MHR

RESEARCH ARTICLE

W. X. WU, ET AL. [2005] MED HYPOTHESES RES 2: 283-294.

STIMULATION OF PLACENTAL AND ENDOMETRIAL, BUT NOT MYOMETRIAL PROSTAGLANDIN H SYNTHASE-2 BY REPEATED MATERNAL DEXAMETHASONE ADMINISTRATION

WEN XUAN WU*, DAVID HOWE, KAUSHIK CHAKRABARTY, VALTA COLLINS, JAMES ROSE AND PETER W. NATHANIELSZ

DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, WAKE FOREST UNIVERSITY BAPTIST MEDICAL CENTER, WINSTON SALEM, NC 27157 (W.X.W., K.C., V.C.); WAKE FOREST UNIVERSITY SCHOOL OF MEDICINE, DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, WINSTON-SALEM, NC 27157 (J.R.); UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER AT SAN ANTONIO, SAN ANTONIO, TX 78229 (P.W.R.), USA; AND DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, THE QUEEN MOTHER'S HOSPITAL, YORKHILL, GLASGOW G3 8SJ, UK (D.H.D.).

ABSTRACT. OBJECTIVE: To determine whether maternal dexamethasone administration in amounts inadequate to produce labor after 48 hours has a differential effect on stimulation of prostaglandin H synthase (PGHS)-2 mRNA and protein expression in different fetal and maternal intrauterine tissues. METHODS: Saline or three courses (one course per week) of dexamethasone were administered to pregnant sheep commencing at 103, 110 and 117 dGA for two days per course (4 x 2 mg doses at 12 hour intervals). Maternal and fetal placenta, myometrium and endometrium were collected from saline and dexamethasone treated ewes (N = 6 in each group) 48 hours after the last injection of the third course of dexamethasone. Maternal and fetal placental, myometrial and endometrial total RNA and proteins were extracted and analyzed by Northern and Western blot and in situ hybridization for PGHS-2 mRNA and protein. Data were analyzed by Anova. RESULTS: After three courses of maternal dexamethasone, PGHS-2 mRNA and protein were significantly increased in maternal and fetal placenta and endometrium, but remained low in myometrium. CONCLUSIONS: Induction of fetal and maternal placental and endometrial but not myometrial PGHS-2 provides further evidence for a tissue specific action of glucocorticoids on intrauterine PGHS-2 in pregnant sheep.

*ADDRESS ALL CORRESPONDENCE TO: DR. WEN XUAN WU, DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, WAKE FOREST UNIVERSITY BAPTIST MEDICAL CENTER, 1ST MEDICAL CENTER BLVD, WINSTON SALEM, NC 27106, USA.
PHONE: 336-716-1651. FAX: 336-716-6937. E-MAIL: wen_xuan_wu@hotmail.com.

• MEDICAL HYPOTHESES AND RESEARCH • THE JOURNAL FOR INNOVATIVE IDEAS IN BIOMEDICAL RESEARCH •

1. INTRODUCTION

Intrauterine prostaglandin (PG) production plays a central role in the preparation and completion of the process of parturition. Prostaglandin H synthase (PGHS) is the central enzyme [1,2] that controls the intrauterine PG biosynthetic pathway [3-5]. Two forms of PGHS exist, however PGHS-2 is considered to be responsible for the stimulation of PG production by agonistic ligands [1,2]. Tissue-specific ontogenic expression of PGHS-2 has been demonstrated in myometrium, endometrium, and placenta during late gestation and spontaneous term labor. Both fetal placenta and maternal endometrium display an increased level of PGHS-2 expression during late ovine gestation when the estradiol levels are still low, whereas myometrial PGHS-2 remains low throughout late stages of gestation and only increases during labor in association with rising levels of estradiol [6]. Indeed, estradiol has been shown to regulate intrauterine PGHS-2 expression in a tissue-specific manner, i.e., the myometrial PGHS-2 is mainly under the control of estrogens in ovariectomized non-pregnant sheep [7] as well as in pregnant sheep [8], and estradiol does not stimulate fetal placental PGHS-2 expression [8]. Inhibition of placental estradiol production results in down-regulation of the PG system in maternal uterine tissues, but not in fetal placenta [9]. All these data suggest that estrogen may be responsible for the labor-associated increase of PGHS-2 expression and PG production from maternal uterine tissues [9-11]. However, the regulatory mechanisms which control gestation-associated rise of placental and endometrial PGHS-2 expression remain unresolved.

The time course of PG secretion into the maternal and fetal plasma in pregnant sheep [12,13] is consistent with the gradual development of PGHS-2 activity in intrauterine tissues during pregnancy. PGE_2 in maternal and fetal plasma increases steadily throughout late gestation [13]. There is a further increase of PGE_2 and $PGF_{2\alpha}$ during glucocorticoid induced preterm labor [5, 14] and spontaneous term labor in association with the rising estradiol [4,14].

The above data suggest that the gradual late gestation-associated rise of fetal placental and endometrial PGHS-2 may result in the increased secretion of PGE_2 in fetal and maternal plasma throughout gestation [5,14], whereas further induction of intrauterine PGHS-2 during labor is responsible for the labor-associated increase of a variety of PGs [9,11]. The above data also raise two questions: (i) What are the mechanisms that control tissue-specific PG production? (ii) What are the mechanisms that control gestational age-specific PG production?

The rise in fetal plasma PG is better associated with the rise in fetal plasma cortisol than the rise in estrogen after 125 dGA. In vitro studies conducted by several groups of researchers [5,15-17] have shown that glucocorticoids directly stimulated PG production in cultured human fetal membranes. In sheep, the abundance of placental PGHS-2 increases before the rise of the placental $P450_{17\alpha}$ hydroxylase activity [18]. Furthermore evidence has also been obtained for the existence of a cortisol-dependent and estradiol-independent mechanism for PGHS-2 induction within trophoblast tissue, leading to elevation of fetal plasma PGE_2 in pregnant sheep [9]. Recently, we have shown that cortisol administration to adrenalectomized fetuses to clamp fetal cortisol at levels present early in the late gestation rise stimulates intrauterine PGHS-2 mRNA and protein expression without inducing labor [11]. Taking together, these findings support an action of cortisol on prostaglandin production mainly in fetal intrauterine tissues by a direct pathway. However the regulatory mechanisms which control the gestation-associated increase of endometrial PGHS-2 expression remain unclear. Since our previous study determined the cortisol regulatory effect on the intrauterine PG system by fetal administration which did not alter cortisol level in maternal plasma [19], the current study was undertaken to determine whether maternal dexamethasone administration in amounts inadequate to produce labor, regulate maternal or fetal placental, endometrial and myometrial PGHS-2 mRNA and protein expression in pregnant sheep.

