Expression of Myometrial Activation and Stimulation Genes in a Mouse Model of Preterm Labor: Myometrial Activation, Stimulation, and Preterm Labor

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ABSTRACT

Myometrial contractions of labor result from an increase in myometrial activation and stimulation. Activation develops through the expression of contraction associated proteins (CAPs), including oxytocin receptors (OTR), connexin-43 (Cx-43), and prostaglandin F_2alpha_ receptors (FP). Stimulation involves increases in contractile agonists including prostaglandin E_2_ (PGE_2_) and prostaglandin F_2alpha_ (PGF_2alpha_), that may result from increases in prostaglandin endoperoxide H synthase (PGHS)-2. A mouse model of preterm birth was used to study gene expression involved in myometrial activation and stimulation. To induce preterm birth, pregnant C57BL/6J mice were intubated with 6 g/kg ethanol on gestational day 16 and were killed every 6 h from treatment until birth. RIA was used to measure uterine PGE_2_ and PGF_2alpha_, while PGHS-2, OTR, Cx-43, and FP messenger RNA levels were measured by ribonuclease protection assay. Increases in CAP mRNA were associated with term and preterm birth. There were differences in stimulation effectors associated with preterm and term birth. Uterine PGF_2alpha_ values were increased only at the time of term birth, but PGE_2_ was elevated during both preterm and term labor. These data suggest that existing levels of PGF_2alpha_ are sufficient for preterm birth when CAP expression is increased, but term labor requires increases in PGE_2_, PGF_2alpha_, and CAPs. The PGHS-2 messenger RNA expression pattern suggests that it is a CAP. (Endocrinology 141: 1718–1728, 2000)

P RETERM DELIVERY is the leading cause of perinatal morbidity and mortality, occurring in 5–9% of pregnancies and accounting for more than 60% of perinatal morbidity and mortality. (1) Although tocolytic therapy has been used for more than four decades, the preterm birth rate has not changed and, in fact, is increasing. To design effective treatments and interventions for preterm birth, mechanisms of both term and preterm birth must be elucidated and understood.

In the myometrium, the switch from a state of relative quiescence during pregnancy to a muscle that is spontaneously active and highly sensitive to the actions of endogenous uterotonins during labor is termed “uterine activation” (2). It is hypothesized that activation of the myometrium results from the coordinated expression of a cassette of “contraction-associated proteins” (CAPs), including actin, myosin, connexin-43, and the receptors for oxytocin and prostaglandins, and that the genes for these proteins may be regulated by a common mechanism (2, 3).

The evidence for a role for CAPs in parturition stems from a number of human and animal studies reporting that significant increases in CAPs are associated with labor at term. In both humans and in rats, a significant increase in myometrial prostaglandin F_2alpha_, receptor (FP) receptor messenger RNA (mRNA) expression is associated with labor at term (4, 5), and myometrial oxytocin receptor (OTR) density increases markedly before parturition in every model in which it has been studied, including the human (6). Connexin (Cx)-43 has been shown to increase with labor onset, (7, 8) possibly as a result of steroid hormone changes (9–12) and uterine stretch (13).

An activated myometrium is able to respond to stimulants such as prostaglandins (PGs). Prostaglandins have both direct and indirect effects on myometrial contractility; they stimulate myometrial contractions, further increase uterine sensitivity to uterotonic agents, synchronize myometrial contractions, (14–16) and alter hormone synthesis (17–19). Although labor is associated with increases in PGs in uterus, blood, and amniotic fluid, (20) the mechanisms that regulate the synthesis of PGs before and during labor remain unclear.

Recent evidence suggests that the inducible form of the prostaglandin synthase (PGS) responsible for production of the prostaglandin H synthase (PGHS)-2, is responsible for production of the uterine contractile agonists, PGE_2_ and PGF_2alpha_, during labor (21–24), and this may be a regulatory mechanism that can be targeted for the prevention and treatment of preterm birth.

