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Progesterone Antagonizes Dexamethasone-Regulated Surfactant Proteins In Vitro

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Abstract

Pregnant women at risk of preterm labor routinely receive glucocorticoids (GCs) and frequently also progesterone. Administration of GCs accelerates intrauterine surfactant synthesis and lung maturation, thereby reducing the incidence of neonatal respiratory distress syndrome; progesterone has the potential to prevent preterm birth. Little is known about possible interactions of GCs and progesterone. Our aim was to clarify whether progesterone can affect dexamethasone (DXM)-regulated expression of surfactant protein A (SP-A), SP-B, and SP-D in lung epithelial cells. H441 cells were exposed to DXM and progesterone and expression of SPs was analyzed by quantitative real-time polymerase chain reaction and immunoblotting. Although progesterone had no direct effect on the expression of SP-B, DXM-mediated induction was inhibited dose dependently on the transcriptional (64 μM [$P < .0001$], 32 μM [$P = .0005$], 16 μM [$P = .0019$]) and the translational level. Furthermore, progesterone inhibited stimulatory effects of other GCs as well. While exogenous tissue growth factor β1 (TGF-β1) inhibited DXM-induced SP-B expression (messenger RNA [mRNA]: $P = .0014$), progesterone itself did not influence TGF-β1 mRNA expression and/or TGF-β1/Smad signaling, demonstrating that TGF-β1 and/or Smad activation is not involved. The inhibitory effect of progesterone could be imitated by the GC and progesterone receptor (PR) antagonist RU-486, but not by the specific PR antagonist PF-02413873, indicating that progesterone acts as a competitive antagonist of DXM. The effect of progesterone on DXM-regulated genes was not specific for SP-B, as expression of SP-A and SP-D mRNAs was also antagonized. The present study highlights a new action of progesterone as a potential physiological inhibitor of GC-dependent SP expression in lung epithelial cells. The clinical relevance of this in vitro finding is currently unknown.

Keywords

RDS, BPD, TGF-β, SP-B, progesterone receptor

Background

Preterm birth before 37 weeks' gestation is the most common cause of death to neonates, with estimates ranging from 5% in European countries to 18% in African countries.[1] To prevent preterm labor or to improve its outcome, pregnant women may receive a variety of medications daily,[2,3] such as steroids like betamethasone (BTM) or dexamethasone (DXM), which have been shown to decrease neonatal morbidity and mortality.[4-6] Especially the administration of prenatal BTM or DXM induces the immature pulmonary surfactant system of preterm infants, thereby reducing frequency and intensity of respiratory distress syndrome (RDS).[6]

Administration of maternal progesterone has been also demonstrated to be beneficial in reducing primary and recurrent preterm birth.[2,7] Progesterone is a steroid hormone like BTM and DXM and naturally important in maintaining pregnancy,[2] and a decline of progesterone levels at the end of the pregnancy is involved in the onset of labor.[2]

Different formulations of progesterone are used to prevent spontaneous preterm birth. Intramuscular hydroxyprogesterone caproate is already approved for prevention of prematurity in singleton pregnancies in patients with prior spontaneous preterm birth.[7] Vaginal progesterone has demonstrated similar efficacy in women with asymptomatic cervical shortening in the midtrimester.[8] On the basis of these benefits, progesterone is implemented to be used in other clinical situations at risk for preterm birth, but with less scientific evidence.[7]

Like DXM and BTM, progesterone also reduces morbidity and mortality of preterm infants, lowers the incidence of RDS, and reduces the need for mechanical ventilation and intensive care unit admissions.[7] Furthermore, replacement of

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progesterone in preterm infants to maintain high intrauterine levels of progesterone has been shown to be associated with tendency toward a reduced incidence of bronchopulmonary dysplasia.[9,10]

Until now, little is known about possible positive or negative interactions of progesterone and BTM/DXM in the pregnant women and/or the infant. Especially, the effect on the pulmonary surfactant system in preterm infants after a simultaneous application of DXM/BTM and progesterone is undefined.

