BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **238,** 838–841 (1997)
ARTICLE NO. RC977397

The Prostaglandin E_2 and $F_{2\alpha}$ Receptor Genes Are Expressed in Human Myometrium and Are Down-regulated during Pregnancy

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Received August 17, 1997

Prostaglandin (PG) E_2 and $PGF_{2\alpha}$ are believed to play important roles in the myometrial contraction and the initiation of labor. Myometrial contraction by these prostanoids is mediated mainly through EP_3 and FP, which are specific receptors to PGE_2 and $PGF_{2\alpha}$, respectively. During normal pregnancy, uterine myometrium are relaxed until term. To explore the involvement of EP_3 and FP in the myometrial relaxation during pregnancy, we examined the EP_3 and FP gene expressions in nonpregnant and pregnant myometrium obtained by hysterectomy for gynecological diseases. In all samples examined, expressions of EP_3 and FP genes were detected. During pregnancy, the expression of EP_3 gene in human myometrium was significantly reduced, to 60% of that in nonpregnant myometrium. The expression of FP gene in human myometrium also decreased during pregnancy to 55% of that in nonpregnant myometrium. In the myometrium from the nonpregnant women taking combined oral contraceptives, the gene expressions of EP_3 and FP were not significantly changed as compared to those in nonpregnant controls. The down-regulation of EP_3 and FP during pregnancy may play a role in the relaxation of myometrium and thus in the maintenance of normal pregnancy in humans.

Prostaglandin (PG) E_2 and $PGF_{2\alpha}$ play important roles in the myometrial contractions, cervical ripening and initiation of parturition in humans (1). It is reported that the prostaglandin receptors EP_2 and EP_4 cause the relaxation of smooth muscle and EP_1, EP_3 and FP cause smooth muscle contraction (2). Among receptors, EP_3 and FP receptors which is specific to PGE_2 and $PGF_{2\alpha}$ respectively, are present in human myometrium (3). During normal pregnancy, the uterine myometrium does not contract until term, although the volume of uterine contents increases markedly during pregnancy. Thus, it is plausible that there exist some mechanisms to block the uterine contraction during pregnancy. However, the regulatory mechanisms for uterine contraction by prostanoids during pregnancy remain mostly unknown.

The usefulness of PGE_2 and $PGF_{2\alpha}$ is widely approved in the clinical field. It is well known that administration of the inhibitor of cyclooxygenase, such as indomethacin, inhibits uterine contraction and labor (4). Some researchers reported that prostanoids cause oxytocin release from the pituitary (5). Other investigators found that prostanoids lower the myometrial threshold to oxytocin (6). Recently, it has been reported that in FP knockout mice, spontaneous labor did not commence even one week after term, and that oxytocin receptors did not express even at term (7). Recently, we cloned human EP_3 and found the existence of multiple isoforms of EP_3, produced by alternative splicing (8). It was previously reported that PGE_2 and $PGF_{2\alpha}$ binding sites in human myometrium were decreased during pregnancy (9). However, it remains unclear whether or not the PG receptors are down-regulated at the transcription level during pregnancy. To address these issues including hormonal regulation, we performed Northern blot analyses for EP_3 and FP receptors, using the human myometrial tissues obtained from pregnant and nonpregnant women, and those taking combined oral contraceptive pills.

Abbreviations: $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGE_2, prostaglandin E_2; FP, prostaglandin $F_{2\alpha}$ receptor; EP_3, prostaglandin E_2 receptor subtype 3; EP_4, prostaglandin E_2 receptor subtype 4; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; kb, kilobases.

MATERIALS AND METHODS

Subjects. Uterine tissues were obtained from premenopausal women who had received total hysterectomy for gynecological rea-

