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Prostaglandin E_2 Receptor Expression in Fetal Baboon Lung at 0.7 Gestation After Betamethasone Exposure

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ABSTRACT: The fetal lung produces and metabolizes prostaglandin (PG) E_2. *In vitro* PGE_2 induces surfactant production *via* E prostaglandin $(EP)_1$ and cyclic adenosine monophosphate (cAMP)–coupled EP (EP_2 and EP_4) receptors. Glucocorticoids alter PG function and increase lung function in preterm neonates. We hypothesized that fetal exposure to maternally administered betamethasone (βM) enhances fetal lung EP_1 and cAMP-coupled EP receptor expression. Pregnant baboons were injected intramuscularly (i.m.) with either βM ($n = 7$) or saline [control (CTR); $n = 8$] at 0.7 gestation. Fetal lungs were removed at cesarean section 48 h after the first injection. We determined mRNA levels, protein localization and abundance for all four PGE_2 receptors by real-time polymerase chain reaction (PCR), immunohistochemistry, and Western blot. EP receptors were widely distributed in bronchiolar epithelium, bronchiolar smooth muscle, and endothelium and media of blood vessels, but not alveoli. Compared with CTR, βM exposure resulted in a twofold EP_2 mRNA decrease ($p < 0.05$) in male fetuses only. EP_1, EP_3, and EP_4 receptor mRNA levels were unaffected. Western blot analysis showed no alteration in EP receptor protein expression. In summary, this is the first demonstration of the four EP receptors in fetal lung. The only change after 48-h βM exposure was a gender-specific decrease in EP_2 receptor mRNA. (***Pediatr Res* 61: 421–426, 2007**)

βM is widely administrated to pregnant women at risk of preterm delivery because it has been demonstrated that glucocorticoids reduce the risk of respiratory distress syndrome, intraventricular hemorrhage, and perinatal death in preterm newborns (1). The beneficial effects of antenatal glucocorticoids on postnatal lung function of preterm newborns are attributed to the ability of βM to induce fetal lung maturation, mostly by increasing the synthesis of surfactant components. Indeed, one single course of βM at 123 d of gestation in sheep enhances surfactant-associated protein (SP)-A, SP-B, and SP-C mRNA levels in lung tissue (2) and SP-B (3) and saturated phosphatidylcholine (4) concentrations in lavage fluid 48 h after maternal treatment.

PGE_2 is produced (5,6) and metabolized in the fetal lung (6,7). PGE_2 tranduces its signal *via* seven-transmembrane domain, G protein–coupled receptors, called EP receptors (8). The EP receptor family has been further classified into four subtypes, EP_1, EP_2, EP_3, and EP_4 (9), differing in their structure, ligand-binding affinities, and signal transduction pathways (8). EP_1 and EP_3 receptors, linked to the Ca^{2+}/phospholipase C pathway, cause smooth muscle contraction (8), whereas EP_2 and EP_4 receptors, coupled to the cAMP/adenylate cyclase pathway, induce relaxation of smooth muscle (8). In the lung, PGE_2 functions as a bronchiolar tone modulator. EP_1 and EP_3 receptors mediate bronchoconstriction indirectly through activation of neuronal pathways (10), whereas bronchodilatation results from direct activation of EP_2 receptors on airway smooth muscles (10–12). Furthermore, PGE_2 induces SP-A synthesis by human fetal lung explants *via* the cAMP pathway (13) and phosphatidylcholine secretion *via* EP_1 receptor in rat cultured alveolar type II cells (14).

The first aim of this study was to evaluate the presence and determine cellular localization of EP receptor expression in the fetal baboon lung. Glucocorticoids have been shown to alter PG synthesis and metabolism (15,16); however, no data exist relating to potential alterations in PG receptor–mediated function. To address potential effects of glucocorticoid on PG receptors, we hypothesized that βM enhances EP_1 and cAMP-coupled EP (*i.e.* EP_2 and EP_4) receptor expression in the fetal lung. Randomized trials in pregnant women have demonstrated that the major benefit of tocolytic therapy is a 48-h prolongation of the pregnancy (17,18), which provides just enough time for protective measures, *i.e.* transfer to a tertiary neonatal care center and induction of fetal lung maturation by corticosteroid. Therefore, we examined the effects of 48-h exposure to βM administered to pregnant baboons at 0.7

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Abbreviations: βM, betamethasone; **Ct,** threshold cycle; **CTR,** control; **EP,** E prostaglandin; **MUC5AC,** mucin 5 subtype AC; **PG,** prostaglandin; **SP,** surfactant-associated protein

gestation on fetal lung EP receptor mRNA levels and protein expressions.

