Hormonal regulation of PGE$_2$ and COX-2 production in rabbit uterine cervical fibroblasts

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Sato, T., H. Michizu, K. Hashizume, and A. Ito. Hormonal regulation of PGE$_2$ and COX-2 production in rabbit uterine cervical fibroblasts. J Appl Physiol 90: 1227–1231, 2001.—Prostaglandins (PGs) play an important role in the regulation of uterine function such as inflammatory cytokines, sex hormones, and proteases. Our laboratory demonstrated that, in rabbit uterine cervical fibroblasts, progesterone and $17\beta$-estradiol suppressed COX-2 mRNA expression (6). A recent study with PGF receptor-deficient mice by Tsuboi et al. (37) indicates that COX-2 is closely associated with the occurrence of parturition but that COX-1 is induced much earlier and kept at a high level in uterine tissue until the initiation of parturition.

Uterine cervical ripening is a typical event with dramatic connective tissue remodeling (17, 24) and is regulated by various endogenous factors such as inflammatory cytokines, sex hormones, and proteases. Our laboratory demonstrated that, in rabbit uterine cervix, progesterone and $17\beta$-estradiol suppress the production of matrix metalloproteinases (MMPs), which play an important role in the degradation of extracellular matrix at the onset of parturition (14, 32). Our laboratory also demonstrated that an augmented production of interleukin (IL)-1 and IL-8 is observed in pregnant rabbit uterine cervixes (15, 38) and that the production of IL-8 is transcriptionally suppressed by progesterone in rabbit uterine cervical fibroblasts (16). Therefore, it is proposed that the sex hormone-mediated suppression of MMP and cytokine production is a key mechanism for maintaining the uterus during pregnancy in rabbit. Moreover, previous studies indicate that PGE$_2$ production during gestation is involved in cervical ripening in sheep and ovine (25, 28), but the hormonal regulation of PG biosynthesis in uterus is controversial. It has been reported that estradiol decreases both PGF$_{2\alpha}$ and PGE$_2$ by suppressing COX-2 mRNA expression, whereas progesterone increases PGF$_{2\alpha}$ secretion in bovine endometrial cells (41). In addition, in bovine myometrium, progesterone but not $17\beta$-estradiol suppresses COX-2 mRNA expression (6). Furthermore, Kim et al. (21) reported that progesterone suppresses COX-1 but not COX-2 expression in baboon endometrium.

In the present study, we demonstrated that IL-1$\alpha$ increased the biosynthesis of PGE$_2$ and that the augmented PGE$_2$ level was suppressed by both progesterone and $17\beta$-estradiol in rabbit uterine cervical fibroblasts treated with interleukin-1$\alpha$ (IL-1$\alpha$), the level of PGE$_2$ was augmented in a time- and dose-dependent manner. The IL-1$\alpha$-augmented PGE$_2$ level was almost completely suppressed by progesterone and $17\beta$-estradiol at the physiological concentration (0.01 $\mu$M), whereas a slight decrease in the basal level of PGE$_2$ was observed in the cervical fibroblasts treated with both hormones at a pharmacological concentration (1 $\mu$M). In addition, the level of PGE$_2$ augmented by IL-1$\alpha$ was due to the increase of cyclooxygenase (COX) activity, which was inhibited by progesterone and $17\beta$-estradiol as well as by indomethacin and a specific COX-2 inhibitor, NS-398, but not by the well-known COX-1 inhibitor, aspirin. Furthermore, progesterone and $17\beta$-estradiol suppressed the IL-1$\alpha$-augmented COX-2 production but not the constitutive production of COX-1 in rabbit uterine cervical fibroblasts. These results suggest that progesterone and $17\beta$-estradiol prevent the initiation of labor by inhibiting PGE$_2$ production after the suppression of COX-2 production during pregnancy in the rabbit.

pregnancy; uterine contraction; parturition; labor; prostaglandin $E_2$; cyclooxygenase-2

PROSTAGLANDINS (PGs) play an important role in the regulation of uterine function such as parturition during the reproductive process. An increased level of prostanooids such as PGE$_2$ and PGF$_{2\alpha}$ causes the myometrial contractility that initiates labor at term (26, 27). Suppression of PG levels results in delay of labor in humans and animals (22, 34, 40). The production of PGs is regulated by cyclooxygenase (COX)/prostaglandin endoperoxide H synthase (PGHS), and two types of the enzyme, COX-1/PGHS-1 and COX-2/PGHS-2, have been characterized in mammalian cells (8, 13). COX-1 is constitutively expressed in most tissues and cultured cells, and COX-2 is an inducible enzyme in response to inflammatory cytokines and growth factors (4, 20, 31, 35). Therefore, COX-1 and COX-2 may participate in parturition by increasing prostanoid metabolism (36, 39, 42).
blands. Furthermore, these hormones suppressed the production of COX-2, but not COX-1, in the IL-1α-treated uterine cervical fibroblasts.

