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Induction of *c-Jun* mRNA without Changes of Estrogen and Progesterone Receptor Expression in Myometrium During Human Labor

To elucidate the endocrine mechanism of human parturition, the expression of *c-Jun* and *c-Fos* mRNA were examined in relation to estrogen receptor (ER) and progesterone receptor (PR) in human myometrium. *c-Jun* mRNA was detected in all myometrial tissues (n=5) during labor but not before labor (n=5) and in oxytocin-resistant postterm pregnancy (n=3). *c-Fos* mRNA was detected in only one myometrial tissue from a woman in labor. The distribution and intensity of immunostaining for ER and PR were semiquantitatively scored. During the late pregnancies, no significant difference was seen in the receptor scores for myometrial ER and PR between the patients who experienced labor and those who did not. Receptor scores for ER and PR were significantly lower in postterm pregnancy than in late pregnancy, regardless of the labor status. These data suggest that there are no changes in ER and PR in human myometrium during parturition. On the other hand, postterm pregnancy is associated with low ER and PR. c-Jun, induced during labor without changes in ER and PR, may play a role as a signaling mechanism in human myometrium.

Key Words: *Myometrium; Receptor, estrogen; Receptor, progesteron; Genes, c-Jun; Genes, c-Fos; Labor*

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INTRODUCTION

The mechanism of human parturition is very complex and is not yet fully understood. In nonprimate mammals such as the rat and the sheep, the end of pregnancy is associated with a fall in maternal progesterone concentration (1, 2). Progesterone withdrawal along with estradiol activates multiple pathways including the stimulation of the synthesis of prostaglandin, oxytocin (3, 4), and oxytocin receptor (5), which contributes to the initiation of labor. In men and other primates, however, the same pathways are activated at the end of pregnancy, despite the fact that there is no decrease in the concentration of plasma and myometrial progesterone (6).

As receptors are necessary for steroid hormone action (7), an increase in the number of estrogen receptor (ER) and/or decrease in progesterone receptor (PR) has been thought to be a way of achieving effective estrogen dominance without absolute increase in estrogen concentrations in human myometrium during pregnancy. The results of previous studies on ER and/or PR in myometrium of pregnant women are controversial. Some authors could not detect their presence (8, 9), while others reported a decrease in myometrial PR (10) or an increase in myometrial ER (11) at the onset of labor in women at term pregnancies.

c-Jun and *c-Fos* are members of the immediate early genes and their products are primarily expressed in uterine smooth muscle cells. Their protein products form the activator protein-1 (AP-1) which participates in the induction of gene transcription (12). In rats, estrogen increases the transcription of the connexin-43 gene, one of the contraction-associated proteins, through the putative estrogen response elements or by increasing the expression of c-Fos and c-Jun acting through the putative AP-1 sites (13, 14). In spite of these studies on the changes in c-Fos and c-Jun levels in the uterine tissues of pregnant rats, little is known about their changes in relation to the events of parturition in human pregnancy (15). Up to now, no information is available on the changes in *c-Fos* and *c-Jun* mRNA in human myometrium at parturition.

This study aimed to examine the expressions in *c-Jun* and *c-Fos* mRNA in relation to ER and PR expression in human myometrium during the late pregnancy around labor. It revealed induction of *c-Jun* mRNA in women

during labor. In contrast, immunohistochemical study for ER and PR showed no changes in their levels between patients who experienced labor and those who did not.

MATERIALS AND METHODS

Materials

Myometrial tissue was obtained during cesarean section from ten late pregnant women with and without spontaneous labor (n=5, each) and three postterm pregnant women (n=3). Myometrial tissue was also procured from one nonpregnant woman who had undergone hysterectomy for a gynecologic disease (CIN III) during the luteal menstrual phase. This study was approved by the Institutional Review Board for Clinical Research at Samsung Medical Center and informed consent was given by the participating patients.