Although it is clear that parturition involves both myometrial activation as well as stimulation, the initiating processes remain poorly defined, and the relationship between myometrial activation and myometrial stimulation has not been well studied. Thus, this study was designed to determine gestational age-dependent changes in myometrial activation and stimulation associated with term and preterm birth in a mouse model, as well as to begin to understand the relationships between CAPs and uterine PGs. We hypothesized that the expression of myometrial activation genes (OTR, FP, Cx-43) would increase at both preterm and term
labor relative to gestational age-matched controls or earlier in gestation and that 2) myometrial stimulation genes and effectors (e.g. PGHS-2 and prostaglandins) would increase at both term and preterm labor relative to gestational age-matched controls or earlier in gestation.

Materials and Methods

Subjects

Nulliparous 10- to 12-week-old female C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) were used in this experiment. A timed breeding procedure was employed to precisely control the time of insemination. Two females were placed into a cage with four males from 0900 h until 1000 h each morning. After this time, females were removed and examined for the presence of a seminal plug. Successfully bred females were weighed and individually housed in a separate temperature and humidity-controlled room, with a 12-h light, 12-h dark cycle (lights on at 0600 h). Seminal plug day was designated as GD 0 and represented the beginning of the gestational period.

Animal model of premature labor

An earlier experiment in our laboratory indicated that treatment of C57BL/6 mice on GD 16 with 6 g/kg alcohol induced preterm labor, with an incidence rate of 93%. (25) This was the treatment regime used for the present study. Blood alcohol levels of dams treated with 6 g/kg alcohol on GD 16 measured 0.38 ± 0.02 g/dl, (25) and this dose of ethanol is the threshold dose for teratogenicity in this mouse strain.

Treatment

Pregnant dams were assigned to a sampling time point in either the term birth group (untreated dams, n = 8) or the preterm birth group (ethanol-treated dams, n = 8). Previous data indicated that dams treated with isocaloric sucrose as a vehicle for alcohol did not differ in uterine prostaglandin levels when compared with untreated dams, (25) so untreated dams were used as the control group in this study. Sampling time-points were GD 14, 15, 16, 16.5, 17, 17.5, 17.75, 18, 18.25, 18.5, and 19, and the time of term labor for the untreated dams, and GD 16, 16.25, 16.5, 17, 17.25, 17.5, and the time of preterm labor in the ethanol-treated group. Both term and preterm labor were defined as delivery of the first pup. The normal length of gestation in this mouse strain is 19.3 ± 0.1 days and alcohol-induced preterm delivery occurs on GD 17.5 ± 0.1 (25).

Tissue collection

Dams were killed at the appropriate time point by decapitation. The abdominal wall of the mother was opened and both horns of the uterus were exposed. Incisions were made through the uterine tissue, just below each ovary. The uterus was removed from the dam by separation from the fascia with scissors, leaving the fetuses and the uterine wall intact. Fetuses, placentas, and fetal membranes were removed from the uterus through incisions in the uterine wall and killed immediately. Uterine tissue was removed, snap frozen in liquid nitrogen, and stored at −70 C until processed.

Prostaglandin extraction, RIA and protein measurement

Prostaglandin F$_2$a and Prostaglandin E$_2$ protein extraction and measurements were performed according to methods described previously. (26) Frozen uterine tissue was pulverized using a prechilled mortar and pestle. The uterine tissue was ground to a fine powder under liquid nitrogen. RNA pellets were resuspended in 20 μl of RNA buffer (100% ethanol). Following centrifugation at 14 000 × g for 10 min, the supernatant was evaporated and samples were reconstituted in 1 ml PBS for PGF$_2$a and 500 μl of PVP buffer (55 mm Tris, 154 mm NaCl, 0.1% polyvinylpyrrolidone, pH 7.4) for PGE$_2$. At this point, PGE$_2$ samples were measured by RIA. Reconstituted PGF$_2$a samples were acidified with 95 μl of 2.7% formic acid (vol/vol) and extracted into ethyl acetate.