Pulmonary surfactant lines the inner epithelium of the alveoli and consists of 90% lipids and 10% proteins. It stabilizes the surface of the air–blood barrier, reduces surface tension, and thereby improves gas exchange.[11] Although other surfactant-associated proteins are postulated,[12] the 4 main surfactant proteins (SPs) are designated SP-A, SP-B, SP-C, and SP-D,[13] of which only the hydrophilic proteins SP-A and SP-D have immunological properties and are secreted independently from the remaining components of surfactant.[13] The major role of the most important and essential SP, SP-B, is to promote the formation and stability of the surfactant monolayer.[11] Surfactant protein B is mainly synthesized in alveolar type II and club cells, the main producers of surfactant.[14] Surfactant protein B expression is altered in different lung diseases, especially in RDS of preterm infants, thereby contributing to pathogenesis.[11,15] The identification of neonates with refractory RDS due to genetic absence of SP-B highlights its importance for the functionality of the surfactant film and the maintenance of lung compliance.[15] Synthesis of pulmonary surfactant, essential for lung maturation, starts between 22 and 28 weeks of gestation. Treatment with surfactant, including SP-B, has substantially increased survival rates of preterm infants in the last decades.[16] For this reason, identification of the substances that modulate SP-B expression (positively or negatively) remains an important area of investigation.[11] Dexamethasone is described as a known inducer of SP-B in lung epithelial cells,[17] whereas tissue growth factor β1 (TGF-β1) is known as an inhibitor of SP-B expression in lung epithelial cells mediated by activation of the Smad signaling pathway.[18-20] In addition, TGF-β1 and DXM are able to induce connective TGF, which is involved in airway remodeling.[21-23] In the present study, we investigated whether the endogenous female sex steroidal hormone progesterone independently or in combination with the glucocorticoid (GC) DXM is able to affect SP-B expression in lung epithelial cells.

Methods

Reagents

Dexamethasone, BTM, hydrocortisone, budesonide, progesterone, and mifepristone (RU-486) were purchased from Sigma-Aldrich (St Louis, Missouri). The progesterone antagonist PF-02413873 was kindly provided by Pfizer (New York City, New York).[24] Recombinant TGF-β1 was obtained from R&D Systems (Thermo Fisher Scientific, Waltham, Massachusetts).

Cells

Airway epithelial cells NCI-H441 (H441),[25] a human lung adenocarcinoma cell line with characteristics of bronchiolar club epithelial cells, were purchased from ATCC (LGC Standards, Teddington, United Kingdom). H441 cells were cultured in RPMI 1640 (Sigma-Aldrich) with additional 5% fetal bovine serum (Thermo Fisher Scientific), 100 U/mL penicillin, and 100 μg/mL streptomycin (Sigma-Aldrich). Experiments with TGF-β1 were performed in serum-free medium. Incubation was carried out at 37°C in a humidified atmosphere with 5% CO_2.

Cell Viability Assay

H441 cell viability after exposure to progesterone (32 μM), TGF-β1 (10 ng/mL), DXM (1 μM), RU-486 (10 μM), and/or PF-02413873 (150 nM) was evaluated after 1 to 3 days using methylthiazolyldiphenyl-tetrazolium bromide (MTT). Cells were seeded in 6-well plates (Greiner, Frickenhausen, Germany) and treated as described. After washing with Dulbecco phosphate-buffered saline (DPBS; Sigma-Aldrich), 1 mL DPBS containing 1.2 mM MTT was administered to the cells, and the plates were incubated at 37°C for 30 minutes. The MTT medium was removed, 500 μL isopropanol was added to the wells, and plates were gently rocked for 5 minutes at room temperature to dissolve purple formazan. Optical density was measured in triplicates in 96-well plates (Greiner) with a MR 5000 microplate reader (Dynatech, Santa Monica, California) at 550 nm. Untreated cells were considered 100% viable and used as reference. No toxic effects could be found for progesterone (32 μM), TGF-β1 (10 ng/mL), DXM (1 μM), RU-486 (10 μM), and/or PF-02413873 (150 nM; data not shown).