Gestational period from the last menstrual period was determined by the first trimester crown-rump length and/or second trimester biparietal diameter. Patients were grouped on the basis of gestational age into late pregnancy (33-40 weeks of gestation) or postterm (above 42 complete weeks of gestation). In all patients in labor, labor began spontaneously and its progress was assessed (16). External tocodynamometers were used to monitor uterine contractions; contractions in every woman who experienced labor showed more than three contractions per 10 min, and each of which lasted more than 1 min, thus oxytocin was not infused. For these women, labor pain started within 12 hr of cesarean section and cervical dilatations were less than 4 cm at operation. Indications for cesarean sections are listed in Table 1. All three postterm patients underwent cesarean section because labor was not induced with the use of vaginal prostaglandin E_2 suppository and oxytocin infusion for two successive days. We considered these patients had some defects in the mechanism of labor.

Myometrial tissues, each weighing less than 5 grams, were excised from the upper portion of a low segment uterine incision. In women who had previous cesarean section, care was taken to avoid scar tissue and to exclude serosa. Myometrial tissues were dissected free of the decidua, measured into about 1 gram, snap frozen in liquid nitrogen, and stored at -70℃.

Northern blot analysis

Each of the frozen tissues was pulverized in liquid nitrogen. Total RNA was extracted in Trizol® (Gibco-BRL, U.S.A.) as recommended by the manufacturer. Total RNA (30 μg per lane) from each tissue were denatured and were separated on 1% formaldehyde agarose gel. RNA was transferred to a nylon membrane by capillary blotting and cross linked by ultraviolet irradiation. Membranes were prehybridized at 42℃ in Hybrisol® I (Oncor, U.S.A.). The 1.8 Kb *Eco*R I-*Eco*R I fragment of mouse *c-Jun* cDNA inserted in pGEM 2 (17) and the 2.8 Kb *Pst*I-*Pst*I fragment of *v-Fos* cDNA inserted in pIBI 30 (18) were labeled with alpha-^{32}P-dCTP by means of the random priming method (Rediprime™, Amersham Life Science, UK). Hybridization with purified probe was carried out overnight at 42℃. Membranes were washed sequentially at room temperature in 2×SSC, 0.2% SDS and 1×SSC, 0.2% SDS. Depending on the radioactivity

Table 1. Demographic profiles and classification of women included in this study based on clinical courses

	Labor status	n	Age (y)	Gestational age	Cervical dilation at operation	Diagnosis
Nonpregnant		1	36			CIN III
Late pregnancy	No labor	5	29	35	Closed	Breech, PROM
			32	34	Closed	Preeclampsia
			24	38	Closed	Psychologic reason
			38	39	Closed	Repeated cesarean
			26	38	Closed	Breech, PROM
	Labor	5	29	33	3	Repeated cesarean
			29	34	2	Repeated cesarean
			31	38	1	Repeated cesarean
			29	40	4	Fetal distress
			27	40	3	Psychologic reason
Postterm		3	29	42	Closed	Failed induction
			23	42	Closed	Failed induction
			26	42	Closed	Failed induction

CIN, cervical intraepithelial neopasia; PROM, premature rupture of membrane

of the membrane, it was washed at 65℃ in 0.5×SSC and 0.2% SDS. X-ray film was exposed to the membrane with intensifying screens at -80℃. Membranes were stripped of c-Jun or c-Fos probe by heating to 95℃ in 0.2×SSC and 0.5% SDS before rehybridization with a β-actin probe (CLONTECH, U.S.A.) (19). Hybridization signals of c-Jun and β-actin were measured by densitometry.

Immunohistochemistry

Immunohistochemical detection of ER and PR was performed on paraffin-embedded sections using monoclonal antibodies to ER (Estrogen receptor IV Ab-3 [Clone AER320], Neomarkers, Fremont, CA) and PR (PR10A9, Immunotech, France). Briefly, myometrial tissues were fixed in 10% formalin and embedded in paraffin. Tissue sections were mounted on slides coated with 0.1% poly-L-lysine. After deparaffination and rehydration, tissue sections were microwaved in 10 mM citrate buffer. The sections were blocked with normal blocking serum and incubated overnight with specific monoclonal antibody, which was either Estrogen receptor IV Ab-3 at dilution of 1:50 or PR10A9 without dilution. Tissue sections were treated in 0.1% Triton X-100, and then they were incubated with 1:100 diluted biotinylated secondary antibody. After incubation in streptoavidin buffer, the antibody complexes were visualized by incubation with stable DAB chromogen® (Research Genetics, U.S.A.) for 5 min. Sections were counterstained with Autohematoxylin® (Research Genetics, U.S.A.), dehydrated and mounted.