2. MATERIALS AND METHODS

2.1. CARE AND USE OF ANIMALS

Mature Rambouillet-Columbia crossbred ewes mated on a single occasion only and carrying a fetus

of known gestational age (term 148 days) were used. Experimental procedures were approved by the Cornell University Institutional Animal Care and Use Committee. The Cornell facilities are approved by the American Association for the Accreditation of Laboratory Animal Care.

2.2. MATERNAL DEXAMETHASONE INJECTION

Dexamethasone (2 mg) or vehicle was administered intramuscularly for two days to pregnant ewes as a course of four injections at 12h intervals. Treatment was given at 103-104, 110-111 and 117-118 dGA. The dose of dexamethasone was chosen following extensive trials in which 2, 4 and 6 mg were administered without progesterone coverage. Delivery within 48 h of receiving the first four injection courses occurred in 84 and 100% of animals in the 4 and 6 mg groups, respectively (N = 6 per group). All animals in the 2 mg group delivered at term.

2.3. TISSUE COLLECTION

All ewes underwent Caesarean section under halothane anesthesia on 119 dGA, 48h after the last injection of the third course. Maternal and fetal placenta, myometrium and endometrium were collected from control (N = 6) and dexamethasone (N = 6) treated groups. Tissues were flash frozen in liquid nitrogen for Northern and Western blot analysis and slowly frozen on dry ice for in situ analysis. Myometrium was also collected from five animals in spontaneous labor.

2.4. TOTAL RNA PREPARATION AND NORTHERN BLOT ANALYSIS

Total RNA from placentome, endometrium and myometrium was prepared as described previously [7]. The RNA purity and recovery of each tissue were determined by UV spectrophotometer (260 and 280 nM). Samples of total RNA (40 µg/lane) were denatured in 17.4% (v/v) formaldehyde, 50% (v/v) formamide, 20 mM 3-(N-morpholino)propanesulfonic acid, 5mM sodium acetate and 1mM EDTA, pH 7.0 for 5 min at 65ºC and separated on a 1% (w/v) agarose/0.66 M formaldehyde gel. Ethidium bromide stained ribosomal RNA (rRNA) bands were visualized (UV) to ensure that RNA degradation had not occurred and an equal amount of RNA was loaded into each lane. After electrophoresis, RNA was transferred to a nylon membrane (Gene Screen Plus, New England Nuclear, Dupont, Wilmington, DE) by capillary blotting for 24 h in 10X SSC (1X SSC is 0.15 M NaCl and 0.015M Na Citrate, pH 7.0). Pre-hybridization (>1 h) and hybridization (>18 h) were carried out at 65C. The probe concentration was ~1 x 10^6 cpm per mL of hybridization buffer, which consisted of 50% (v/v) deionized formamide, 50 mM sodium phosphate, 0.8 M NaCl, 2% (w/v) sodium dodecyl sulphate (SDS), 100 µg salmon sperm DNA/ml, 20 µg tRNA/ml and 1X Denhardt's (50X = 1% solution of bovine serum albumin, Ficoll, and polyvinylpyrrolidone). Membranes were washed twice for five minutes in 2X SSC and 0.1% SDS at 65ºC temperature and twice for 15 minutes in 0.1X SSC and 0.1% SDS at 65ºC. The same membranes were re-probed for 18S.

2.5. IN SITU HYBRIDIZATION

Frozen sections (4-µm thick) cut onto commercially prepared poly-L-lysine coated slides (Sigma Chemical Co. St. Louis, MO) were fixed in freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer (20 minutes), washed twice in 0.1 M phosphate buffer, immersed in triethanolamine-HCl (3.71 g of TEA, 2 mL of 6 M NaOH and 198 mL of water), pH 8.0 and then TEA and acetic anhydride (0.25%) for 10 minutes. They were then washed in 2X SSC for 5 minutes and briefly in 70% ethanol and allowed to air dry. The specimens were incubated for 2 hours in a humidified container (55ºC) with 70 µL of pre-hybridization buffer (50% deionized formamide, 0.3 M NaCl, 20mM Tris-HCl (pH 8.0), 5mM EDTA, 10 mM sodium phosphate buffer (pH 8.0), 1X Denhardt's solution), and for at least 16 hours in hybridization buffer (i.e. pre-hybridization buffer plus probe (1 x 10^6 cpm per specimen in 70 µL). Control slides were hybridized in the presence of an excess of unlabelled antisense or with labeled sense RNA. Slides were then washed three times in 4X SSC and 4 mM dithiothreitol (DTT, sigma); three times in NTE buffer (0.5 M NaCl, 10mM Tris-HCl and 5 mM EDTA, pH 8.0) at 37ºC (second NTE wash was for 30 min with 30 µg/mL Ribonuclease A); 2X SSC and 1 mM DTT; 0.1X SSC and 1 mM DTT at 60ºC; and finally 0.1X SSC at room temperatures. The

slides were then dehydrated in a graded series of ethanol plus 0.3 M ammonium acetate. They were air dried and exposed to autographic films for 2-4 days and then dipped in emulsion (Kodak NTB2, VWR, So. Plainfield, NJ) and exposed for 1-4 weeks at 4C. Following developing and fixing they were counter-stained with haematoxylin and eosin, mounted and covered with a glass cover slip.

2.6. SYNTHESIS OF PROBES

Our cloned ovine PGHS-2 cDNA in pCR II vector (Invitrogen), which include promoters for phage polymerases SP-6 to produce antisense probe and T-7 to produce sense probe, was linearized by an appropriate restriction enzyme. The antisense and sense riboprobes were synthesized using a commercial kit (MAXIscript, Ambion, TX) labeled with [α-^{32}P]UTP for Northern or [α-^{35}S]UTP for in situ (NEN Life Science). We have validated our cloned PGHS-2 riboprobes in our previous study [8]. Only the PGHS-2 antisense probe generated the specific signals at the expected molecular weight, ~4.5 kb [8].

2.7. SOLUBILIZED CELL MEMBRANE EXTRACTION AND WESTERN BLOT ANALYSIS

Solubilized cell membrane extracts from myometrium, endometrium and maternal and fetal placenta were prepared as described previously [7,8]. The protein concentration was determined by the method of Bradford (Bio-Rad Laboratories). The extracted protein samples from different groups in each tissue were separated on the same gel and stained with coomassie blue to analyze IgG heavy and light chains before performing Western blot analysis. We only used protein samples which did not yield significant differences in these control proteins. The solubilized proteins (50 μg/lane) were then separated on 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred to a nylon membrane (Imobilon, Millipore Corp., Bedford, MA), using a Bio Rad transfer blot cell. Western blot analysis was performed as described previously [7,8]. The protein bands were visualized using an enhanced chemiluminescence Western blotting detection kit (ECL, Amersham Life Sciences, Arlington Heights, IL). The molecular sizes of the proteins were determined by running standard molecular weight marker proteins (BioRad) in an adjacent lane. Chemiluminescence signals were analyzed and quantified with the scanner and data were analyzed with a densitometry program-Scan Analysis and quantified against an arbitrary scale in the plot.