MATERIALS AND METHODS

Animals and tissue collection. All procedures were approved by the Cornell University Animal Use and Care Committee where the studies were conducted. Fifteen pregnant baboons (*Papio cynocephalus*) of 12.1 ± 0.4 kg body weight [mean ± standard error of the mean (SEM)] were maintained for at least 6 wk in individual cages in sight of at least one other animal in rooms with controlled light/dark cycles (14 h light/10 h dark) and had free access to water and food (Teklad 25% protein primate diet 2055, Harlan, Madison, WI). Animals interacted at least twice a day with the same caregiver and were provided with fresh fruits and vegetables. Four injections of 87.5 $\mu g \cdot kg^{-1}$ body weight βM (celestone phosphate; Schering, Kenilworth, NJ; $n = 7$) or of the equivalent volume of saline ($n = 8$) were administered i.m. 12 h apart beginning at 134 ± 1 days gestational age (dGA) (mean ± SEM; 0.73 of gestation equivalent to 28 wk of gestation in humans). This dose of βM (175 $\mu g \cdot d^{-1}$) is equivalent to the clinically used dose regimen (12 $mg \cdot day^{-1}$) over two consecutive days when weight adjusted to a 70-kg pregnant woman. Forty-eight hours after the first injection, animals were premedicated with 7.5 $\mu g \cdot kg^{-1}$ buprenorphine (Reckitt & Coleman, Richmond, VA) and 12.5 $\mu g \cdot kg^{-1}$ glycopyrrolate (Robinul, Baxter, Deerfield, IL) i.m. Ketamine (10–15 $mg \cdot kg^{-1}$, KetaFlo, Abbott, North Chicago, IL) was administered i.m., and general anesthesia was initiated with halothane and maintained with 1.5% halothane (Halothane, Halocarbon, River Edge, NJ) in 1–2 $L \cdot min^{-1}$ O_2. Fetuses were exteriorized from the uterus at cesarean section and euthanized by exsanguination while still connected to the placenta and under halothane general anesthesia. Fetal lungs were immediately removed, rinsed in saline, and either frozen in liquid nitrogen or fixed in 10% formalin. Mothers recovered from surgery in individual cages and were treated with 15 $\mu g \cdot kg^{-1} \cdot d^{-1}$ buprenorphine in two equal i.m. injections for 3 d and given antibiotic treatment with clavamox orally, 30 $mg \cdot kg^{-1} \cdot d^{-1}$, in equally divided doses twice daily in peanut butter for 5 d. After recovery in individual cages, the mothers were returned to the social context of group cages.

Immunohistochemistry. Sections 5 μm thick were cut from the paraffin-embedded tissues, mounted on ProbeOn Plus microscope slide (Fisher Scientific, Pittsburgh, PA), deparaffinized in xylene, and rehydrated in a graded ethanol series. Immunohistolocalization of EP receptor proteins was performed using Vectastain Elite ABC kit according to the manufacturer's protocols (Vector Laboratories, Burlingame, CA). Endogenous peroxidase activity was removed by immersing sections in 0.3% hydrogen peroxide in methanol. Antigen was retrieved by microwaving at full power for 10 min in DAKO target retrieval solution (pH 6.20) (DAKO Corporation, Carpinteria, CA). All sections were blocked 30 min with 10% normal goat serum in phosphate-buffered saline (PBS) and then incubated for 1 h at room temperature with the primary rabbit polyclonal anti-human EP receptor antibodies (Cayman Chemicals, Ann Arbor, MI, catalog nos. 101740, 101750, 101760, 101775) and used at 1:250 in DAKO antibody diluent with background reducing components (DAKO Corporation). Sections were washed in PBS and then further incubated with the second antibody (biotinylated goat anti-rabbit IgG; Vector Laboratories) diluted 1:200 in 2% goat serum in PBS for 30 min at room temperature. Antigens were localized using 3,3′-diaminobenzidine in PBS for 8 min. Finally, tissues were counterstained with hematoxylin and mounted. Negative controls included slides incubated without the primary antibody and sections incubated with preimmune rabbit serum instead of primary antibody.