0006-291X/97 $25.00

sons such as uterine cervical cancer, ovarian cancer and uterine myoma. We obtained written informed consent from all the patients who underwent hysterectomy. Myometrial samples were collected from nonpregnant women (age; 41.8 ± 2.6 yr, mean ± SE, n = 5), and pregnant women in the first trimester (age; 33.3 ± 2.9 yr, gestational age; 8-11 weeks, n = 6), second trimester (30 yr, 16 weeks, n = 1), and third trimester (30.3 ± 1.3 yr, 31-38 weeks, n = 3). The indications for total hysterectomy in pregnant women were cervical cancer (carcinoma *in situ*, 5 patients; cervical cancer stage Ia, 1 patient), advanced ovarian cancer (1 patient), and complications of giant myoma (3 patients). In addition to the above mentioned 5 nonpregnant women, 4 nonpregnant women with uterine leiomyoma (41.0 ± 3.0 yr) were administered with combined oral contraceptive pills for more than 3 weeks until the time of total hysterectomy to alleviate prolonged genital bleeding and consequent iron-deficiency anemia. After hysterectomy, normal myometrial tissues were excised, and their histological characteristics were confirmed microscopically. The composition of the combined oral contraceptive pill was ethynodiol acetate 1 mg/ethinylestradiol 0.05 mg (G.D. Searle & Co., Chicago, IL), and 1 tablet per day was administered for over 3 weeks.

Northern blot analysis. Samples stored at −80°C were broken into pieces, and total RNA was extracted by homogenizing with a Polytron homogenizer in Trizol reagent (Gibco BRL, Gaithersburg, MD). Poly(A)$^+$ RNA was prepared by a commercial oligo(dT) selection method (PolyATtract mRNA Isolation System, Promega, Madison, WI). Northern blot analyses were performed as described (10,11). Briefly, poly(A)$^+$ RNA was electrophoresed on a 1.4% agarose gel containing 2% formaldehyde, transferred to a nylon membrane (Biodyne, Pall BioSupport, Glen Cove, NY), and UV cross-linked. Hybridization proceeded at 42°C overnight in hybridizing solution containing 50% formamide, 4 × SSC, 5 × Denhardt's solution, 0.5% SDS, and 75 μg/ml denatured salmon sperm DNA with a ^{32}P-labeled probe. A human FP receptor cDNA fragment (−21 to 1077) was prepared by PCR amplification from human genomic DNA(12). A 2.5-kb *Eco*RI/*Xba*I fragment of human EP_3 receptor cDNA (8) was used as a probe. Membranes were washed twice at 55°C in 0.5 × SSC and 0.1% SDS for 20 min, then autoradiographed at −80°C. The membranes were rehybridized with ^{32}P-labeled human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (Clontech, Palo Alto, CA) for control. Autoradiograms were analyzed by densitometry (Advantec DM-303, Advantec Toyo, Tokyo, Japan).

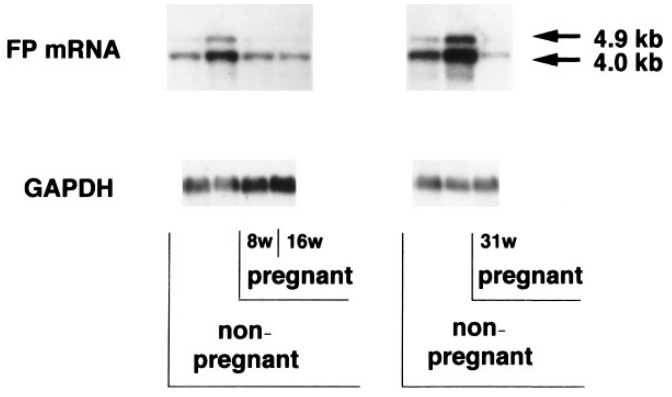

FIG. 1. Northern blots for FP mRNAs from human uterine myometrium. Poly(A)$^+$ RNA samples (2 μg/lane), isolated from pregnant or nonpregnant myometrium, were electrophoresed and hybridized with a human cDNA probe for the FP receptor, and with a probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a control. Representative cases are shown. **Left:** nonpregnant myometrium (**nonpregnant**) and first and second trimester (**pregnant**) (8 weeks, 16 weeks). **Right:** nonpregnant myometrium (**nonpregnant**) and third trimester (**pregnant**) (31 weeks).