2.8. ANTIBODIES FOR PGHS-2 USED FOR WESTERN BLOT ANALYSIS

A rabbit polyclonal antibody for PGHS-2 raised against a synthetic peptide corresponding to the C terminal region of human PGHS-2 (Oxford Biomedical Research, Inc. Oxford, MI) was used at 1:1000 dilution and incubated at 4ºC for 20 h. PGHS-2 antibody has been characterized in our previous study [7,8]. A horseradish peroxidase-conjugated donkey anti-rabbit IgG was incubated with the membranes at room temperatures for 1 h.

2.9. STATISTICAL ANALYSIS

Comparison of 3 or more means was made by analysis of variance and multiple post hoc comparisons with Tukey's method for 95% confidence interval of pairwise differences. Statistical significance was assumed at the 5% level. Data are presented throughout as MEAN ± S.E.M.

3. RESULTS

3.1. PGHS-2 MRNA

After three courses of maternal dexamethasone administration, PGHS-2 mRNA analyzed by Northern Blot analysis was significantly increased in maternal and fetal placenta and endometrium (FIG 1). In contrast PGHS-2 mRNA was barely detectable in the myometrium in either the control or dexamethasone treated groups by Northern blot analysis (FIG. 1), however PGHS-2 mRNA was abundant in the myometrium during spontaneous term labor (FIG. 2). PGHS-2 mRNA was localized in glandular epithelial cells of endometrium and maternal placenta, smooth muscle cells of myometrium and trophoblast cells in fetal placenta (FIG. 2 AND 3). The specific hybridization of PGHS-2 antisense probes with PGHS-2 mRNA was more abundant in endometrium, maternal and fetal placenta collected from dexamethasone treated

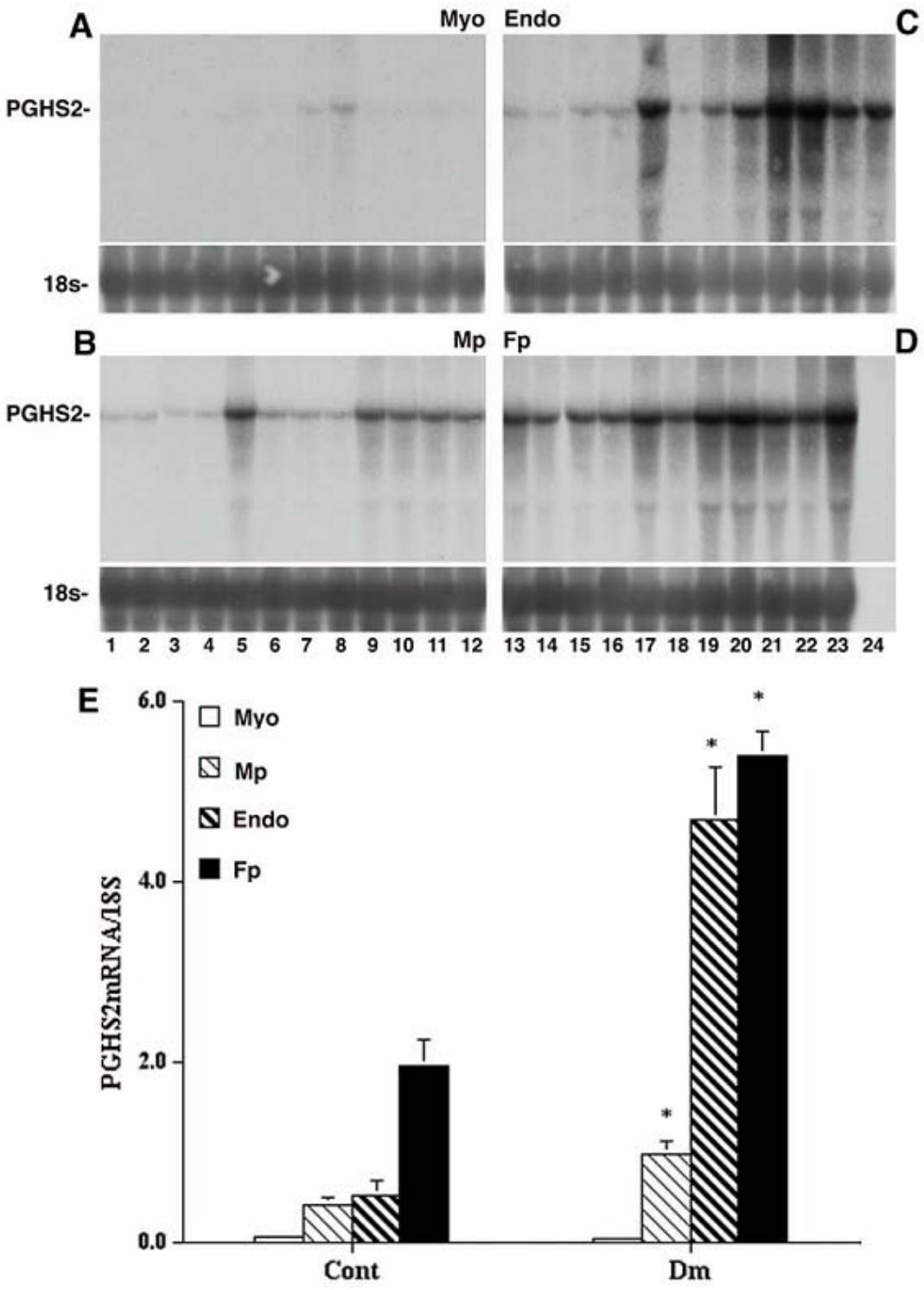

FIGURE 1. NORTHERN BLOT ANALYSIS OF PGHS-2 MRNA IN THE MYOMETRIUM (MYO, A), MATERNAL PLACENTA (MP, B), ENDOMETRIUM (ENDO, C) AND FETAL PLACENTA (FP, D) COLLECTED FROM CONTROLS (CONT, LANES 1-6 AND 13-18), DEXAMETHASONE (DM) TREATED PREGNANT SHEEP (LANES 7-12 AND 19-24 OR 19-23 FOR D. Note that 18S denotes the 18S-rRNA in each corresponding lane. E. Densitometric analysis of Myo, Endo, Mp and Fp PGHS-2 mRNA to 18S ratio in Cont and Dm treated groups (N = 6 in each group; N = 5 for Fp). PGHS-2 mRNA increased significantly in Endo, Mp and Fp in Dm group, but not in Myo. (* $P < 0.05$ compared with Cont). Data are presented as MEAN ± S.E.M.

