and E_1 in circulating blood are rapidly metabolized in lung and liver (Hamberg and Samuelsson, 1971; Granström, 1972) it seems to be very hard to detect the changes in the levels of the primary PGs, mainly PGF and E, in peripheral plasma and serum in relation to human labor. Meanwhile, it is conjectured that the accumulation of $\text{PGF}_{2\alpha}$ in amniotic fluid might result partly from the slow degradation of PGF in amniotic fluid and partly from increased the production of PGF in relation to the uterine contractions.

The values of PGs measured by our RIA were of the order of nanogram, while the normal plasma and serum levels of PGs reported by other laboratories ranged from about 10 pg/mL to 10 ng/mL. These discrepancies seem to be attributed to the difference of antisera used and the variation in sampling technique. Besides, it should be pointed out that the concentrations of PGs may, in general, represent higher levels

than those expected due to the possible formation of PGs during the collection and isolation of plasma, since it has been shown that platelets are able to form endoperoxide intermediates rapidly, which are then converted into PGE and F (Smith and Willis, 1971; Smith *et al.*, 1973).

It is, therefore, obvious that the estimation of the major metabolites, i.e. 15-keto-13, 14-dihydro PGF_{2α}, 15-keto-13, 14-dihydro PGE₂ and the urinary metabolites may be more reliable for monitoring the production of PGF_{2α} and E₂. The report by Gréen *et al.* (1974) using gas-chromatography-mass spectrometry showed that the concentration of 15-keto-13, 14-dihydro PGF_{2α} in plasma was increased to 10- to 30-fold during active labor compared with that in the last month of pregnancy preceding labor.

In conclusion, the present results reported here especially concerning PGF levels in amniotic fluid clearly indicate that PGF_{2α} endogenously formed may be strongly involved in human labor, although the main sites of PG production have been unknown yet.

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