Tissue sample preparation for protein and total RNA. Approximately 0.1 g of lung tissue was homogenized in 1 mL of RIPA buffer [50 mmol/L Tris-HCl, pH 7.4, NP40 1%, NaCl 150 mmol/L, sodium dodecylsulfate (SDS) 0.1%, sodium deoxycholate 0.25%] supplemented with a cocktail of protease inhibitors. Samples were centrifuged at 12,000 × *g* for 5 min to pellet cellular debris. Protein extracts were quantified using a modified Bradford technique with BSA as a standard. To obtain total RNA, 0.1 g of lung tissue was homogenized in Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA) and extracted per manufacturer's directions. Briefly, total RNA preparations were recovered by phenol chloroform extraction, isopropanol precipitation, and ethanol washing. RNA concentrations and purities were determined by measuring absorbance at 260 and 280 nm.

Reverse transcription and real-time PCR. Five micrograms of total RNA were reverse transcribed using 100 ng of random primers in the presence of 50 units of Superscript II reverse transcriptase (Invitrogen Life Technologies). Fifty nanograms of cDNA were then subjected to real-time PCR in 25 μL of iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA) containing 250 nmol/L of specific primers designed for human EP receptors (Table 1) performed with the iCycler (Bio-Rad Laboratories). Primers were designed to amplify a sequence corresponding to the fourth and fifth transmembrane domains for EP_2, to the first and second transmembrane domains for EP_4, and to the sixth and seventh transmembrane domains for EP_1 and EP_3 receptors. Primer sequences for EP_3 receptor were common to all splice variants. Conditions of real-time PCR in regard to annealing temperature (Table 1) were optimized by performing gradient PCR for each set of primers. A three-step run protocol was used: (i) denaturation (95°C for 5 min), (ii) amplification and elongation repeated 40 times (95°C 10 s, annealing temperature for 30 s, 72°C for 30 s), (iii) melting curves (55°–95°C with a heating rate of 0.5°C every 10 s and a continuous fluorescence measurement). 18S rRNA was used as a nonregulated reference gene using commercially available universal primers (Ambion, Austin, TX). For mucin 5 subtype AC (MUC5AC) (gene bank access number AJ001402), total RNA (50 ng) was reverse transcribed in a 100 μL reaction using a High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). A negative control reverse-transcription reaction with reverse transcriptase but no RNA was included. Complementary DNA synthesis was followed by real-time-PCR using MUC5AC gene specific primers provided by the manufacturer (Applied Biosystems), TaqMan Universal PCR master mix (Applied Biosystems), and the target cDNA performed with the ABI Prism 7900 Sequence Detection System (Applied Biosystems). A three-step run protocol was used: (i) denaturation (95°C for 10 min), amplification and elongation repeated 40 times (95°C for 15 s, annealing and extension 60°C for 1 min), (iii) melting curves (55°–95°C with a heating rate of 0.5°C every 10 s and a continuous fluorescence measurement). Samples were analyzed in triplicate. Specificity of the desired PCR products was documented with high-resolution gel electrophoresis and by melting curve analysis. The product-specific melting curves showed only single peaks and no primer-dimer peaks or artifacts. Negative controls including *Taq* polymerase and primers but lacking cDNA were included for each primer pair. Real-time PCR products were size fractionated on agarose gel to ensure that the correct size product was amplified. These samples resulted in a difference of at least eight cycles (<0.4% contamination) of the threshold cycle (Ct) (cycle number at which the fluorescence increases appreciably above the background fluorescence) compared with the reverse transcriptase–containing samples. For relative quantification of gene expression, the comparative Ct method was used (described in User Bulletin 2 for ABI PRISM 7700 Sequence Detection System). Using this approach, the endogenous control (18S rRNA) Ct values were subtracted from the genes of interest Ct values to derive a ΔCt value. The relative expression of the genes of interest was then evaluated using the expression $2^{-\Delta\Delta Ct}$ where the value for $\Delta\Delta$Ct was obtained by subtracting the ΔCt of the calibrator from each ΔCt using the lowest ΔCt value for each gene as a calibrator. Results are expressed as fold change relative to the calibrator and presented as mean ± SEM of