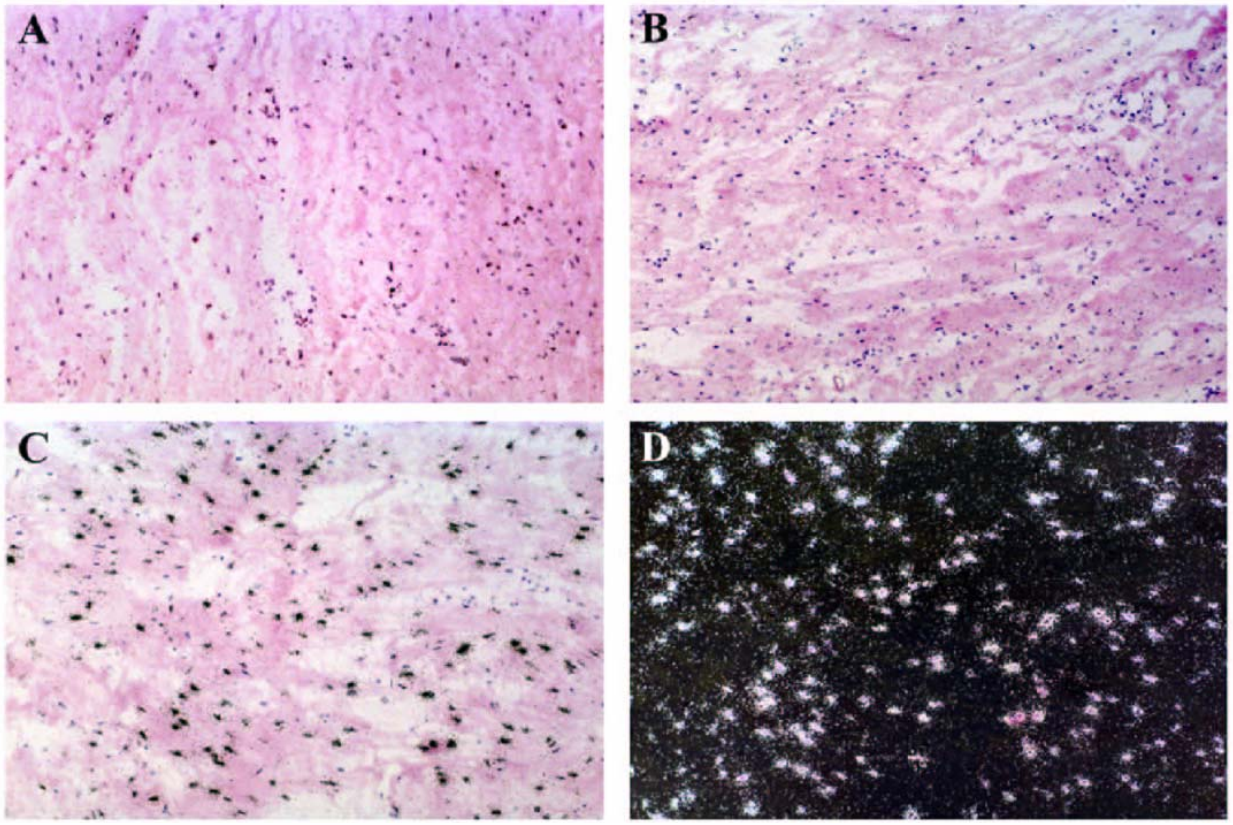

FIGURE 2. IN SITU HYBRIDIZATION ANALYSIS OF PGHS-2 MRNA IN THE MYOMERIUM FROM CONTROL (A), DEXMETHASONE-TREATED (B), SPONTANEOUS TERM LABOR (C-BRIGHT FIELD AND D-DARK FIELD) ANIMALS. Note: Black grains are not readily apparent in A and B, indicating the absence of specific localization of PGHS-2 antisense probe to the myometrium. In contrast, abundant black grains are associated with the myometrial cells in C and D, indicating an induction of PGHS-2 mRNA in the myometrium during labor.

animals (FIG. 3). The hybridization signals for PGHS2 mRNA were absent when PGHS-2 antisense probe was replaced by sense probe (data not shown).

3.2. PGHS-2 PROTEIN

To test whether the mRNA changes were carried to the protein level, Western blot analysis was employed to evaluate PGHS-2 enzyme protein level in ovine fetal and maternal placenta, endometrium and myometrium. As expected, the change in PGHS-2 enzyme protein paralleled the changes in PGHS-2 mRNA observed by Northern blot analysis (FIG. 1). PGHS-2 protein significantly increased in the fetal and maternal placenta and endometrium (FIG. 4 AND 5). PGHS 2 protein was not detectable in the myometrium (FIG. 4).

4. DISCUSSION

We present the first in vivo evidence that maternal administration of glucocorticoid at a dose inadequate to induce labor resulted in increased PGHS-2 expression in the endometrium, maternal and fetal placenta, but not myometrium. Our current study is an extension of our previous study [11]. The previous study utilized fetal adrenalectomy to isolate the effect of fetal cortisol from other fetal adrenal steroids. In contrast, our current study maintained the fetal hypothalamic-pituitary-adrenal axis intact thereby retaining the components of the fetal-placental unit. In both situations glucocorticoid at a dose inadequate to induce onset of labor produced tissue-specific PGHS-2 induction in the endometrium and placental tissues. Secondly, our current study delivered exogenous glucocorticoid by