**MATERIALS AND METHODS**

**Cell culture and treatment.** Uterine cervical fibroblasts were prepared from Nippon White rabbits at a gestational age of 23 days and maintained in culture in MEM (Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (BioWhittaker, Walkersville, MD), as described previously (14, 15, 16, 32, 38). In all experiments, cells up to the fourth passage were used. After achieving confluence, the cells were washed once with MEM supplemented with 0.2% lactalbumin hydrolysate (Sigma Chemical, St. Louis, MO) and then treated with progesterone, 17β-estradiol, indomethacin, aspirin (Sigma Chemical), and NS-398 (Calbiochem-Novabiochem, La Jolla, CA) in the presence or absence of recombinant human IL-1α (2 × 10^7 U/mg) (a generous gift from Dainippon Pharmaceutical, Osaka, Japan) in the same medium for 24 h. The harvested culture media were stored at −20°C until use.

**PGE2 assay.** PGE2 contents in the culture media were measured by radioimmunoassay as described previously (33). An aliquot (50 μl) of the harvested culture medium was incubated with ^3H^PGE2 (DuPont NEN, Boston, MA) and PGE2 antibody (PerSeptive Diagnostics, Cambridge, MA) in a total volume of 160 μl for 18 h at 4°C, and then 500 μl of 0.025% dextran-25% charcoal-0.9% NaCl were added to remove the unbound ^3H^PGE2. After incubation for 15 min at 4°C, the reaction mixture was subjected to centrifugation and the radioactivity in the resultant supernatant was counted. The amount of PGE2 was calculated from a standard curve performed concomitantly using authentic PGE2.

**COX activity measurement.** COX activity was determined that the hormonal suppression of PGE2 production in rabbit uterine cervical fibroblasts (Table 1), suggest similarly inhibited by progesterone and 17β-estradiol (1.3-fold) was increased by 10 ng/ml of IL-1α (column 3). The augmentation of PGE2 level by IL-1α was also time dependent; a twofold increase was observed within 2 h, and then the induction reached a plateau for 6–10 h (Fig. 1B). In addition, IL-1α-augmented PGE2 level was completely diminished by a broad COX inhibitor, indomethacin (10 μM), and by a COX-2-specific inhibitor, NS-398 (1 μM), but not by aspirin (1 μM), which is a well-known COX-1 inhibitor (Fig. 2). We furthermore confirmed that the conversion of exogenous arachidonic acid to PGE2 was enhanced by IL-1α and that the COX activity was no longer detected in rabbit uterine cervical fibroblasts treated with NS-398 and indomethacin but was detected in those treated with aspirin (data not shown). Thus these results suggest that IL-1α-augmented PGE2 level is dependent on COX-2 activity in rabbit uterine cervical fibroblasts.

**Progesterone and 17β-estradiol suppress the level of PGE2 and COX-2 activity in rabbit uterine cervical fibroblasts.** As shown in Fig. 3, progesterone and 17β-estradiol decreased the IL-1α-augmented PGE2 level in rabbit uterine cervical fibroblasts and the suppression was found to be significant at 0.01 μM (columns 3 and 6). A slight decrease in the basal level of PGE2 by both hormones was also observed at the relatively high concentration (1 μM) (columns 9 and 10). Furthermore, the IL-1α-augmented COX-2 activity (1.3-fold) was similarly inhibited by progesterone and 17β-estradiol in rabbit uterine cervical fibroblasts (Table 1), suggesting that the hormonal suppression of PGE2 production is due to the inhibition of COX-2 activity by progesterone and 17β-estradiol.