Transfection and Promoter Assays

The CAGA elements were originally found in the promoter region of plasminogen activator inhibitor 1 and are known to be activated after binding of the Smad3/4 complexes and after TGF-β1 binding to the TGF-β receptor.[26] $(CAGA)_{12}$-luc (2 μg) and Renilla luciferase control reporter vector (phRL-TK; 5 ng) were transfected into H441 cells, seeded in 6-well plates, using 6 μg of linear polyethylenimine (molecular weight 25 000; Polysciences Inc, Warrington, Pennsylvania). Transfection medium (Opti-MEM; Thermo Fisher Scientific) was changed to Opti-MEM with additional 0.2% fetal bovine serum after 2 hours. Twenty-four hours after transfection, cells were treated as indicated. Sixteen hours later, luciferase activity was measured by the dual luciferase assay system (Promega Biotech Inc, Madison, Wisconsin) according to the manufacturer's instruction using a Berthold MiniLumat LB 9506 luminometer (Bad Wildbach, Germany). Firefly luciferase activity was normalized to the activity of Renilla luciferase under control of the thymidine kinase promoter of phRL-TK. Results are given as relative increase compared to controls. All values were obtained from experiments carried out in triplicates and

repeated at least 3 times. The error bars indicate standard deviation (SD).

RNA Extraction and Real-Time Polymerase Chain Reaction

For RNA extraction, 3×10^5 H441 cells were seeded on 6-well plates (Greiner) and grown at 37°C. Cells were washed with DPBS and treated as indicated. After the appropriate time, cells were washed again, and total RNA was isolated using NucleoSpin RNA Kit (Macherey-Nagel, Dueren, Germany) according to the manufacturer's protocol. Total RNA was eluted in 60 µL nuclease-free H_2O (Sigma-Aldrich) and stored at −80°C until reverse transcription. For real-time PCR (RT-PCR), 1 µg of total RNA was reverse transcribed using high capacity complementary DNA (cDNA) reverse transcription kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Upon analysis by quantitative real-time PCR (qPCR), first-strand cDNA was stored at −20°C.

Quantitative Real-Time PCR

For detection of human SP-A, SP-D, TGF-β1, and GAPDH messenger RNA [mRNA], cDNA was analyzed using 12.5 µL iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, California), 0.5 µL deionized H_2O, and 10 pmol of each forward and reverse primer, respectively. Primers for detection of SP-A, SP-D, TGF-β1, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA were hSP-Afwd 5′-GAGAAATGCCATGTCCTCCT-3′, hSP-Arev 5′-TAGATGAGCTGGAAGCCCTG-3′, hSP-Dfwd 5′-GGCTTCCAGATGTTGCTTCTC-3′, hSP-Drev 5′-CTGTGCCTCCGTAAATGGT-3′, hTGFβ1fwd 5′-AATTCCTGGCGATACCTC-3′, hTGFβ1rev 5′-TAGTGAACCCGTTGATGTC-3′, hGAPDHfwd 5′-CCATGGAGAAGGCTGGGG-3′, and hGAPDHrev 5′-CAAAGTTGTCATGGATGACC-3′, respectively. In case of SP-B and β-actin mRNA, probes (Thermo Fisher Scientific) were used as described.[27] Real-time PCR was performed on an ABI Prism 7500 Sequence Detection System (*Taq*Man) as described.[27] Melt curve analyses were performed to verify single PCR products for detection with SYBR Green. Results were normalized to those of β-actin or GAPDH and mean fold changes were calculated by the $\Delta\Delta C_T$ method.[28]