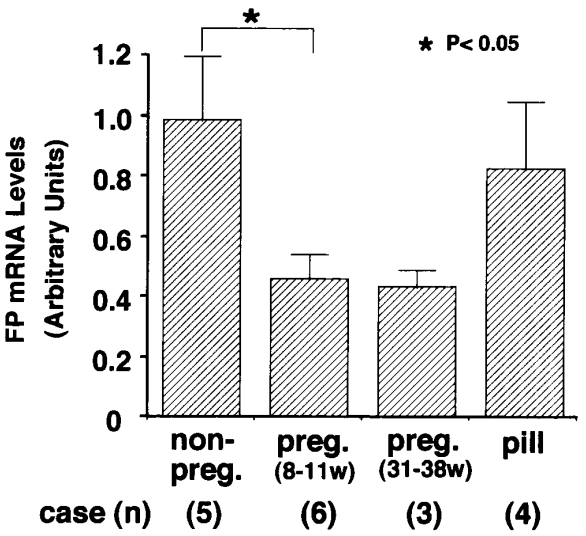

FIG. 2. Relative FP mRNA levels in human myometrium. Myometrium were obtained from nonpregnant women (**nonpreg.**), pregnant women (**preg.**)(8−11 or 31−38 weeks), and women taking oral contraceptive pills (**pill**). The relative FP receptor mRNA level in each tissue was determined as the ratio of FP receptor mRNA/GAPDH mRNA measured by densitometry. Each bar represents the mean ± SE of the relative FP receptor mRNA level in arbitrary units. The arbitrary unit is defined by assuming that the relative FP receptor mRNA level of the nonpregnant myometrium is 100. *, $P < 0.05$.

Statistical analysis. Values are expressed as the mean ± SE. Statistical analysis was performed using analysis of variance followed by Scheffe's test. Difference with $P < 0.05$ was regarded as significant.

RESULTS AND DISCUSSION

FP Receptor in Human Myometrium

To examine the down-regulation of the EP_3 and FP receptors at the message level, Northern blots were performed using the cDNA of the human EP_3 and FP receptor as a probe.

In all samples, messages for the FP receptor (4.9 and 4.0 kb) were detected (Fig. 1). Main band is 4.0 kb. In the myometrium during pregnancy, the level of FP receptor mRNA expression was significantly reduced as compared to that in normal controls (nonpregnant myometrium), and it was not decreased in the myometrium obtained from women who were given combined oral contraceptive pills (Fig. 1). When samples from early and late pregnancy were compared, the reduction of the FP mRNA levels was the same in both period (Fig. 1). Figure 2 summarizes comparisons among gene expressions of the FP receptor relative to those of GAPDH in all subject groups. The relative expression levels of FP mRNA in the myometrium of pregnant

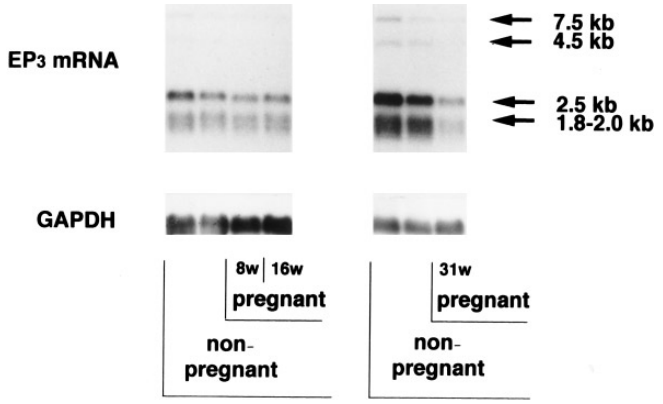

FIG. 3. Northern blots for EP_3 mRNAs from human uterine myometrium. Poly(A)$^+$ RNA samples (2 μg/lane), isolated from pregnant or nonpregnant myometrium, were electrophoresed and hybridized with a human cDNA probe for the EP_3 receptor, and with a probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a control. Representative cases are shown. **Left:** nonpregnant myometrium (**nonpregnant**) and first and second trimester (**pregnant**) (8 weeks, 16 weeks). **Right:** nonpregnant myometrium (**nonpregnant**) and third trimester (**pregnant**) (31 weeks).

women in the first trimester were significantly reduced to 55 % of that in nonpregnant women. Although not significant in this study, such down-regulation was also recognized in the late gestation period as well as in the early period. However, the myometrial FP mRNA expression levels in the women taking combined oral contraceptive pills were similar to those in nonpregnant women.

EP_3 Receptor in Human Myometrium

In all samples, messages for the EP_3 receptor (7.5, 4.5, 2.5 and 2.0-1.8 kb) were detected (Fig. 3). Main band is 2.5 kb. In the myometrium during early pregnancy, the level of main EP_3 mRNA expression (2.5 kb) was significantly reduced as compared with the normal controls (nonpregnant myometrium). However, such down-regulation of EP_3 mRNA expression was not observed in the myometrium obtained from women who were given combined oral contraceptive pills (Fig. 3). When samples from late pregnancy were compared with those from early pregnancy, there was a reduction of the EP_3 mRNA level similar to that in early pregnancy (Fig. 3). Figure 4 summarizes a comparison of the gene expressions of the EP_3 relative to those of GAPDH among all subject groups. The relative expression level of EP_3 mRNA in the myometrium of pregnant women in the first trimester was significantly reduced to 60 % of that in nonpregnant women. On the other hand, the relative expression of EP_3 mRNA in the women taking combined oral contraceptive pills were similar to those in nonpregnant women (Fig. 4).