After evaporation, samples were reconstituted overnight in 500 μl of PVP buffer then measured by RIA. Extraction recovery values were 89.6 ± 1.2% for PGF$_2$a and 88.43 ± 0.6% for PGE$_2$.

Both antibodies were obtained commercially (PE Biosystems, Inc., Mississauga, Ontario, Canada). Cross-reactivity of the PGF$_2$a antibody is reported to be 100% with PGF$_2$e, but there is not cross-reactivity with other prostaglandins. The PGE$_2$ antibody cross-reacts 50% with PGF$_2$e, but there is less than 0.001% cross-reactivity with other prostaglandins. Procedural blanks were assayed with the samples and extraction recoveries were determined for all samples and blanks. Sample values were corrected for recovery and blank values were subtracted, and sample values are expressed as the mean of duplicate measures. The PGF$_2$a and PGE$_2$ assays were sensitive to 5 pg and the intra and inter-assay coefficients of variation measured 8.9% and 4.5%, respectively, when measuring 200 pg for PGF$_2$a and were 3.4% and 12.6%, respectively, when measuring 200 pg for PGE$_2$. PGs were expressed as pg/mg protein.

RNA extraction

RNA was extracted as previously described (27). Briefly, frozen uterine tissue was ground to a fine powder under liquid nitrogen. RNA reagent (Trizol: Life Technologies, Inc., Burlington, Onatrio, Canada), was added to 0.05 g of frozen sample and the tissue was homogenized on ice. Following chloroform extraction and isopropanol precipitation, RNA pellets were washed with 75% ethanol. The RNA pellet was dissolved in TE buffer (10 mm Tris, pH 8.0 + 1 mm EDTA, pH 8.0) and RNA was quantified through spectrophotometric analysis. The integrity of the RNA was also confirmed by agarose electrophoresis.

Generation of RNA probes

The pGT-PGHS-2 construct for generating PGHS-2 RNA probes was prepared as previously described. (27) The pGT-FP construct used for FP RNA probe synthesis was generated by standard methods. More specifically, a 222 nt FP receptor fragment (GenBank Accession no. D17433, nucleotides 859-1080) was generated by standard RT-PCR methods (FP forward primer: 5′-TCTTGGTTGCTCTTCTGCTG-3′, reverse primer 5′-GCTGTTCACAGGTCACTG-3′) and cloned into pGEMT. Primers were chosen so that the forward primer was located in exon 1 of the mFP gene and the reverse primer was located in exon 2.

The pBSKOTR1.0 construct used for making the OTR probe was a generous gift from Dr. Louis Muglia (Washington University Medical Center, St. Louis, MO), and the pCRII-Cx-43 construct for making the Cx-43 probe was provided by Dr. Stephen J. Lye (University of Toronto, Toronto, Ontario, Canada). The pTRI-Cyclophilin-Mouse construct used for making the cyclophilin probe was a generous gift from Dr. Louis Muglia (Washington University Medical Center, St. Louis, MO).

Linearized pGT-PGHS-2, pGT-FP, pBSKOTR1.0, pCRII-Cx-43, and pTRI-Cyclophilin-Mouse were transcribed to make mouse PGHS-2, FP, OTR, Cx-43, and cyclophilin antisense RNA probes. Sense RNA probes were also generated for use as a negative control. RNA controls were made as previously described (27). Briefly, 1 μg of linearized plasmid was transcribed with either T7, T3, or SP6 RNA polymerase under the following conditions: 5 μCi/ml α-32P-CTP; 0.5 mm each of rATP, rGTP, and rTTP; 10 mm DTT; 40 mm Tris (pH 7.9); 6 mm MgCl$_2$; 2 mm spermidine; 10 mm NaCl; 1.2 U/μl RNasin Ribonuclease inhibitor; 0.1 μg/μl linearized DNA template; and 2 U/μl T7, T3, or SP6 polymerase.