Table 1. *Primer sequences used in real-time PCR experiments*

Receptors (gene bank access number)	Sequences (5′-3′) and positions	Product size	Annealing temperature (°C)
EP_1 (L22647)	F 974-CGG TAT CAT GGT GGT GTC R 1172-CAA GAG GCG AAG CAG CAG TTG	199	55
EP_2 (U19487)	F 814-CTC AAC CTC ATC CGC ATG R1137-ACG CAT TAG TCT CAG AAC AG	324	62
EP_3 (X83857)	F 1078-CTG TCG GTC TGC TGG TCT C R1365-GTC GCT CCA CAT CAA GGT TG	288	58
EP_4 (NM_000958)	F 421-TGA GAA GCC GAA GAT TTG R 768-CAT ACC AGC GTG TAG AAG	348	55

F, forward; R, reverse.

three to eight animals. Data were compared using a two-tailed Student *t* test; $p < 0.05$ was considered significant.

Western blot analysis. Western blots were run under denaturing and reducing conditions. Protein extracts (30 μg) were dissolved vol/vol in 2× Tris glycine SDS sample buffer (126 mmol/L Tris-HCl, 20% glycerol, 4% SDS, 0.005% bromophenol blue, pH 6.8) (Invitrogen Life Technologies) supplemented with 10 mmol/L dithiothrietol (DTT) and heated at 95°C for 5 min before analysis on a 7.5% SDS-polyacrylamide gel electrophoresis. After migration, proteins were transferred to a polyvinyldene fluoride (PVDF) membrane (Amersham, Piscataway, NJ). Membranes were blocked in 10% nonfat dried milk powder in Tris-buffered saline-Tween 20 (TBS-T) (Tris 10 mmol/L, NaCl 150 mmol/L, Tween-20 0.1%, pH 7.6) for 1 h. For immunodetection, blots were incubated with rabbit polyclonal antibodies against human EP receptors (Cayman Chemicals, Ann Arbor, MI) for 90 min at a dilution of 1:1000 in TBS-T containing 5% nonfat dried milk powder. After three washes in TBS-T, blots were incubated with horseradish peroxidase–linked goat anti-rabbit antibodies (Santa Cruz Biotechnologies, Santa Cruz, CA) for 45 min in TBS-T containing 5% nonfat dried milk powder at a dilution of 1:2000. Immunoreactive proteins were detected using a chemiluminescent detection system (Amersham ECL reagents; Amersham) per manufacturer's directions. Control experiments were performed by incubating the membranes with anti-human EP receptor antibodies preabsorbed on the related blocking peptides to which the antibodies were prepared (Cayman Chemicals, catalog nos. 301740, 301750, 301760, 301775). Quantification of the immunoreactive bands was performed using the software Un-Scan-It (Silk Scientific, Orem, UT). Data are presented as mean ± SEM. Differences in the means between the CTR and βM groups were assessed using the Mann-Whitney test. The difference was considered significant when $p < 0.05$.

RESULTS

Specificity of rabbit polyclonal anti-human EP receptor antibodies for fetal baboon lung. The specificity of the different anti-human polyclonal antibodies for all four baboon EP receptor proteins was assessed by Western blot analysis. As shown in Figure 1, distinct proteins of different apparent molecular masses were identified by the various rabbit anti-human EP receptor antibodies. We observed a protein band of approximately 67 kD by staining with anti-EP$_1$ antibody, a 68-kD band with the anti-EP$_2$ antibody, a 60-kD band with the anti-EP$_3$ antibody, and a 63-kD band with the anti-EP$_4$ antibody. Furthermore, preabsorbing the primary antibodies with the related blocking peptides abolished the 67-, 68-, 60-, and 63-kD signals.