**RESULTS**

**IL-1α increases PGE2 level in rabbit uterine cervical fibroblasts.** When rabbit uterine cervical fibroblasts were treated with IL-1α, the level of PGE2 increased in a dose-dependent manner (Fig. 1A). The maximal effect was observed at 0.5 ng/ml of IL-1α (column 3). The augmentation of PGE2 level by IL-1α was also time dependent; a twofold increase was observed within 2 h, and then the induction reached a plateau for 6–10 h (Fig. 1B). In addition, IL-1α-augmented PGE2 level was completely diminished by a broad COX inhibitor, indomethacin (10 μM), and by a COX-2-specific inhibitor, NS-398 (1 μM), but not by aspirin (1 μM), which is a well-known COX-1 inhibitor (Fig. 2). We furthermore confirmed that the conversion of exogenous arachidonic acid to PGE2 was enhanced by IL-1α and that the COX activity was no longer detected in rabbit uterine cervical fibroblasts treated with NS-398 and indomethacin but was detected in those treated with aspirin (data not shown). Thus these results suggest that IL-1α-augmented PGE2 level is dependent on COX-2 activity in rabbit uterine cervical fibroblasts.

**Western blot analysis for COX-1 and COX-2.** To monitor the production of COX-1 and COX-2, the cells were pretreated with progesterone and 17β-estradiol for 24 h and then treated with the hormones in the presence or absence of IL-1α for 24 h. The cells were washed once with Ca^2+ - and Mg^2+ -free PBS and then lysed with 50 mM Tris-HCl (pH 7.5)-2% SDS-10% glycerol-5% 2-mercaptoethanol. The lysate was boiled for 12 min and then mixed with trichloroacetic acid at the final concentration of 3.33%. After centrifugation, the precipitated proteins were separated by SDS-polyacrylamide gel electrophoresis using 10% acrylamide gel and electrotransferred onto a nitrocellulose membrane. The membrane was reacted with monoclonal anti-murine COX-1 or anti-murine COX-2 antibody (Cayman Chemical, Ann Arbor, MI), which was then complexed with horseradish peroxidase-conjugated rabbit anti-mouse IgG (Sigma Chemical). Immunoreactive COX-1 and COX-2 were visualized with enhanced chemiluminescence-Western blotting detection reagents (Amersham Life Science, Tokyo, Japan) using Image Analyzer LAS-1000 plus (Fuji Photo Film, Tokyo, Japan).

**Statistical analysis.** Data were analyzed by Student’s t-test; P < 0.05 was considered to be statistically significant.
Different from control, P

Table 1. Progesterone and 17β-estradiol inhibit IL-1α-induced COX-2 activity in rabbit uterine cervical fibroblasts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>COX-2 Activity, pg PGE$_2$ ml$^{-1}$ min$^{-1}$ (% of IL-1α)</th>
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<tbody>
<tr>
<td>Control</td>
<td>35.63 ± 0.31 (80.8)</td>
</tr>
<tr>
<td>IL-1α (10 ng/ml)</td>
<td>44.09 ± 0.18 (100.0)*</td>
</tr>
<tr>
<td>IL-1α (10 ng/ml) + progesterone (0.01 μM)</td>
<td>37.33 ± 0.32 (84.7)§</td>
</tr>
<tr>
<td>IL-1α (10 ng/ml) + progesterone (0.1 μM)</td>
<td>37.08 ± 0.18 (84.1)§</td>
</tr>
<tr>
<td>IL-1α (10 ng/ml) + 17β-estradiol (0.1 μM)</td>
<td>35.94 ± 0.51 (81.5)§</td>
</tr>
<tr>
<td>IL-1α (10 ng/ml) + 17β-estradiol (0.01 μM)</td>
<td>39.59 ± 0.56 (89.8)§</td>
</tr>
<tr>
<td>IL-1α (10 ng/ml) + 17β-estradiol (0.1 μM)</td>
<td>38.20 ± 0.15 (86.6)§</td>
</tr>
<tr>
<td>IL-1α (10 ng/ml) + 17β-estradiol (1 μM)</td>
<td>37.33 ± 0.48 (84.7)§</td>
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Values are means ± SD. Rabbit uterine cervical fibroblasts at the third passage in 24-well multiplate were pretreated with progesterone or 17β-estradiol and then treated again in the presence or absence of interleukin-1α (IL-1α; 10 ng/ml) for 24 h. At the end of period, exogenous arachidonic acid (30 μM) was added after replacement of fresh culture medium and then incubated for 10 min. Prostaglandin E$_2$ (PGE$_2$) converted from exogenous arachidonic acid was measured by radioimmunoassay as described in MATERIALS AND METHODS. COX-2, cyclooxygenase-2. *Significantly different from control, P < 0.05. **Significantly different from control, P < 0.001. †Significantly different from IL-1α treatment, P < 0.001.