Western Blot Analysis

H441 cells were rinsed with ice-cold Tris-buffered saline and incubated in 100 µL lysis buffer consisting of cell lysis buffer (Cell Signaling Technologies, Danvers, Massachusetts), Complete Mini protease inhibitor cocktail tablets, and PhosStop phosphatase inhibitor cocktail tablets (Roche, Basel, Switzerland), and 0.1 mM phenylmethane sulfonyl fluoride (PMSF; Merck KGaA, Darmstadt, Germany) for 10 minutes on ice. The lysate was cleared by centrifugation at 30 000 *g* for 10 minutes, and the supernatant was used for Western immunoblotting analysis. Protein concentrations were determined using the Bradford assay (Bio-Rad, Richmond, California). Equal amounts of cellular protein were loaded and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 10% to 12% Bis-Tris gels and electrophoretically transferred to polyvinylidene difluoride blotting membranes (Amersham Pharmacia Biotech, Piscataway, New Jersey). Membranes were blocked in 5% bovine serum albumin for 1 hour at room temperature and successively incubated with primary antibodies overnight at 4°C. Blots were probed with primary antibodies to mature SP-B (WRAB-48604; Seven Hills Bioreagents, Cincinnati, Ohio) and β-actin (926-42212; LI-COR Inc, Lincoln, Nebraska), followed by staining with corresponding IRDye secondary antibodies (LI-COR Inc) for 1 hour at room temperature. Specific protein bands were visualized using an Odyssey Infrared Imaging System (LI-COR Inc). Accumulated signals were quantified using Image Studio Lite v5.0.21 (LI-COR Inc).

Data Analysis

Results are given as means (SD). Data were analyzed using 1-way analysis of variance with Sidak multiple comparisons test. A P value $\leq .05$ was considered significant. All statistical analyses were performed using Prism version 6 (GraphPad Software, San Diego, California).

Results

Effect of Progesterone on SP-B Expression in Lung Epithelial Cells

To study a possible direct effect of progesterone on the expression of SP-B mRNA in lung epithelial cells, we incubated H441 cells with different concentrations of progesterone (0.3 to 64 µM) for 12 hours (Figure 1A) and for different time intervals (6 to 48 hours; Figure 1B). Progesterone alone had only minor effects on SP-B mRNA expression (Figure 1A and B) and no effects on mature SP-B expression (Figure 2B).

Effect of Progesterone on DXM-Induced SP-B Expression in Lung Epithelial Cells

To examine the effect of progesterone on DXM-induced SP-B upregulation, we incubated H441 cells with different concentrations of progesterone in the presence of DXM (10 µM) for 12 hours (mRNA analysis) or 24 hours (protein analysis). Progesterone significantly diminished DXM-mediated, 14 (4)-fold ($P < .0001$), upregulation of SP-B mRNA at 64 µM ($P < .0001$), 32 µM ($P = .0005$), and 16 µM ($P = .0019$; Figure 2A). Inhibitory effect of progesterone on DXM-mediated upregulation of SP-B was also present on the protein level as shown by Western blot (Figure 2B). Induction of SP-B mRNA by various GCs (BTM, hydrocortisone, budesonide) was also significantly inhibited by progesterone (Figure 2C). These results indicate that progesterone is able to inhibit GC-induced SP-B expression in lung epithelial cells in a dose-dependent manner.