Fetal baboon lung distribution of EP receptor proteins. Immunohistochemistry demonstrated the expression of all four EP receptor proteins in control fetal baboon lung (Fig. 2). The EP$_1$, EP$_2$, EP$_3$, and EP$_4$ receptor proteins were mainly expressed in the bronchiolar epithelium (Fig. 2*B–F*). EP$_2$ receptor was further localized in bronchiolar smooth muscle (Fig. 2*D*), whereas EP$_3$ receptor was also detected in both the endothelium and vascular smooth muscle of the blood vessels (Fig. 2*E*). Inconsistent light staining of the alveoli was observed in three of the CTR and two of the βM-treated animals. Absence of detectable immunoreactive protein when the primary antibody was replaced by preimmune rabbit serum confirmed the specificity of the staining in the different tissue compartments (Fig. 2*A*).

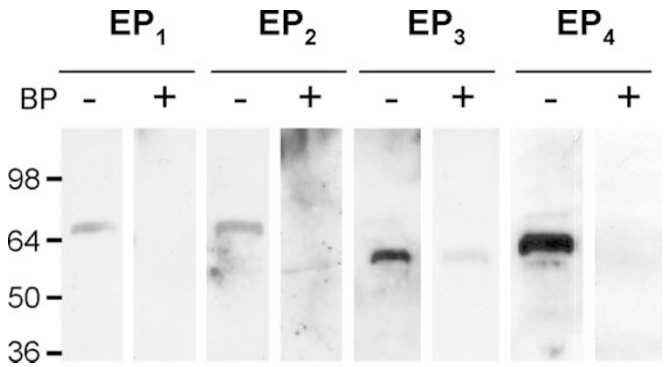

Figure 1. Specificity of rabbit polyclonal anti-human EP receptor antibodies in fetal baboon lung. EP receptor expression in fetal baboon lung was assayed by Western blot analysis which demonstrated only one single band. Preabsorption of the primary antibodies with the related blocking peptide (BP) abolished the specific signals. BP absent (−), BP present (+).

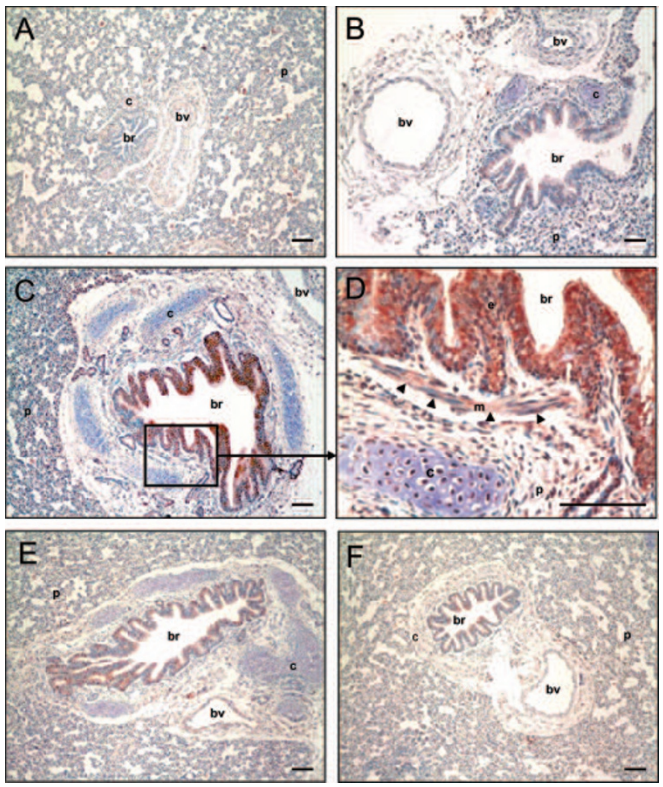

Figure 2. Fetal lung distribution of EP receptor proteins. EP$_1$ (*B*), EP$_2$ (*C*, *D*), EP$_3$ (*E*), and EP$_4$ (*F*) were localized in bronchiolar epithelium. EP$_2$ was further identified in bronchiolar smooth muscle (*D*, *arrowheads*), whereas EP$_3$ was also expressed in blood vessels (*E*). Replacement of primary antibodies by preimmune rabbit serum demonstrated the absence of specific staining (*A*). br, bronchi; bv, blood vessel; c, cartilage; e, epithelium; m, smooth muscle; p, parenchyma. Scale bar: 50 μm.