Following transcription, the DNA template was digested by adding 24 U RNasin Ribonuclease inhibitor, and 60 U DNase I. Following phenol/chloroform extraction, the RNA was precipitated with 100% ethanol, using yeast transfer RNA (tRNA) as carrier.

RNA pellets were resuspended in 20 μl formamide RNA buffer (80% formamide, 1 mm EDTA, pH 8.0, 0.1% bromophenol blue, 0.1% xylene cyanol), denatured by boiling for 5 min, placed on ice, and electrophoresed on a 6% polyacrylamide, 8 μm urea gel. Full-length transcripts (PGHS-2: 437 nt, Cx-43: 389 nt, FP: 573 nt, Cx-43: 389 nt, Cyclophilin: 165 nt) were cut out of the gel and eluted in 400 μl elution buffer (2 mM ammonium acetate, 1% SDS, and 25 μg/ml yeast tRNA) for 3 h at 37°C. RNA was then precipitated from the supernatant with 100% ethanol. Following centrifugation at 14 000 × g for 10 min, the
RNA pellet was resuspended in hybridization buffer (80% formamide, 40 mM PIPES, pH 6.4, 400 mM NaCl, and 1 mM EDTA). Two microliters were counted in a liquid scintillation counter to determine incorporation.

**RNase protection assay**

Twenty micrograms of total mouse uterine RNA was hybridized to 5 × 10⁶ cpm of the appropriate probe in 30 µl hybridization buffer for 16 h at 55°C. Yeast tRNA was also processed in a similar manner as a negative control. Levels of the constitutively expressed cyclophilin were also measured to verify uniform abundance of mRNA in the different samples. Hybridizations of sample RNA with sense RNA probes served as another negative control. Following hybridization, samples were digested with 2.3 µg/ml ribonuclease A and 300 U ribonuclease T1 in 300 µl ribonuclease digestion buffer (10 mM Tris-Cl, pH 7.5, 300 mM NaCl, 5 mM EDTA) for 30 min at 30°C. Ribonucleosomes were removed by treatment with 25 µg/ml proteinase K in the presence of 0.6% SDS for 20 min at 37°C. Samples were centrifuged for 10 min at 14,000 × g; resuspended in 6 µl formamide RNA buffer and electrophoresed on a 6% acrylamide, 8 m urea gel. The gel was dried and analyzed by autoradiography. Protected bands were the following sizes: PGHS-2, 362 nt; OTR, 301 nt; FP, 223 nt; Cx-43, 294 nt; and Cyclophilin, 103 nt.

**Statistical analysis**

All data were analyzed by separate one-way ANOVA. When a significant F value was obtained, time or treatment effects were differentiated by using Tukey’s test. Tests between treatments at a single time point were performed by the Student’s t test. Significance was achieved when P ≤ 0.05.

**Results**

**CAPs**

**Uterine PGF₂α receptor (FP) mRNA expression**

The profile of uterine FP receptor mRNA expression is shown in Fig. 1. Analysis of uterine FP mRNA expression over time for alcohol-treated and control dams in separate one-way ANOVAs revealed main effects of time in control [F (7, 33) = 17.31, P < 0.001] and alcohol-treated [F (4, 17) = 4.13, P < 0.05] dams. Posthoc analysis suggested that FP mRNA levels at the time of preterm and term labor were significantly elevated over values measured on GD 16 (P < 0.05), and preterm levels were significantly elevated when compared with the gestational age-matched controls on GD 17.75 (t₀ = −4.35, P = 0.002). Again, there were no significant differences between OTR mRNA levels associated with preterm and term labor (t₀ = 1.75, P = 0.004).