The intensity and distribution of specific staining were evaluated visually using a modification of the semiquantitative analysis (20), as follows:

$$\text{Receptor score} = \sum_{i=0}^{i=3} P(i) \times i$$

where i is intensity of staining from 0 (no staining) to 3 (very intense staining) and $P(i)$ is the percentage of stained cells in category i (0-100%). At least three separate cohorts of 100 cells in different high power fields were assessed. The final receptor score was obtained by calculating the mean of the cohorts.

Statistical analysis

Continuous variables were presented throughout as the mean±SEM. Intraobserver and interobserver variations of the receptor score were assessed by the method of limits of agreement. Kruskal Wallis test with Dunn procedure and Pearson correlation analysis were used for calculating the significance. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

Maternal demographic and clinical descriptions are given in Table 1. The average age of the patients was 29.1 (range 23-38) years and they were all in their third trimester of pregnancy.

There were no differences in the yield of RNA per gram of tissue over increasing gestational ages or between women in labor and those not in labor. Northern blot analysis using *c-Jun* and *c-Fos* probe revealed mRNA species 2.7 Kb and 2.2Kb in size respectively. Fig. 1 shows a representative result of Northern blot analysis of *c-Jun* and *c-Fos* mRNA in human myometrium. The hybridization signals for *c-Jun* mRNA in myometrium from five women with spontaneous onset of labor were positive compared to the absence in other women who did not go into labor and those who failed to induce labor at 42 weeks' gestation (Fig. 1). This result was also statistically significant when hybridization signals of c-Jun/β-actin were compared among study groups. As shown in Fig. 1, the signal of *c-Jun* was significantly higher during labor compared to those before labor and in postterm pregnancy. There was, however, only one positive signal for *c-Fos* mRNA in myometrial tissue from a woman with spontaneous onset of labor. These

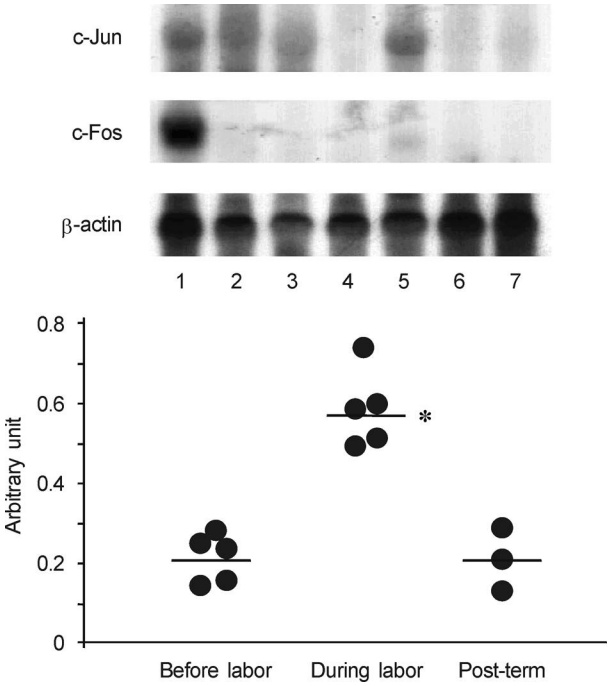

Fig. 1. Representative view of Northern blot for *c-Jun* and *c-Fos* mRNA. 1, positive control (MCF7); 2, nonpregnant; 3, preterm with labor; 4, preterm without labor; 5, term with labor; 6, term without labor; 7, postterm. The signal of *c-Jun* is significantly higher during labor compared to those before labor and in postterm pregnancy (*, $p<0.05$).