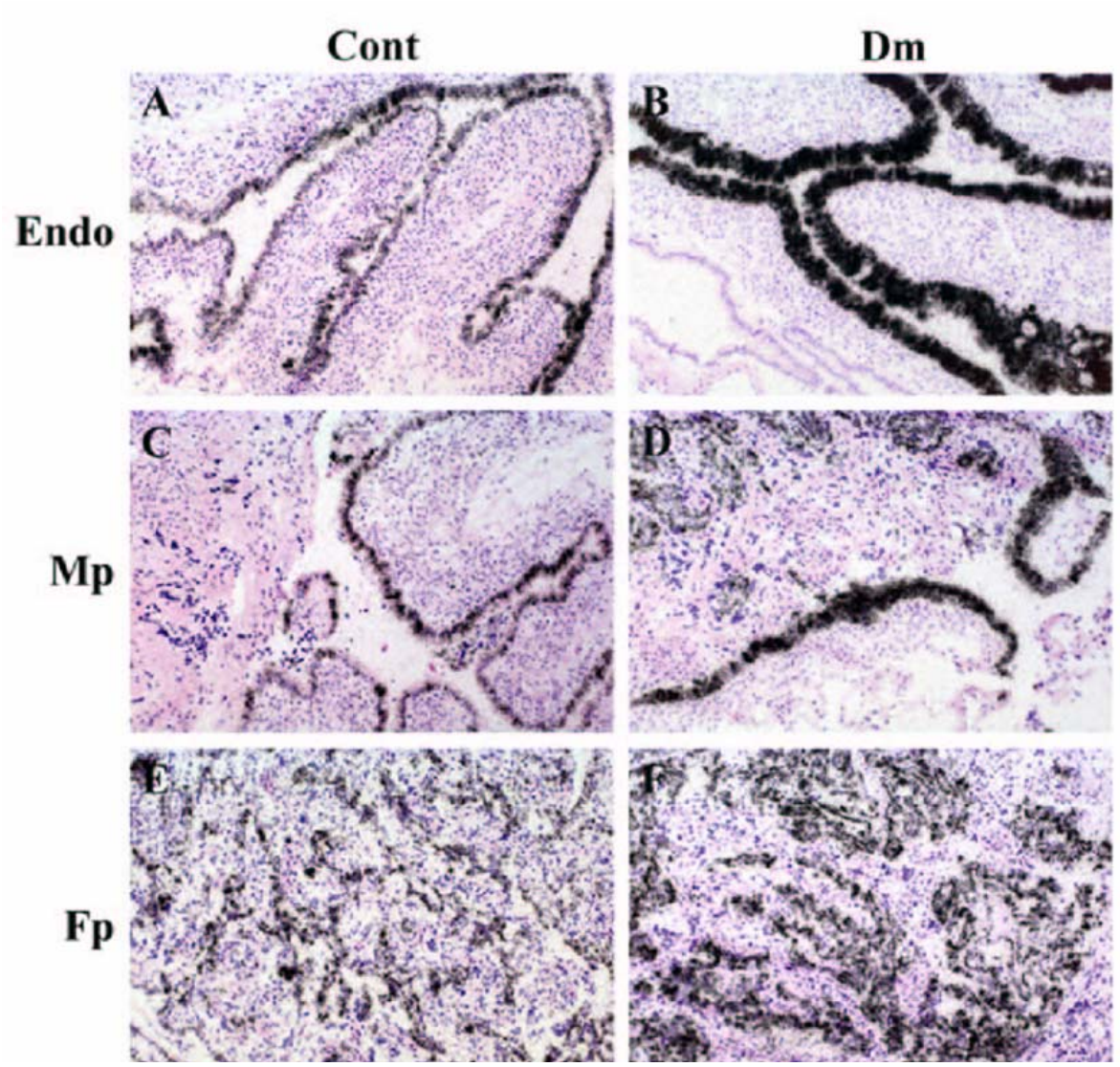

FIGURE 3. IN SITU HYBRIDIZATION ANALYSIS OF PGHS-2 MRNA IN THE ENDOMETRIUM (ENDO), MATERNAL PLACENTA (MP) AND FETAL PLACENTA (FP) COLLECTED FROM CONTROL (CONT, A, C, E) AND DEXAMETHASONE TREATED ANIMALS (DM, B, D, F). Note: the black grains formed by specific hybridization of PGHS-2 antisense probes with PGHS-2 mRNA were more abundant in Endo, Mp and Fp collected from Dm-treated animals.

maternal administration, whereas the previous studies by others and us have administered glucocorticoid directly to the fetus [9,11]. Not only does the experimental design we use more closely mimic the clinical situation, but also maternal administration is essential to differentiate gluco-corticoid effects in maternal and fetal tissues. The observations reported here are the first evidence to show that maternally administered glucocorticoid is able to induce PGHS-2 expression in maternal tissues in a distinct tissue-specific manner. Endometrium, but not the myometrium showed a response to glucocorticoid which supports the part of our hypothesis that attributes a direct effect to cortisol, namely that circulating cortisol at late gestation levels induced placental and endometrial PGHS-2, while in contrast labor-associated increase of estradiol is the cause for myometrial PGHS-2 induction as we reported previously [6,7]. These two studies support each other by providing similar conclusions from different approaches toward understanding cortisol regulation of PGHS-2 associated with two separate stages of pregnancy--late gestation and during labor in pregnant sheep.