**Connexin-43**

ANOVA indicated that there was a main effect of sampling time on uterine Cx-43 mRNA expression in control [F (7, 27) = 4.64, P = 0.003] as well as alcohol-treated [F (4, 13) = 3.39, P = 0.04] dams (Fig. 3). Tukey’s Test revealed that in control dams, Cx-43 levels remained at prelabor levels until term, when they were significantly higher (P < 0.05) than expression levels associated with GD 16. Dams in preterm labor had uterine Cx-43 mRNA expression levels that were significantly elevated by GD 17.5 when compared with prelabor values on GD 16 (P < 0.05). High levels persisted until preterm labor (t₀ = −7.48, P < 0.001). Levels associated with preterm labor were significantly higher than levels of control dams matched for gestational age (P < 0.05). Again, there were no significant differences between Cx-43 mRNA expression levels associated with term and preterm labor (t₀ = −1.74, P = 0.157).

**Uterine stimulants**

**Uterine PGHS-2 mRNA expression**

Analysis of PGHS-2 mRNA expression over time (Fig. 4) for alcohol-treated and control dams using separate one-way ANOVAs indicated that uterine PGHS-2 mRNA expression increased significantly over time in both groups [Control: F (8, 41) = 2.54, P = 0.029; Alcohol: F (7, 38) = 5.35, P < 0.001]. Posthoc analysis suggested that PGHS-2 mRNA levels were significantly elevated over prelabor (GD 16) values at the time of preterm and term labor (ps <0.05). There were no significant differences between PGHS-2 mRNA expression during preterm and term labor [t₁₁ = −0.925, P = 0.375], and preterm levels were significantly higher than levels from gestational age-matched control dams (t₀ = 2.91, P = 0.02).

**Uterine PGF₂α**

Separate one-way ANOVAs were used to analyze uterine PGF₂α levels in the two treatment groups. Results revealed that uterine PGF₂α levels increased over time in both alcohol-treated [F (7, 55) = 4.57, P < 0.001] and control dams [F (8, 68) = 3.91, P < 0.001, Fig. 5]. Posthoc analyses suggested that dams in preterm and term labor had PGF₂α levels that were significantly higher than prelabor values measured on GD 16 (P < 0.05). More specifically, alcohol-treated dams had elevated levels by GD 17.5 and untreated dams had elevated levels by GD 18.5. Further, uterine PGF₂α levels associated with preterm and term labor were similar (t₁₁ = −1.622, P = 0.127), but preterm levels were not significantly higher than levels from gestational age-matched control dams (t₀ = 1.49, P = 0.89).

**Uterine PGE₂**

Figure 6 illustrates the uterine PGE₂ levels over gestation in alcohol-treated and control dams. Mean uterine PGE₂ levels were one-twentieth those of PGF₂α, and uterine PGE₂...
levels in the two treatment groups were analyzed by separate one-way ANOVAs. In the control dams, there was an increase in uterine PGE2 levels over gestation \( [F(8, 81) = 3.511, P = 0.002] \), but while there was an apparent increase in mean levels in the ethanol-treated females, this effect was at the borderline for nonsignificance \( [F(7, 64) = 2.134, P = 0.054] \).

Posthoc analysis by the Student’s \( t \) test revealed that dams in preterm labor had significantly higher uterine PGE2 levels than gestational age-matched controls \( (t_{17} = 2.49, P = 0.02) \), but there was not a statistically significant difference between uterine PGE2 levels associated with term birth and preterm birth \( (t_{22} = 0.36, P = 0.72) \).