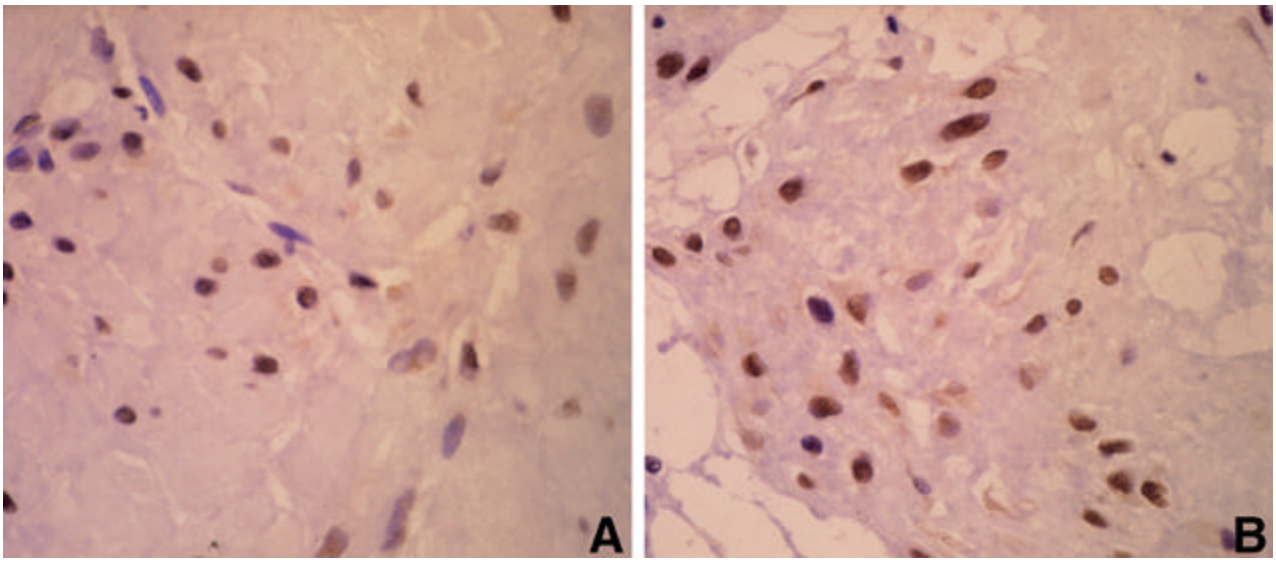

Fig. 2. Immunohistochemical staining for progesterone receptor in myometrium (×400). A: myometrium of term pregnancy without labor, B: myometrium of term pregnancy with labor. The semiquantitative measurements of nuclear PR do not show significant difference before and during labor.

results suggest the induction of c-Jun mRNA during labor in the myometrium.

Immunostainings for PR in myometrium were localized in the nucleus during late pregnancy (Fig. 2), but those for ER were diffuse and faint over the whole cell (Fig. 3). The semiquantitative measurements of nuclear PR and ER (receptor score) did not show any significant difference in myometrial tissues from women with and without labor. Receptor scores for ER in myometrium from late pregnant women who experienced labor, late pregnant women who did not experience labor and postterm women who were oxytocin-resistant were 41±4.25, 35.4±2.66 and 12±3. Receptor scores for PR were 68.2±3.79, 85.6±6.90, and 22.3±5.04 in that order. Intraobserver limits of agreement of receptor score were from -16.9 to 12.4 and from -29 to 7.5 for ER and PR respectively. Interobserver limits of agreement of receptor score were from -32.9 to 18.1 and from -34.4 to 27.4 for ER and PR respectively. This result showed that women who experienced labor did not show signifi-