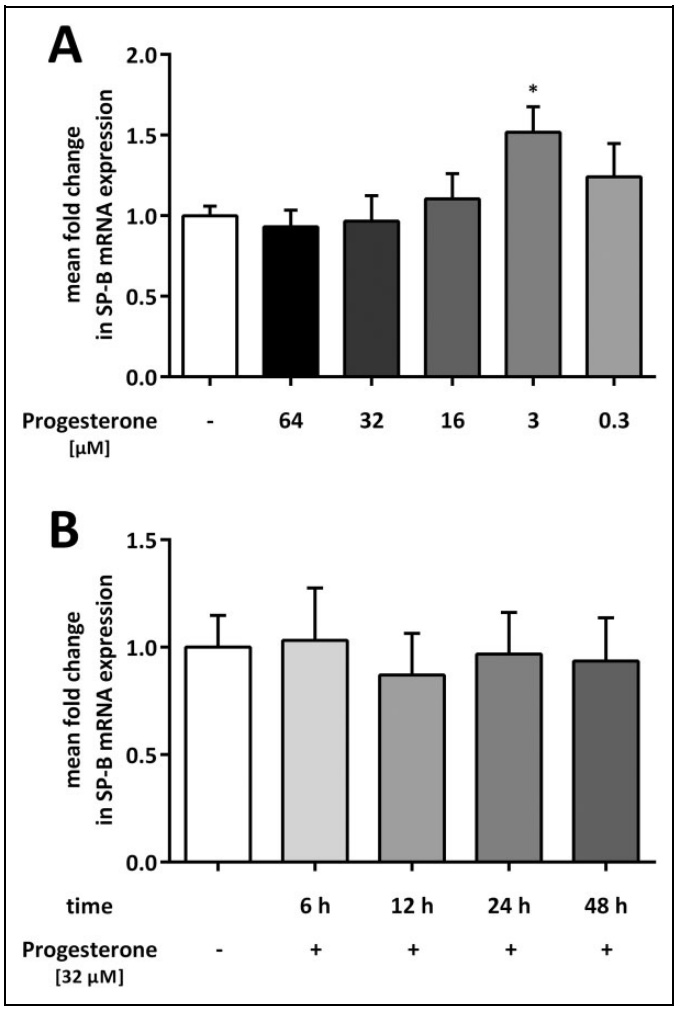

Figure 1. Effects of progesterone on SP-B mRNA expression in lung epithelial cells. H441 cells were incubated with different concentrations of progesterone (0.3 to 64 μM) for 12 hours (A) or for different time points (6 to 48 hours) with 32 μM progesterone (B). Real-time PCR of SP-B mRNA was performed after different time point as indicated. Relative mRNA levels of SP-B were calculated by normalizing signals to detected GAPDH mRNA. Differences compared to untreated cells were calculated. Means (SD) of at least n = 3 independent experiments are shown. *$P < .05$ compared to untreated cells. GAPDH indicates glyceraldehyde 3-phosphate dehydrogenase; mRNA, messenger RNA; PCR, polymerase chain reaction; SD, standard deviation; SP, surfactant protein.