EP receptor and MUC5AC mRNA levels in fetal baboon lungs. As evidenced by real-time PCR, compared with CTR, βM induced a significant twofold decrease in EP$_2$ receptor mRNA levels. Other EP receptor mRNA levels were not affected by treatment (Fig. 3*A*). After correcting the analysis for gender, βM provoked a decrease in EP$_2$ receptor mRNA levels only in males (Fig. 3*B* and *C*).

To check for adequate amounts of bronchiolar tissue in both groups, we looked at mRNA levels of a large airway specific marker, MUC5AC. Real-time PCR evidenced no significant difference in levels of MUC5AC mRNA between CTR and βM groups (Fig. 4).

EP receptor protein expression in fetal baboon lungs. Western blot analysis failed to demonstrate any significant difference in EP receptor expression between the CTR and βM groups (Fig. 5), even after correction for gender (data not shown).

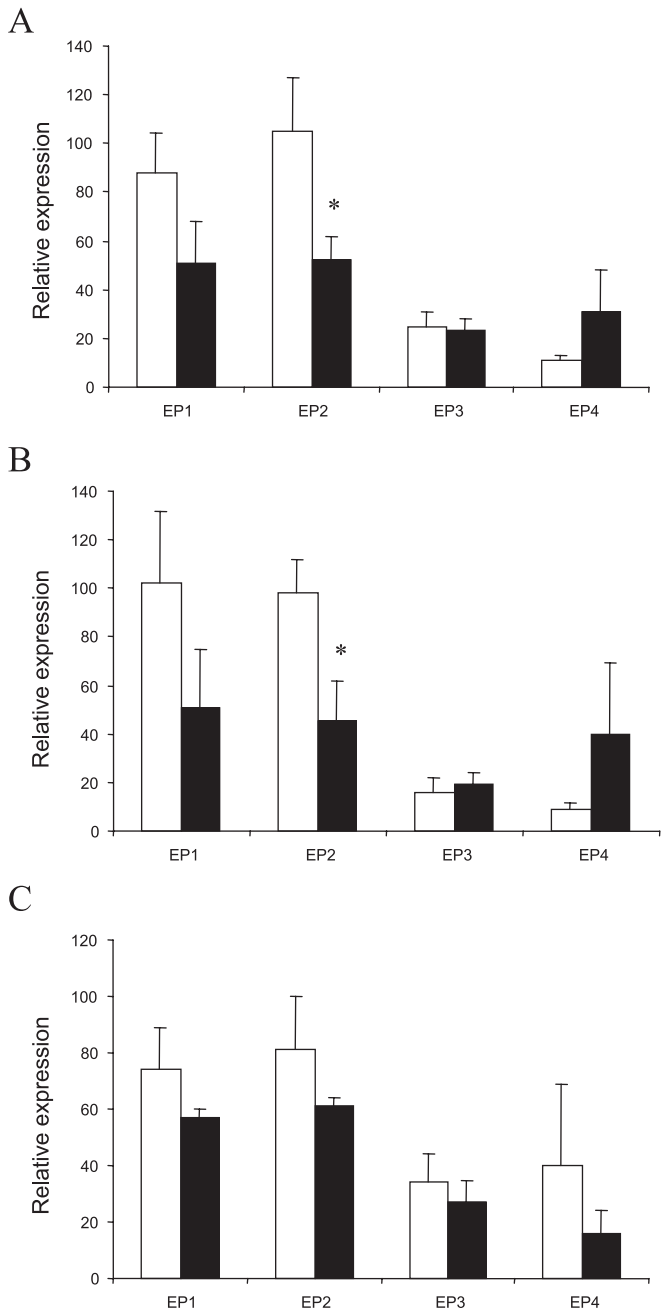

Figure 3. EP receptor mRNA levels in fetal baboon lung. EP receptor mRNA levels in fetal baboon lung [*A*: both sexes combined CTR ($n = 8$), βM ($n = 7$), *B*: males CTR ($n = 4$), βM ($n = 4$), *C*: females CTR ($n = 4$), βM ($n = 3$)] were assayed by real-time PCR. Data are expressed as fold change relative to the calibrator, presented as mean ± SEM, and compared using a two-tailed Student *t* test. $^*p < 0.05$ was considered significant. CTR (*open columns*), βM (*solid columns*).