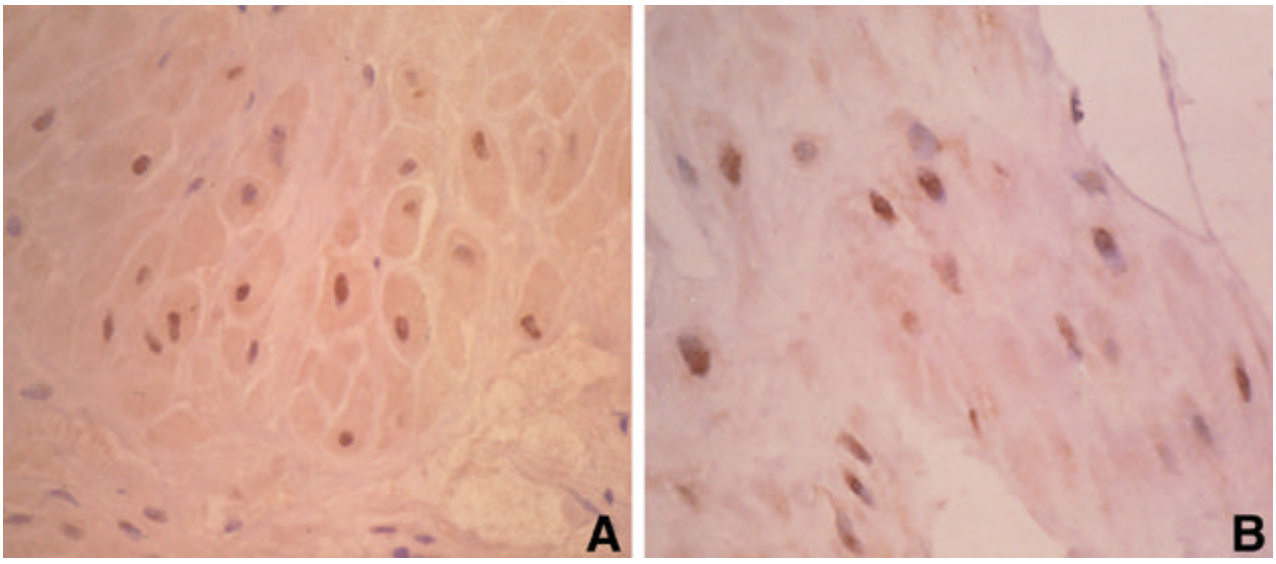

Fig. 3. Immunohistochemical staining for estrogen receptor in myometrium (×400). A: myometrium of term pregnancy without labor, B: myometrium of term pregnancy with labor. The semiquantitative measurements of nuclear ER do not show significant difference before and during labor.

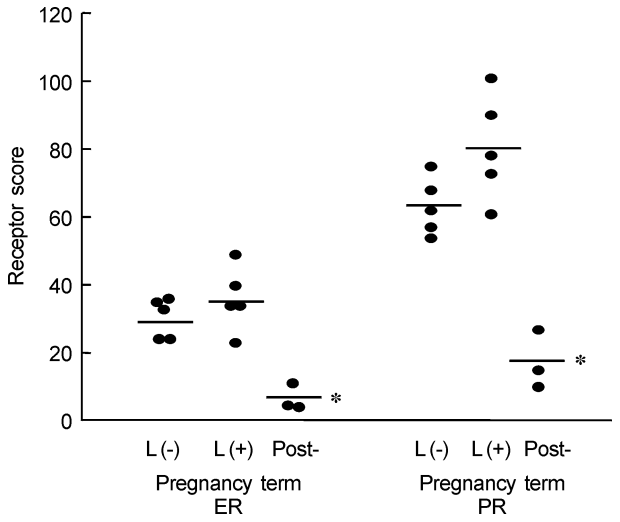

Fig. 4. Receptor scores for myometrial ER and PR in different gestational period and labor status. In postterm pregnancy, receptor scores for ER and PR are significantly lower compared to those in late pregnancy, regardless of the labor status. L, labor (*, $p<0.05$).

cant changes in receptor score for ER and PR than those who did not (Fig. 4). There was, however, a significant correlation between the receptor scores for ER and PR in myometrium during pregnancy (r=0.974, p=0.0001). On the other hand, receptor scores for ER and PR in myometrium from postterm oxytocin-resistant women were significantly lower compared to those from women in late gestation (Fig. 4).

DISCUSSION

The data of previous studies (8-11, 21) on changes in the number of ER and/or PR in myometrium of pregnant women have been controversial. This may be due to the differences in the methodology employed for detection of ER or in the way labor progressed among the subjects recruited. In animal studies, uterine contractions were monitored by electromyography, but this was not possible in human research. It is almost impossible to define the onset of labor in humans. Patients who went into labor were confined to those whose first labor pain occurred within 12 hr of cesarean section and were not in the active phase, as assessed by tocodynamometer and cervical dilatation (less than 4 cm). We thought that this would provide information on the biologic processes occurring in the myometrium around the onset of labor.