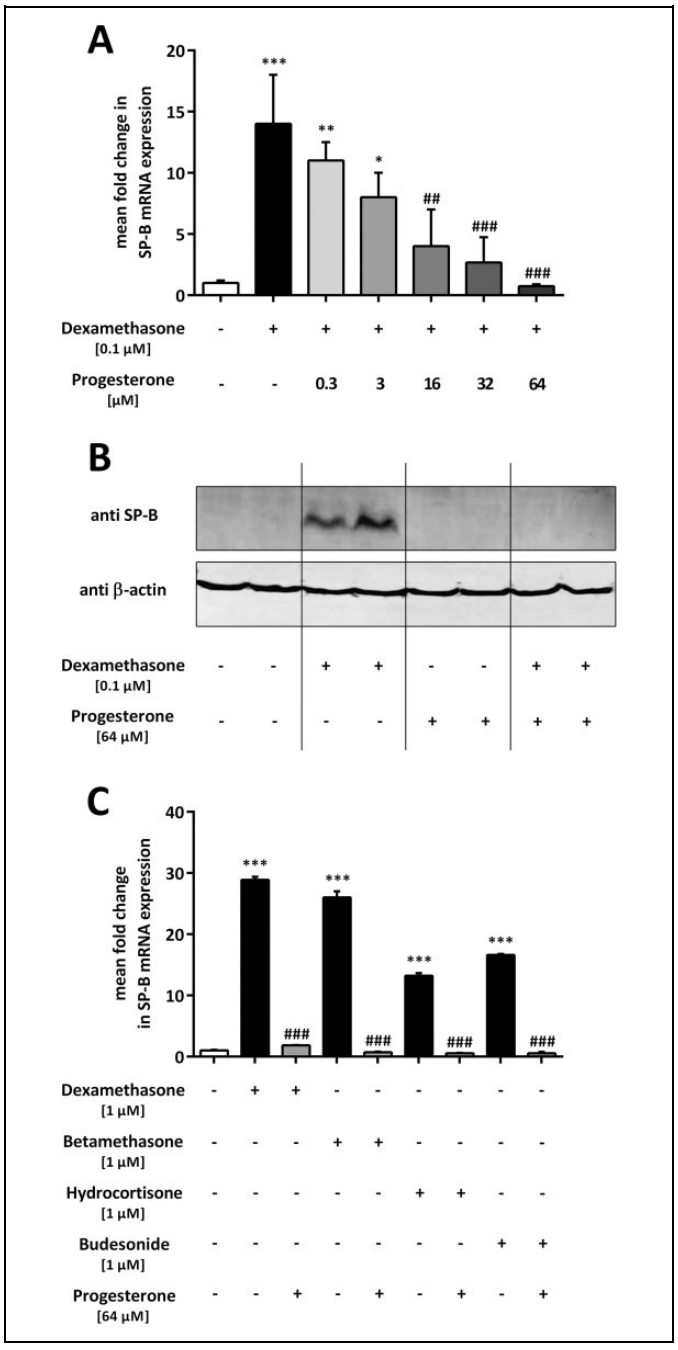

Figure 2. Progesterone inhibits DXM-induced SP-B expression in lung epithelial cells. H441 cells were incubated with DXM (10 μM) (A) or other various glucocorticoids (C) and with or without progesterone in different concentrations as indicated. Real-time PCR of SP-B mRNA was performed after 12 hours (A + C) and Western blot analysis after 24 hours (B). Relative mRNA levels of SP-B were calculated by normalizing signals to detected GAPDH mRNA. Differences compared to untreated cells were calculated. Means (SD) of at least n = 3 independent experiments are shown. B, Representative immunoblots against SP-B and β-actin of at least n = 3 independent experiments are shown. *$P < .05$, **$P < .01$, and ***$P < .001$ compared to control cells; ###$P < .01$ and ####$P < .001$ compared to cells treated with only DXM or another glucocorticoid. DXM indicates dexamethasone; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; mRNA, messenger RNA; PCR, polymerase chain reaction; SD, standard deviation; SP, surfactant protein.

Effect of TGF-β1 on DXM-Induced SP-B Expression in Lung Epithelial Cells

H441 cells were treated with DXM (10 μM) and TGF-β1 (10 ng/mL) or progesterone (32 μM). Surfactant protein B mRNA was measured after 12 hours and mature SP-B expression after 24 hours. Tissue growth factor β1 reduced DXM-induced upregulation of SP-B mRNA ($P = .0014$) similar to progesterone (Figure 3A). These results for SP-B mRNA expression could be confirmed on the protein level by Western blot analysis for mature SP-B (Figure 3B). These data demonstrate that TGF-β1 is able to inhibit DXM-induced SP-B expression.

Effect of Progesterone on TGF-β1 Expression and Smad Activation in Lung Epithelial Cells

To reveal if the inhibitory effect of progesterone on DXM-induced SP-B expression (Figure 2) is mediated by progesterone-induced TGF-β1 synthesis in H441 cells, we measured TGF-β1 mRNA levels in the presence of progesterone and/or DXM. We did not find a significant effect of progesterone and/or DXM on TGF-β1 mRNA synthesis in H441 cells (Figure 4A). To analyze a possible effect of progesterone on Smad-signaling in lung epithelial cells, a TGF-β1-sensitive $(CAGA)_{12}$-luciferase construct was transfected into H441 cells. Treatment with TGF-β1 induced a significant increase of reporter gene activity in transfected cells compared to that in untreated and transfected cells (12 [4]-fold, $P = .0034$), whereas progesterone (64 μM) had no effect on activation of Smad signaling (Figure 4B). These results demonstrate that the observed inhibitory effect of progesterone on DXM-induced SP-B expression is not mediated by progesterone-induced TGF-β1 mRNA expression or an activation of Smad signaling by TGF-β1.

Involvement of the GC Receptor in the Inhibitory Effect of Progesterone on DXM-Induced SP-B Expression

To investigate a possible involvement of the GC receptor in the observed inhibitory effect of progesterone on DXM-induced SP-B expression, we analyzed the additional presence of RU-486 and PF-02413873, both being progesterone receptor (PR) antagonists but only RU-486 antagonizing GCs at the receptor level.[24] While RU-486 was able to antagonize the effect of DXM on SP-B expression, PF-02413873 showed no effect and did not modify the inhibitory effect of progesterone (Figure 5). Hence, the inhibitory effect of progesterone on DXM-induced SP-B expression is not mediated by PRs, but by competition between progesterone and DXM for the GC receptor binding site.