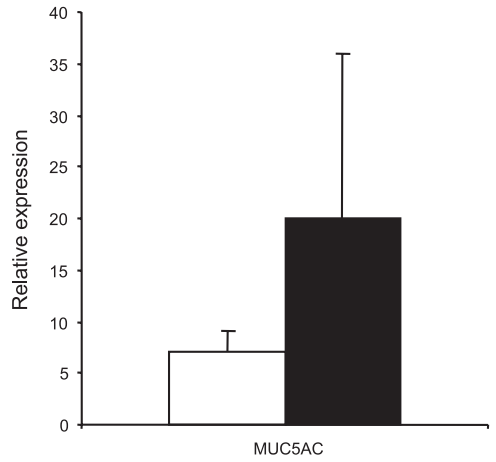

Figure 4. MUC5AC mRNA levels in fetal baboon lung (both sexes combined). MUC5AC mRNA levels in fetal baboon lung were assayed by real-time PCR. Data are expressed as fold change relative to the calibrator and presented as mean ± SEM (CTR, $n = 5$; βM, $n = 5$). The difference between the groups was not significant. CTR (*open column*), βM (*solid column*).

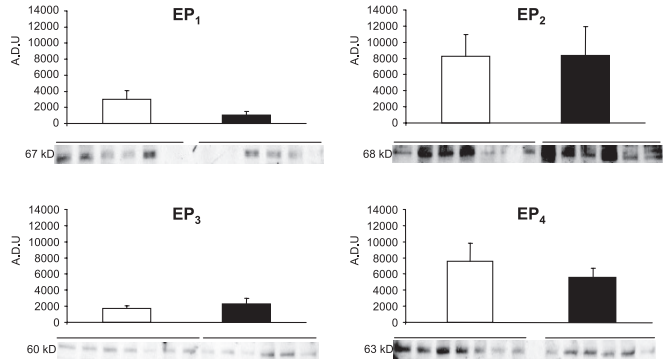

Figure 5. EP receptor protein expression in fetal baboon lungs (both sexes combined). EP receptor expression in fetal baboon lung was assayed by Western blot analysis. Data are presented as mean ± SEM [EP_1: CTR ($n = 6$), βM ($n = 6$); EP2: CTR ($n = 7$), βM $n = 6$; EP3: CTR $n = 7$, βM $n = 6$; EP4: CTR $n = 7$, βM ($n = 6$)]. Differences in the means between the CTR and βM groups were assessed using the Mann-Whitney test. There were no significant differences. A.D.U., arbitrary density unit. CTR (*open columns*), βM (*solid columns*).

DISCUSSION

Until recently, the lack of EP receptor antibodies has prevented any attempt to study the expression of EP receptors at the protein level in the lung of either adult or developing mammals. In the fetus, the expression of EP receptors in the lung has been investigated either by binding studies (19) that have demonstrated the functional presence of the receptors but not their cellular location, or by Northern mRNA analysis (20) that does not provide information either on cellular localization or protein expression. The recent development of EP receptor antibodies enables localization as well as examination of glucocorticoid regulation of EP receptor protein expression in the tissues that compose the fetal lung.

In this study, we first checked the specificity of the available rabbit anti-human EP receptor antibodies in baboon lung by performing Western blot analysis. We evidenced single bands for EP_1, EP_2, EP_3, and EP_4 receptor of 67, 68, 60, and 63 kD, respectively, in the molecular weight range already reported in human (21) and rat (22) tissues. The specificity of the immunoreactive detection was further confirmed by the absence of signal when the primary antibodies were preabsorbed with the antigen used to raise the antibody.