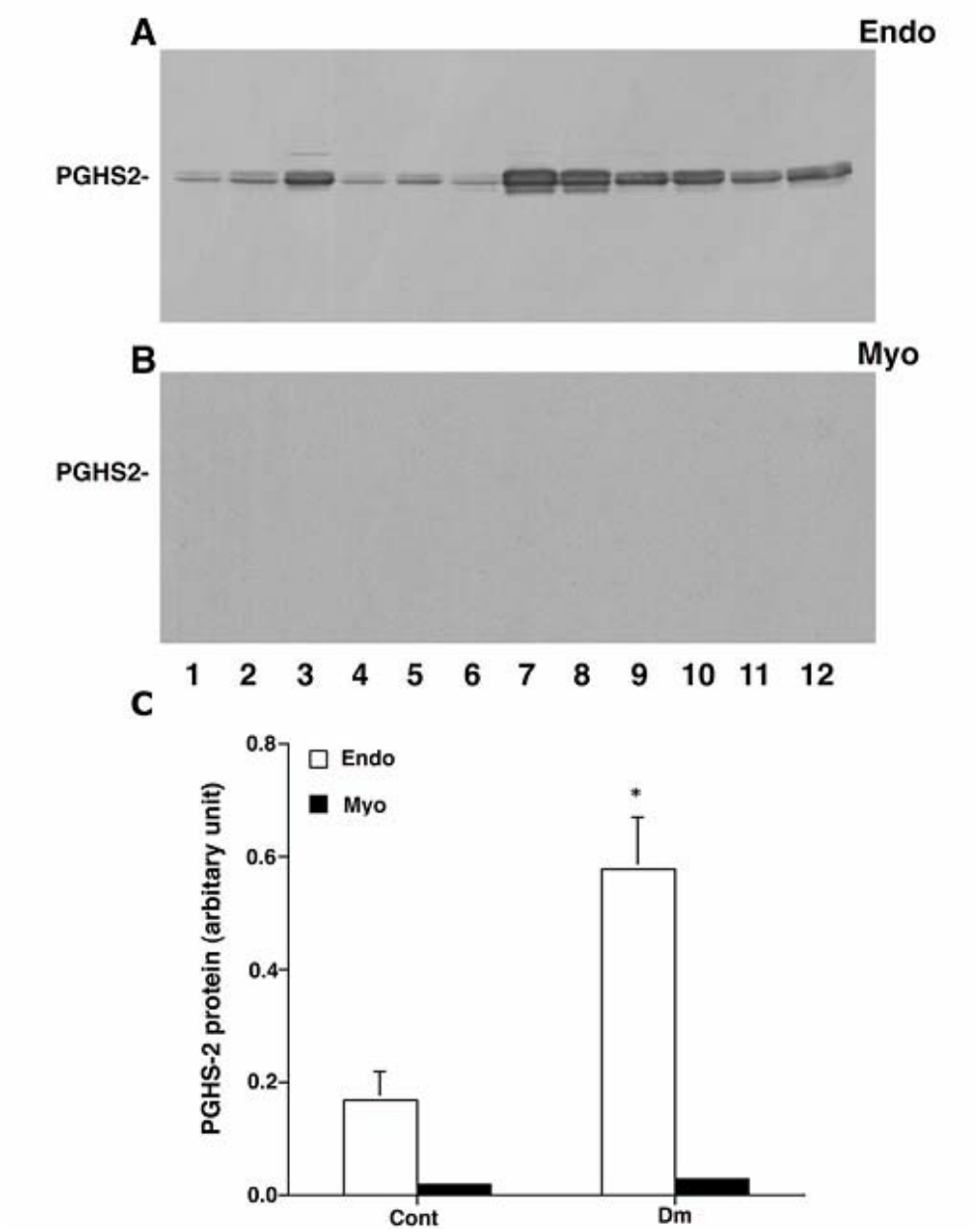

FIGURE 4. WESTERN BLOT ANALYSIS OF PGHS-2 PROTEIN IN ENDOMETRIUM (ENDO, A) AND MYOMETRIUM (MYO, B) COLLECTED FROM CONTROL (CONT, LANES 1-6) AND DEXAMETHASONE TREATED EWES (DM, LANES 7-12). (C) Densitometric analysis of Endo and Myo PGHS-2 protein in control and Dm-treated ewes (N = 6 in each group). PGHS-2 increased significantly in Endo in the Dm group but was not detectable in Myo in either control or Dm-treated animals. * $P < 0.05$ compared with the control ewes. Data are presented as MEAN ± S.E.M.

This is additional in vivo evidence that these lower levels of glucocorticoid infusion with or without intact fetal hypothalamic-pituitary-adrenal axis through either fetal or maternal administration, resulted in increased PGHS-2 expression in the endometrium and placenta prior to labor.

Because of the key role of PGHS in the biosynthetic pathway of PGs and because of the rapid catalytic inactivation of PGHS, this enzyme is a logical control point and a rate-limiting step in the formation of PGs. Two isoforms of PGHS exist, however considerable evidence indicates that

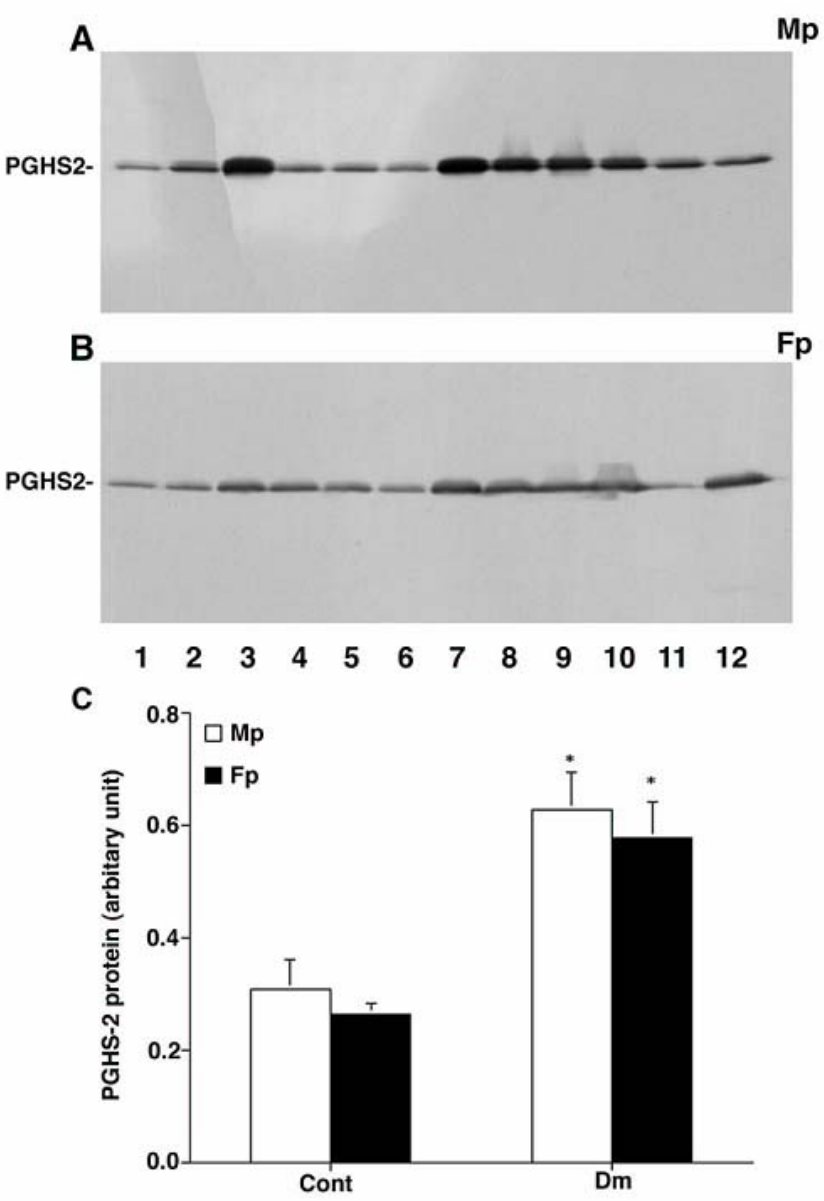

FIGURE 5. WESTERN BLOT ANALYSIS OF PGHS-2 PROTEIN IN MATERNAL PLACENTA (MP, A) AND FETAL PLACENTA (FP, B) COLLECTED FROM CONTROLS (CONT, LANES 1-6) AND DEXAMETHASONE TREATED EWES (DM, LANES 7-12). C. Densitometric analysis of Mp and Fp PGHS-2 protein in Cont and Dm-treated groups (N = 6 in each group). PGHS-2 increased significantly in Mp and Fp in Dm animals. * $P < 0.05$ compared with control ewes. Data are presented as MEAN ± S.E.M.

PGHS-2 is the inducible form in intrauterine tissues responsible for increased production of PGs in late gestation and during labor [3,8,10,11,20]. For this reason we have concentrated on PGHS-2 in the current study.

PGs are produced throughout pregnancy, however PGs, particularly PGE2, starts to increase in late gestation well before the onset of labor in pregnant sheep [13] and pregnant women [14,21]. Over the decades most studies have focused on the labor-associated increase of PGs in intrauterine tissues, but it is poorly understood as to the gestation-associated increase of PGs. Previous studies [12,13] have shown that PGE_2 in fetal plasma was increased throughout late gestation in sheep, and this increase may be an important signal

for the development and maturation of fetal hypothalamic-pituitary-adrenal axis [12,13,22] and hence a variety of critical fetal organs.