Effect of Progesterone on Other DXM-Regulated Genes in Lung Epithelial Cells

To evaluate whether progesterone is able to influence other DXM-regulated SP genes in H441 cells, we analyzed the effect of progesterone and DXM on SP-A and SP-D mRNA levels. Dexamethasone led to a reduction of SP-A mRNA-levels (0.2 [0.04]-fold, $P < .0001$), as well an increase of SP-D mRNA levels (2.4 [0.2]-fold, $P < .0001$) in H441 cells. The DXM-regulated expression of these 2 genes was again antagonized by progesterone at the given concentration (Figure 6A and B). These data demonstrate that progesterone is also able to antagonize other DXM-regulated genes in lung epithelial cells.

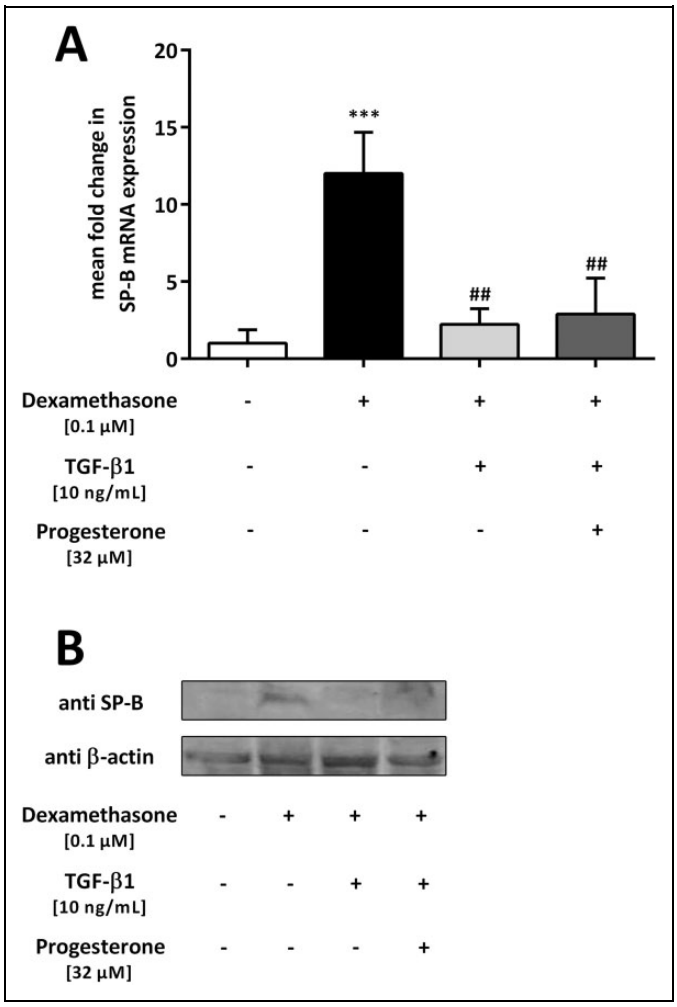

Figure 3. Tissue growth factor β1 inhibits DXM-induced SP-B expression in lung epithelial cells. H441 cells were incubated with DXM (10 μM) and TGF-β1 (10 ng/mL) or progesterone (32 μM) in combinations as indicated. Real-time PCR of SP-B mRNA was performed after 12 hours (A) and Western blot analysis against SP-B after 24 hours (B). Relative SP-B mRNA levels were calculated by normalizing signals to detected GAPDH mRNA and differences compared to untreated cells were calculated (A). Means (SD) of at least n = 3 independent experiments are shown. B, A representative immunoblot against SP-B and β-actin is shown. ***$P < .001$ compared to control cells; ##$P < .01$ compared to cells treated with DXM. GAPDH indicates glyceraldehyde 3-phosphate dehydrogenase; DXM, dexamethasone; PCR, polymerase chain reaction; mRNA, messenger RNA; SD, standard deviation; SP, surfactant protein.