**Discussion**

This study was designed to compare and contrast mechanisms of term and preterm birth in terms of myometrial activation and stimulation in a mouse model. The first part of the hypothesis stated that myometrial activation and stimulation genes would be expressed similarly at term and pre-...
term labor, and the data support this hypothesis. Messenger RNA relative abundance for FP, OTR, and Cx-43 was greater at preterm birth when compared with gestational age-matched controls and similar to elevated levels at term. However, the second part of the hypothesis was not true. Prostaglandin F$_2\alpha$ levels in the uterus were not elevated at preterm birth over gestational-age matched control dams, suggesting that mechanisms of preterm birth are different than those of term birth. Principally, in this model, the CAP genes and PGHS-2 mRNA are expressed, but there is no increase in PGF$_2\alpha$ levels. Hence, these data suggest that myometrial activation is the main mechanism responsible for...
enhanced uterine contractile activity and labor at preterm birth, rather than elevated levels of stimulatory prostaglandins. Further, these data suggest that PGHS-2 should be included as a CAP, and confirm the possibility that there are common mechanisms of gene regulation.

CAP mRNA expression patterns paralleled each other. For example, FP, OTR, and Cx-43 mRNA levels were relatively constant across gestation in uterine tissue from control dams and increased over prelabor (GD 16) levels by the time of term labor. By GD 17.5 in EtOH-treated dams, however, levels were already maximally elevated, and OTR mRNA expression peaked by GD 17. Thus, CAP mRNA expression

Fig. 3. Uterine connexin-43 (Cx-43) mRNA expression. The top panel illustrates a representative ribonuclease protection assay (RPA) with n = 2/group. The histogram in the was generated from the densitometric analysis from three separate RPAs (n = 4–6/group). *, Significantly different from GD 16 at α = 0.05; data are shown as mean ± SEM.
increased only at the time of term labor, but was elevated in alcohol-treated dams before the onset of preterm labor. However, the sampling time closest to the onset of term labor was GD 18.5, approximately 19 h from the mean time of term labor onset. Sampling closer to labor was impossible, given that the gestational length for control dams in this study...
varied between 18.6 and 19.8 days. Thus, it must be kept in mind that there is a large span of time between the term labor timepoint and the timepoint immediately preceding it when comparing and contrasting temporal changes associated with mechanisms of term and preterm labor. Thus, these data do not suggest that the pattern of CAP mRNA increases associated with preterm and term labor are different and conclusions cannot be drawn as to when CAPs increase in the uterus of control dams between GD 18.5 and term labor. Indeed, it is possible that CAP mRNA levels are elevated before term labor in a pattern that is similar to that characteristic of preterm labor.

The CAP data from this study parallel data from other laboratories. For example, in humans, FP receptor mRNA expression declined significantly with gestational age in patients not in labor and increased significantly with labor at term, indicating that labor at term is associated with a significant increase in FP receptor expression, consistent with influence on contraction. (5) In other studies, expression of FP receptor in the myometrium of pregnant rats increased significantly from late gestation until delivery, returning to prepartum levels by one day postpartum. (4) Thus, together these studies suggest that myometrial activity at parturition in mice, humans, and in rats may change from an active
quiescent to an active contractile state in concert with an up-regulation of contractile FP receptors.

Many studies report that the number of oxytocin receptors in the pregnant uterus increases dramatically at the time of parturition in humans (6), cows (28), and rats (29, 30) Our data confirm that OTR mRNA is present in mouse uterus and that the changes parallel those measured in other species studied. Cx-43 increases are associated with labor in mice (31), rats (10, 12), and humans (32) at term, and studies suggest that gap junction and Cx-43 levels associated with preterm labor are similar to those measured at term labor in rats (33, 34).

Again, our data confirm these findings in our mouse model of preterm birth.

In terms of myometrial stimulation, the profiles of uterine PGE2 and PGF2α levels were different. While PGF2α levels increased over gestation in both control and alcohol-treated dams, there was not an elevation associated with preterm labor when compared with gestational age-matched controls. However, because levels were elevated at the time of term labor, this suggests that PGF2α increases are not necessary for preterm labor.

There was a trend for uterine PGE2 levels to be higher in uterine tissue of EtOH-treated dams than in the uterus of...
control dams, and by preterm labor, uterine PGE₂ levels were higher than levels from gestational-age matched controls. Whether uterine stimulation by PGE₂ is important for preterm birth is uncertain. The PGE₂ concentrations were one-twentieth of those of uterine PGF₂α, suggesting a minor role in the stimulation of uterine contractions. The necessity for increases in PGE₂ may be related to its role in cervical ripening (35).