In addition to fetal placenta, the endometrium is also involved in increased PGHS-2 expression during late gestation in pregnant sheep [6]. We hypothesize that this gestation related increase in PG production in endometrium is involved in uterine remodeling and cervical ripening in preparation for parturition. The gestation-associated increases in PG production capability by maternal tissues has also been observed in the pregnant baboon [23] and the pregnant women [21]. In late gestation decidua and cervix showed gradual increase of PGHS-2 in the pregnant baboon [23]. In pregnant women PGE_2 production in amniotic fluid and decidua increased throughout late gestation [21,24]. Therefore increased PG production in late gestation is a common feature shared cross species.

We and others have produced extensive data to support an effect of glucocorticoid on regulation of PGHS-2 in intrauterine tissues [9,11,15,17]. Whether these glucocorticoid effects are a direct or secondary effect mediated through other regulatory factors in fetal placental unit merits further study. Glucocorticoid receptors have been localized in the intrauterine tissues [25,26]. It is very likely that glucocorticoids from either the fetus or the mother, acting through the glucocorticoid receptor present in the intrauterine tissues, are involved in regulating gestation-associated increase of PG production from intrauterine tissues. However, our early studies using non-pregnant sheep showed that progesterone treatment increased intrauterine PG synthetic activity and was a prerequisite for the additional stimulating effect of estradiol [7]. In addition, fetal plasma PGE2 [12,13] and intrauterine PGHS-2 [6] start to increase around 90 dGA. This early rise of the intrauterine PG system parallels with the increased progesterone concentration in maternal plasma [27] and is well before fetal plasma cortisol's rise (125 dGA) [28]. This early increase in the PG system can not be attributed to fetal cortisol. Therefore, we must not exclude the role of progesterone in the regulation of the PG system in maternal uterine tissues, particularly before 125 dGA.

In previous studies we [29] and others [30,31] have consistently demonstrated induction of PGHS-2-associated with glucocorticoid induced premature labor and spontaneous term labor in pregnant sheep. In pregnant sheep estradiol is low throughout gestation and only increases prior to labor. Therefore we have hypothesized that estradiol is responsible for the labor-associated increase in PG production from myometrium and other intrauterine tissues. We have conducted a series of studies to test our hypothesis. Firstly we tested our hypothesis using nonpregnant ovariectomized sheep with replacement of estradiol alone or plus progesterone. In nonpregnant sheep we found that estradiol either alone or in combination with progesterone up-regulated myometrial PGHS-2 expression and progesterone did not antagonize estradiol's positive effect on PGHS-2 expression in myometrium or endometrium [7]. It is important to evaluate how far our observations in nonpregnant sheep can be extended to pregnant sheep. During pregnancy, the abundance of PGHS-2 in placenta and endometrium increases gradually during the last third of gestation well before the rise in plasma estrogen that occurs in the final hours of gestation [32]. In contrast, myometrial PGHS-2 remains low throughout late gestation and can not be induced by maternal administration of glucocorticoids if labor is not present (FIG 2). Recently we examined the direct effect of exogenously administered estradiol at a dose resulting in fetal delivery in pregnant sheep at 121 dGA and producing estradiol concentrations in maternal plasma equivalent to that observed during labor. Estradiol stimulated myometrial and endometrial, but not placental PGHS-2 expression [8]. These observations provide firm evidence to support our hypothesis that the gestation-associated increase of PG production is differentially regulated from labor-associated increase of PG. Glucocorticoid is the possible candidate for regulating the gestation-associated rise of PG production in endometrium and placenta particularly after 125 dGA when fetal hypothalamatic-pituitary-adrenal axis is activated, and estradiol is more likely to participate in control of the labor-associated rise in intrauterine PG production, particularly in the myometrium.

There are still some disputes to resolve regarding hormonal control of tissue-specific PG production in late gestation and at labor, for example Whittle et al. determined changes in placental and uterine PGHS-2 expression in the sheep model in which labor is

induced by the administration of cortisol directly to the fetus while at the same time they attenuated estradiol production by maternal infusion of the aromatase inhibitor 4-hydroxyandrostendione. Their data indicate that PG production in trophoblast tissue is under the control of fetal cortisol, whereas PG production in the maternal uterine tissues is regulated by an estrogen-dependent pathway [9]. However, we do observe a rise in endometrial PGHS-2 expression in late gestation well before the onset of labor and while estradiol concentration is still low. Our current study provides evidence for a possible direct stimulation of glucocorticoid on endometrial PGHS-2 expression. In order to examine exogenous glucocorticoid's regulatory effect on intrauterine PGHS-2 expression, we conducted our study at a time in gestation prior to the fetal cortisol rise which occurred around 125 dGA [28,33]. To further confirm whether the effect of glucocorticoid on PGHS-2 expression in maternal tissues is unrelated to labor, we intentionally used dexamethasone at levels inadequate to induce labor in order to maintain the basal level of estrogen concentration in maternal plasma after dexamethasone treatment. Dexamethasone at a dose inadequate to induce labor was able to induce PGHS-2 expression in three of four intrauterine tissues studied, most importantly in the fetal placenta.

In conclusion, the significant stimulation of PGSH-2 in endometrium and placenta, but not in myometrium, by maternal administration of dexamethasone provides further evidence for a tissue-specific direct action of glucocorticoids on intrauterine PGHS-2 in pregnant sheep.

REFERENCES

[1] SMITH WL AND MARNETT LJ [1991] Prostaglandin endoperoxide synthase: structure and catalysis. Biochem Biophys Acta 16: 1-17.

[2] WILLIAMS CS AND DUBOIS RN [1996] Prostaglandin endoperoxide synthase: why two isoforms? Am J Physiol 270: G393-G400.

[3] CHALLIS JRG AND LYE SJ [1984] Parturition. In: KNOBILL E AND NEILL JD (eds). The Physiology of Reproduction, 2nd edition. Raven Press, New York, pp. 985-1031.

[4] LIGGINS GC, FAIRCLOUGH RJ, GRIEVES SA, KENDALL JZ AND KNOX SJ [1973] The mechanism of initiation of parturition in the ewe. Recent Prog Horm Res 29: 111-114.

[5] OLSON DM, ZAKER T AND MITCHELL BF [1993] Prostaglandin synthesis regulation by intrauterine tissues. In: RICE GE AND BRENNECKE SP (eds). Molecular Aspects of Placental and Fetal Membrane Autacoids. CRC Press, Boca Raton, Ann Arbor, pp. 55-59.

[6] WU WX, MA XH AND NATHANIELSZ PW [1999] Tissue-specific ontogenic expression of prostaglandin H synthase 2 in the ovine myometrium, endometrium, and placenta during late gestation and at spontaneous term labor. Am J Obstet Gynecol 181: 1512-1519.