DISCUSSION

The level of prostanoids such as PGE$_2$, PGF$_{2α}$, and thromboxane A$_2$ in plasma and amniotic fluid is known to increase before the onset of labor and is further augmented throughout labor (1–4). MacDonald et al. (23) reported that arachidonic acid initiates labor and accelerates delivery near term, suggesting that the increase of prostanoid production is closely involved in the acceleration of parturition. In the present study, we demonstrated that progesterone and 17β-estradiol
decreased the level of PGE$_2$ in rabbit uterine cervical fibroblasts (Fig. 3). The plasma level of progesterone and 17β-estradiol in rabbit increases from early to late gestation, and then the decrease of the hormone levels correlates with the initiation of delivery at term (2, 12). Therefore, we suggest that progesterone and 17β-estradiol are endogenous suppressors of prostanoid metabolism in uterine cervix during gestation.

The production of PGs is regulated by COXs, and two isotypes, COX-1 and COX-2, have been characterized (8, 13). COX-2 is an inducible enzyme produced in response to inflammatory stimuli, whereas COX-1 is constitutively expressed in various cell lines and tissues (4, 20, 31, 35). COX-2 mRNA and its protein expression in amnion epithelium and decidua are augmented by IL-1β, tumor necrosis factor-α (TNF-α) and epidermal growth factor (10, 19, 30). We demonstrated that IL-1α increased PGE$_2$ level in rabbit uterine cervical fibroblasts (Fig. 1), which was disrupted in the presence of NS-398 and indomethacin but not aspirin (Fig. 2). Furthermore, the IL-1α-induced COX activity was completely inhibited by adding NS-398 and indomethacin (data not shown). In addition, our results showed that IL-1α augmented the production of COX-2, but not COX-1, in the cervical fibroblasts (Fig. 4). Therefore, we suggest that IL-1α induces COX-2 activity along with augmenting COX-2 production in rabbit uterine cervical fibroblasts. Furthermore, it has been reported that inflammatory cytokines such as IL-1 and TNF-α are highly detectable during pregnancy (3, 5, 16). With these findings taken together, we speculate that COX-2 is an enzyme that has a substantial role in the control of uterine contraction and that the uterine cervix may be one of the sources producing prostanoids to cause the onset of labor at term.

Goodwin et al. (9) reported that IL-4 transcriptionally suppresses the expression of COX-2 in human placental trophoblast cells. In addition, recent reports have shown that IL-10 and IL-13 inhibit the biosynthesis of PGE$_2$ in human amnion, chorion, and decidual cells by a mechanism whereby the production of COX-2 may be suppressed (7, 18). However, the involvement of these cytokines in parturition remains controversial. On the other hand, it has been reported that progesterone and 17β-estradiol are significantly detected during gestation (2, 12). In the present study, we demonstrated that progesterone and 17β-estradiol diminished the production of COX-2 and subsequently decreased the level of PGE$_2$ in rabbit uterine cervical fibroblasts (Figs. 3 and 4). Our laboratory’s previous studies also indicate that progesterone and 17β-estradiol inhibit the gene expression and production of MMPs and IL-8 in rabbit uterine cervical fibroblasts (14, 16, 32). Taken together, these results suggest that the suppression of COX-2 production by both hormones may be due to that of its gene expression in rabbit uterine cervical fibroblasts. Moreover, we propose that progesterone and 17β-estradiol are crucial hormones for functionally maintaining the uterus during pregnancy in rabbit.

Recently, several reports indicate discrepancies in the hormonal regulation of PG production. Hedin and Eriksson (11) reported that progesterone inhibits PG synthesis by suppressing COX-2 expression in rat follicles. In contrast, Badawi and Archer (1) reported that both COX-1 and COX-2 expression are augmented by the administration of progesterone and estradiol in ovariectomized rat. On the other hand, although estradiol has been shown to decrease the level of COX-2 mRNA in bovine endometrial cells (41), its gene expression is suppressed by progesterone but not 17β-estradiol in bovine myometrium (6). Taken together with our finding that rabbit COX-2 expression is suppressed by both progesterone and 17β-estradiol, it is suggested that hormonal regulation of COX expression in reproductive tracts may differ depending on animal species and the reproductive condition, such as menstrual cycle or pregnancy.

In conclusion, we demonstrated that progesterone and 17β-estradiol repressed prostanoid metabolism by decreasing the production of COX-2 in rabbit uterine cervical fibroblasts. These results suggest that progesterone and 17β-estradiol substantially participate in preventing the uterine contraction initiated by PGs during gestation.

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