During normal pregnancy, the volume of uterine content increases markedly. However, the uterine myometrium are relaxed and does not contract until term, when the fetus becomes mature enough to survive outside the uterus. Guanylate cyclase and cyclic GMP system in the myometrium or decidual tissue have been proposed as myometrial relaxant (13, 14, 15). On the other hand, PGE_2 and $PGF_{2\alpha}$ as well as their analogues have potent uterine contracting activity, and have been clinically used for the purpose of induction or augmentation of labor. These prostanoids exert their biological function through their specific receptors. EP receptors consists of at least four subtypes, EP_1, EP_2, EP_3 and EP_4 (16). It is reported that EP_2 and EP_4 cause the relaxation of smooth muscle and that EP_1 and EP_3 cause smooth muscle contraction (2). FP also mediates uterine contraction (17). The previous report on the pregnant and nonpregnant human uterus did not discriminate between the EP receptor subtypes and did not examine the expression of mRNA levels (9). Therefore, we investigated changes in the expression of EP_3 and FP mRNAs in pregnant myometrium.

Present study revealed that the human myometrium abundantly expresses EP_3 and FP mRNA. The expressions of EP_3 and FP genes in human myometrium during pregnancy were reduced about 50% as compared to those in nonpregnant myometrium.

The regulatory factor of EP_3 and FP gene expression in human myometrium has not been reported to date. In the present study, the administration of sex steroid hormones did not affect the gene expression of EP_3 and FP receptors (data not shown).

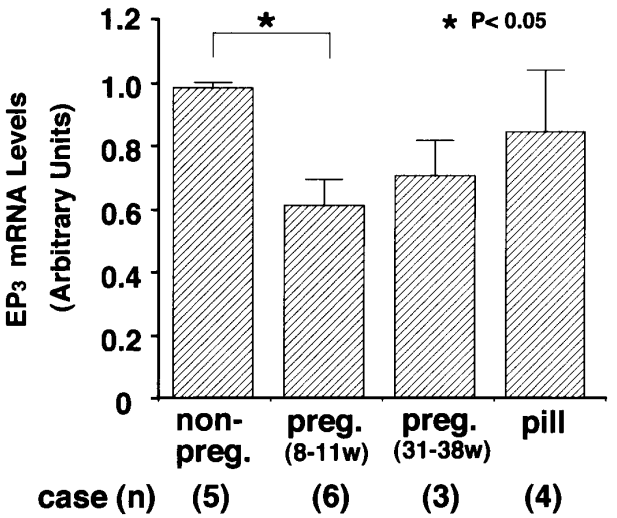

FIG. 4. Relative EP_3 mRNA levels in human myometrium. Myometrial tissues were obtained from nonpregnant women (**nonpreg.**), pregnant women (**preg.**)(8–11 or 31–38 weeks), and women taking oral contraceptive pills (**pill**). The relative EP_3 receptor mRNA level in each tissue was determined as the ratio of EP_3 receptor mRNA/GAPDH mRNA measured by densitometry. Each bar represents the mean ± SE of the relative EP_3 receptor mRNA level in arbitrary units. The arbitrary unit is defined by assuming that the relative EP_3 receptor mRNA level of the nonpregnant myometrium is 100. *, $P < 0.05$.

In conclusion, the present study revealed that the EP_3 and FP receptors in the human myometrium are down-regulated at transcription levels during pregnancy. This down-regulation of EP_3 and FP during pregnancy may play a role in the maintenance of normal pregnancy in humans, since these receptors mediate myometrial contraction by PGE_2 and $PGF_{2\alpha}$, respectively.

It still remains to be elucidated whether the EP_3 and FP receptors are up-regulated at the time of onset of labor. Although it is difficult to obtain the normal myometrium after the onset of spontaneous labor, further study are mandatory to clarify the role of prostanoid receptors in the initiation of labor.

ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (No.08457441, No.09877319), and a grant from Smoking Research Foundation, Japan.

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