There are discrepancies in the literature regarding the role of PGHS-2 in the labor process. Data suggest that PGHS-2 protein levels are not detectable during the latter part of pregnancy in rat myometrium. (36) Our data, however, indicate that both PGHS-2 protein (Cook, I. L., D. H. Sung, K. I. Anderson, and D. M. Olson, unpublished data) and mRNA are present in the homogenate of myometrium plus decidua of the mouse, and suggest that elevations in uterine PGHS-2 may be responsible for elevations in uterine prostaglandins (PGs) associated with term and preterm labor. (25) Studies using other animal models of labor suggest that PGHS-2 plays a significant role in increasing the prostaglandin levels associated with labor in human amnion (24, 37), chorion and decidua (38), and in ovine placenta (39). Thus, it appears that the contributing role(s) of PGHS-2 may be tissue or species dependent.

These data confirm the presence of PGHS-2 mRNA expression in murine uterus and suggest that there is a role for PGHS-2 in murine parturition. In control dams, uterine PGHS-2 mRNA expression was relatively constant across gestation, increasing from prelabor values only at the time of term labor. In EtOH-treated dams, PGHS-2 levels also remained until 6 h before the onset of preterm labor when there was an increase in PGHS-2 mRNA expression over prelabor values. This increase was maximal and persisted until the time of preterm labor onset. Thus, these data suggest that PGHS-2 mRNA expression, like the other CAPs measured in this study, increases prior to the time of preterm labor, but only at the time of term labor.

These data also illustrate that increases in PGHS-2 at the time of preterm labor may underlie PGE₂ changes, but not PGF₂α (since there were no increases in PGF₂α at preterm labor compared with gestational age-matched controls). Some explanations for this include the differential regulation of the specific prostaglandin synthase enzymes to preferentially up-regulate PGE₂ without affecting PGF₂α, or the ability of different cell types to synthesize PGE₂ and PGF₂α in different amounts (e.g. cervical region vs. fundal region of the uterus).

A caveat of this experiment was that measurements were made in total uterine tissue, and delineations were not made between the decidua and the myometrium. It is possible that PG production and CAP expression in decidua and myometrium differ. Production and expression in the decidua may be involved more with the manufacture of uterine stimulants [i.e. activation of OTR increases PG production (40) and activation of PG receptors increases oxytocin (41)] rather than playing a direct role in uterine contractility. Thus, the identification of the independent roles of the decidua and the myometrium in PG production and CAP expression would lead to a more complete understanding of the relationship between myometrial activation and stimulation associated with term and preterm labor.

In summary, the results from this study suggest that the mouse may provide a powerful model to study preterm birth and its mechanisms because myometrial stimulation and activation processes appear to parallel those of other species, including the human. A reliable mouse model will offer the advantage of genetic manipulation to target the role(s) of specific CAPs and their regulation.

Most importantly, this study suggests that preterm labor is not merely accelerated term labor, but rather that the mechanisms of preterm and term labor are different. Thus, studying term labor may not be the ideal way to understand the mechanisms of preterm labor, and it is important to exploit the use of preterm labor models to understand mechanisms. Mechanisms of preterm and term labor differ with respect to myometrial stimulation by prostaglandins. Prostaglandin F₂α seems important for myometrial stimulation of term labor, but not for preterm labor. Further, the expression profiles of CAP (OTR, FP, and Cx-43) and PGHS-2 mRNA expression are similar for term and preterm labor. PGHS-2 should be included among the CAPs and may share a common regulator of expression with the other CAPs. Understanding the regulation of CAPs may be the key to preventing preterm labor in the future because attempting to arrest preterm birth by preventing the production of stimulators (prostaglandins) has many negative fetal side effects (42, 43).

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