In this study, immunostainings for myometrial ER and PR in the nonpregnant state were not compared to those during pregnancy, but a previous report (22) showed that PR expression remained essentially constant throughout the pregnancy (4-38 weeks' gestation), but ER expression was initially weak and decreased further as pregnancy advanced. Others (11) noted a positive relationship between ER and PR levels in uterine tissues, and higher ER/PR values in the lower segment of the uterine myometrium in the group with uterine contraction than in the group without. We, however, did not observe any significant difference in the immunostaining for ER or PR in myometrium between women who were with or without labor experience. The receptor scores for ER and PR in myometrium from postterm pregnant women were also significantly lower than those from late pregnant women regardless of labor status, a result that partly reveals the mechanisms involved in prolonged pregnancy. From this result, it seems that the actions of ER and PR may be involved in the regulation of contractile components in myometrium because labor did not begin spontaneously and pharmacologic manipulation failed to induce labor in postterm pregnancy. The effects of pharmacologic agents such as oxytocin and PG on early response gene and steroid receptors are obscure at present. Our in vitro data using myometrial smooth muscle cells, however, indicated that oxytocin and PGE2 did not induce early response genes in pharmacologic dose (data not shown).

It has been shown that the relative abundance of ER mRNA increased by three to fourfold in human fetal membranes and decidua with the onset of labor, while there were no changes in the expression of the PR mRNA (4). Similarly, increases of ER mRNA in the uterine or the fetal tissue were reported during spontaneous or cortisol-induced labor in sheep (23). We could not detect any ER mRNA in pregnant myometrial tissues recruited in this study using Northern blot (data not shown). We observed, however, induction of c-Jun mRNA in all myometrial samples from women who were in labor spontaneously during late gestation. It is interesting to note that postterm oxytocin-resistant myometrial tissues also failed to show induction of c-Jun mRNA. These findings suggest that c-Jun mRNA is induced during labor and may play a role in signaling during human labor. On the other hand, c-Fos was observed only in one myometrial tissue during labor. It was reported that the expressions of c-Jun and c-Fos were cell-type specific in mouse uterus after estrogen stimulation (24). In addition, the expression of c-Jun but not of c-Fos was reported to correlate with contractile protein, connexin-43 in human myometrium (25). From these results and our data, it seemed that the expression of c-Jun was dominant to c-Fos in human myometrium.

How is c-Jun mRNA induced in human myometrium during labor? Several reports have shown that an induction of uterine c-Jun is an estrogen-driven events (26,

27). During pregnancy, myometrium is dominated by progesterone (1, 2, 22), thus c-Jun mRNA cannot be induced by estrogen. One possible explanation for the engagement of estrogen on c-Jun induction might be that progesterone desensitizes the uterus to progesterone action and permits a transient recovery of nuclear ER (28). It was reported that the serum concentrations of progesterone in both the fetal cord and the maternal vein were significantly lower in the oxytocin-resistant dystocia than in spontaneous normal labor (29). Moreover, PR concentrations in myometrium in the lower segment of the uterus were found to be significantly lower in oxytocin-resistant dystocia than during normal labor and before labor, while no significant difference was found in the ER contents (30). The result of our study, however, does not support the hypothesis that induction of c-Jun in human myometrium is an estrogen-driven event at the spontaneous onset of labor.

In general, activation of protein kinase C results in increased expression of c-Jun and c-Fos genes and AP-1 mediated transcription is stimulated. In vitro experiment with phorbol ester stimulation has shown that c-Fos and c-Jun protein levels increased transiently, followed by significant increases in connexin-43 protein levels in primary myometrial cells (31). Diacylglycerol is the major activator of protein kinase C (32) and endothelin induces the c-Jun and c-Fos transcription (33). The signal which induces c-Jun mRNA in human myometrium at the onset of labor is unknown. This question needs further investigations.

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