We demonstrated for the first time the presence of the four EP receptor proteins in the baboon fetal lung. Whereas the four EP receptors are preferentially localized in the bronchiolar epithelium, suggesting a major function for EP receptors in the interactions of the bronchi with the external environment,

EP_2 and EP_3 receptors are also expressed in bronchiolar smooth muscle and blood vessels, respectively. Although cyclooxygenase-2 is undetectable in fetal or newborn lung in sheep, cyclooxygenase-1 has been localized in the lung of the fetal lamb in endothelial cells and in airway epithelium (5). Our results are therefore in accordance with the existing literature emphasizing a possible paracrine role of EP receptors in the control of bronchial and vascular tone (10–12). Although previous pharmacological studies (13,14) have demonstrated *in vitro* the ability of PGE_2 to induce synthesis of surfactant components *via* EP_1 and cAMP-coupled EP (EP_2 and/or EP_4) receptor by alveolar type II cells, suggesting the presence of functional EP receptors in this cell type, there is no literature reporting EP receptor localization in the pneumocyte *in vivo* by immunohistochemistry in either fetal or adult lung. The lack of consistent immunoreactive alveolar EP receptors in most of the animals in our study may reflect the fact that the tissue collections were performed too early in gestation, at a gestational age at which EP receptors might not be yet detectable by immunohistochemistry in this cell type. Alternatively, there may be differences in paracrine and other effects between *in vivo* and *in vitro* conditions. Finally, the absence of alveolar EP receptors does not support a major role, if any, for PGE_2 in the normal process of surfactant protein production at this gestational age as we first hypothesized, based on the results of *in vitro* studies (13,14).

Unexpectedly, 48-h βM exposure resulted in a gender-specific decrease in EP_2 receptor mRNA. This effect could be species and/or tissue dependent because glucocorticoids have no effect on EP_2 receptor expression in both ovine fetal kidney and ductus arteriosus (23,24) at 0.75 gestation. Other sex-dependent effects of prenatal glucocorticoid exposure have previously been reported in rat and guinea pig. Increased fetal exposure to glucocorticoids induces elevated blood pressure (25,26) and alters fetal development of the hypothalamopituitary-adrenal axis (27) in male offspring. These alterations are associated with time- and gender-related changes in both mineralocorticoid and glucocorticoid receptor expression in the hippocampus and the pituitary (25,28,29) indicating time- and sex-dependent effects of prenatal glucocorticoid administration.

The decreased levels of EP_2 receptor mRNA, observed only in males, was not detected at the protein level. Although discrepancies in mRNA and protein changes might result from unstable mRNA, default in translation or increased protein degradation (30), a more likely explanation for the lack of decreased EP_2 receptor protein is that the lung tissue collection was performed too early after the last βM injection. The surfactant system has been shown to require $\geq$4 d to demonstrate protein induction by βM (4,31). Therefore, analysis of tissues for EP receptor expression at a greater time interval may show changes in protein. Moreover, because EP receptors are mainly expressed in bronchial tissue, we checked that the decreased EP_2 receptor mRNA levels after βM exposure were very unlikely due to inadequate amount of bronchial tissue in the samples used for Western blot analysis because real-time PCR demonstrated in both groups similar levels of MUC5AC, a large airway specific marker.

The EP_2 receptor is responsible for PGE_2-induced bronchodilatation (10–12). Therefore, decreased EP_2 receptor expression after βM exposure could decrease PGE_2 ability to provoke bronchodilatation. Interestingly, EP_2 receptor has also been demonstrated to mediate PGE_2-induced inhibition of the differentiation of fibroblasts into myofibroblasts that occurs during tissue repair and lung fibrosis (32). Furthermore, EP_2 receptor is responsible for PGE_2-induced decreased expression of type I collagen mRNA in human embryo lung fibroblasts (33) and in organotypic cultures of mixed fetal rat lung cells (34). This role for EP_2 receptor in the control of lung fibrosis has been further demonstrated in $EP_2^{-/-}$ mice that developed exaggerated fibrotic response to bleomycin compared with wild-type CTRs (35). Thus, βM, *via* its effect on the PGE_2 system, *i.e.* the decreased expression of EP_2 receptor mRNA levels, could favor lung fibrosis by reducing the inhibitory effects that PGE_2 normally exerts *via* EP_2 receptors in this process.

In conclusion, this study localizes EP receptor in the fetal lung for the first time. EP receptors appear to be widely distributed within the fetal lung tissues and predominantly expressed in the bronchiolar epithelium and smooth muscle. βM exposure decreased EP_2 receptor mRNA levels only in male fetuses.

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