[7] WU WX, MA XH, ZHANG Q, OWINY JR AND NATHANIELSZ PW [1997] Regulation of prostaglandin endoperoxide synthase 1 and 2 by estradiol in non-pregnant ovine myometrium and endometrium (in vivo). Endocrinology 138: 4005-4012.

[8] WU WX, THE LATE MA XH, COKSAYGAN T, CHAKRABARTY K, COLLINS V, ROSE J AND NATHANIELSZ PW [2004] Prostaglandin mediates premature delivery in pregnant sheep induced by estradiol at 121 days of gestational age. Endocrinology 154: 1444-1452.

[9] WHITTLE WL, HOLLOWAY AC, LYE SJ, GIBB W AND CHALLIS JRG [2000] Prostaglandin production at the onset of ovine parturition is regulated by both estrogen-independent and estrogen-dependent pathways. Endocrinology 141: 3783-3791.

[10] MYATT L AND MOORE SD [1994] Myometrium and preterm labor: Steroid and prostaglandins. Seminars in Reprod Endocri 12: 298-313.

[11] WU WX, MA XH, UNNO N AND NATHANIELSZ PW [2001] In vivo evidence for stimulation of placental, myometrial, and endometrial prostaglandin G/H synthase 2 by fetal cortisol replacement after fetal adrenalectomy. Endocrinology 142: 3857-3864.

[12] THORBURN GD AND RICE GE [1990] Placental PGE_2 and the initiation of parturition in sheep. In: MITCHELL MD (editor), Eicosanoids in Reproduction. CRC press, Boca Raton, pp. 73-86.

[13] THORBURN GD [1991] The placenta, prostaglandins and parturition: a review. Reprod Fertil Dev 3: 277-294.

[14] OLSON DM, LYE SJ, SKINNER K AND CHALLIS JRG [1985] Prostanoid concentrations in maternal/fetal plasma and amniotic fluid and intrauterine tissue prostanoid output in relation to myometrial contractility during the onset of adrenocorticotropin-induced preterm labor in sheep. Endocrinology 116: 389-397.

[15] ZAKAR T, HIRST JJ, MIJOVIC JE AND OLSON DM [1995] Glucocorticoids stimulate the expression of prostaglandin endoperoxide H synthase-2 in amnion cells. Endocrinology 136: 1610-1619.

[16] WHITTLE WL, PATEL FA AND CHALLIS JRG [1999] Local regulation of prostaglandin production by 11β-hydroxysteroid dehydrogenase type 1 in the human fetal membrane at term. 81st annual meeting, San Diego, pp. 3-463.

[17] SUN K, MA R, CUI X, CAMPOS B, WEBSTER R, BROCKMAN D AND MYATT L [2003] Glucocorticoids induce cytosolic phospholipase A2 and prostaglandin H synthase type 2 but not microsomal prostaglandin E synthase (PGES) and cytosolic PGES expression in cultured primary human amnion cells. J Clin Endocrinol Metab 88: 5564-5571.

[18] GYOMOREY S, LYE SJ, GIBB W AND CHALLIS JRG [2000] Fetal-to-maternal progression of prostaglandin H_2 synthase-2 expression in ovine intrauterine tissues during the course of labor. Biol Reprod 62: 797-805.

[19] UNNO N, WU WX, DING XY, LI C, HING WKHMA AND NATHANIELSZ PW [1998] The effects of fetal adrenalectomy at 110 days gestational age on AVP and CRH mRNA expression in the hypothalamic paraventricular nucleus of the ovine fetus. Dev Brain Res 106: 119-128.

[20] OLSON DM [2003] The role of prostaglandin in the initiation of parturition. Best Pract Res Clin Obstet Gynaecol 17: 717-730.

[21] MACDONALD PC, KOGA S AND CASEY ML [1991] Decidual activation in parturition: Examination of amniotic fluid for mediator of the inflammatory response. Ann N Y Acad Sci 622: 315-330.

[22] YOUNG IR, DEAYTON JM, HOLLINGWORH SA AND THORBURN GD [1996] Continuous intrafetal infusion of prostaglandin E_2 prematurely activates the hypothalamo-pituitary adrenal axis and induces parturition in sheep. Endocrinology 37: 2424-2431.

[23] WU WX, MA XH AND NATHANIELSZ PW [2000] A new concept of the significance of regional distribution of prostaglandin H synthase 2 throughout the uterus during late pregnancy: Investigation in a baboon model. Am J Obstet Gynecol 183: 1287-1295.

[24] SALMON JA AND AMY JJ [1973] Levels of prostaglandin $F_{2\alpha}$ in amniotic fluid during pregnancy and labor. Prostaglandins 4: 523-528.

[25] WU WX, DERKS JB AND NATHANIELSZ PW [1996] Effects of glucocorticoids on estrogen receptor messenger ribonucleic acid in the pregnant ovine myometrium in vivo and in vitro. Biol Reprod 54: 230-241.

[26] LAGESON JM, SPELSBERG TC AND COULAM CB [1983] Glucocorticoid receptor in human placenta: studies of concentration and functional differences of preterm and term tissues. Am J Obstet Gynecol 145: 515-523.

[27] ARTHUR GH, NOAKES DE AND PEARSON H [1996] Pregnancy and its Diagnosis. In: Arthur GH, Noakes DE, Pearson H and Parkinson TJ**.** Veterinary Reproduction and Obstetrics. Sixth Edition, W.B. Saunders Company Ltd, London, 63-109.

[28] CHALLIS JRG AND BROOKS AN [1989] Maturation and activation of hypothalamic-pituitary-adrenal function in fetal sheep. Endocr Rev 10: 182-204.

[29] ZHANG Q, WU WX, BRENNA JT AND NATHANIELSZ PW [1996] The expression of cytosolic phospholipase A_2 and prostaglandin endoperoxide synthase in ovine maternal uterine and fetal tissues during late gestation and labor. Endocrinology 137: 4010-4017.

[30] RICE GE, PAYNE MJ, WONG MH AND THORBURN GD [1992] Immunoreactive prostaglandin G/H synthase content increase in cotyledons during late gestation. Placenta 13: 429-437.

[31] RICE GE, FREED KA, AITKEN MA AND JACOBS RA [1995] Gestational- and labour-associated changes in the relative abundance of prostaglandin G/H synthase-1 and 2 mRNA in ovine placenta. J Mol Endocrinol 14: 2237-2245.

[32] CHALLIS JRG [1971] Sharp increase in free circulating oestrogen in immediately before parturition in sheep. Nature 229: 208.

[33] MAGYAR DM, FRIDSHAL F, ELSNER CW, GLAZ T, ELIOT J, KLEIN AH, LOWE KC, BUSTER JE AND NATHANIELSZ PW [1980] Time-trend analysis of plasma cortisol concentration in the fetal sheep in relation to parturition. Endocrinology 107: 155-159.

RECEIVED ON 11-23-2004.
ACCEPTED ON 12-24-2004.