Discussion

The present study highlights that progesterone at high concentrations in vitro is able to antagonize the stimulatory effect of DXM on SP-expression in lung epithelial cells. Progesterone and DXM are frequently administered simultaneously during and especially toward the end of pregnancy. Therefore, the ability of progesterone to antagonize the effect of GCs may have clinical consequences for preterm infants, especially with regard to the priming effect of GCs on lung function.

Our in vitro data showed no direct effect on the expression of SP-B if progesterone was used independently. The clinical effect of progesterone on the development of RDS is controversial. It has been shown that RDS could be induced by progesterone in preterm rabbits.[29,30] However, reduced plasma

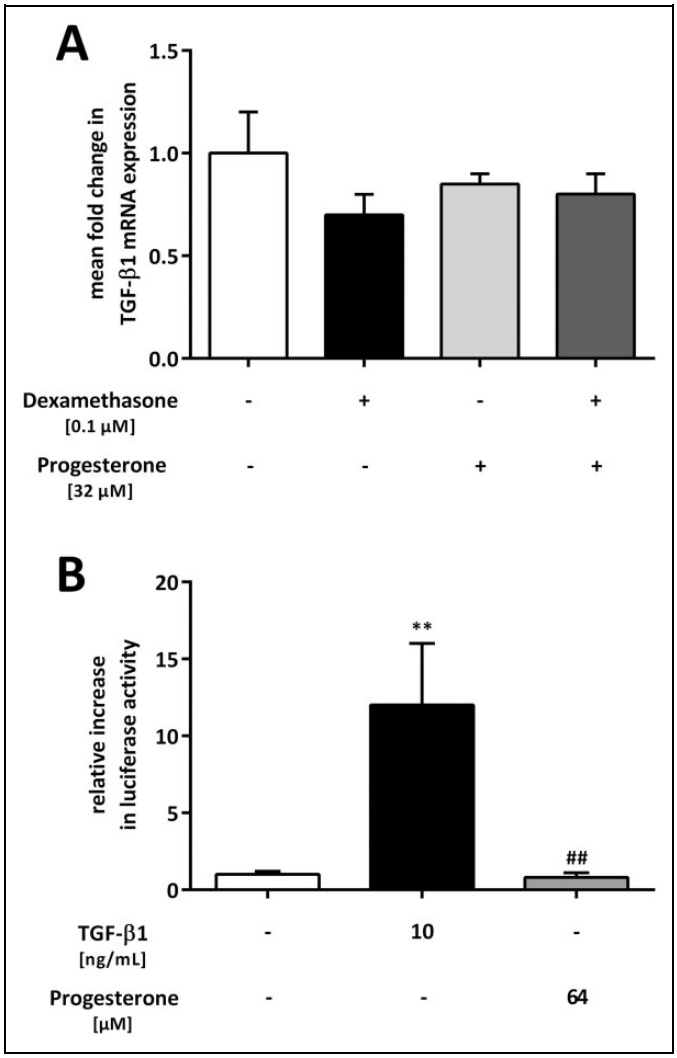

Figure 4. Progesterone has no effect on TGF-β1 mRNA expression and Smad signaling in lung epithelial cells. A, H441 cells were incubated with DXM (10 μM) and/or progesterone (32 μM) for 12 hours. Real-time PCR of TGF-β1 mRNA was performed and relative mRNA levels of TGF-β1 were calculated by normalizing signals to detected GAPDH mRNA. Differences compared to untreated cells were calculated. Means (SD) of at least n = 3 independent experiments are shown. B, The TGF-β1-sensitive $(CAGA)_{12}$-luciferase reporter construct was transiently transfected into H441 cells. After 16 hours, cells were treated with TGF-β1 (10 ng/mL) or progesterone (64 μM). Firefly luciferase activity was normalized to the activity of Renilla luciferase under control of the thymidine kinase promoter. Relative luciferase activity compared to controls is shown. $^{**}P < .01$ compared to control cells; $^{\#\#}P < .01$ compared to cells treated with TGF-β1. DXM indicates dexamethasone; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PCR, polymerase chain reaction; mRNA, messenger RNA; SD, standard deviation; TFG-β1, tissue growth factor β1.

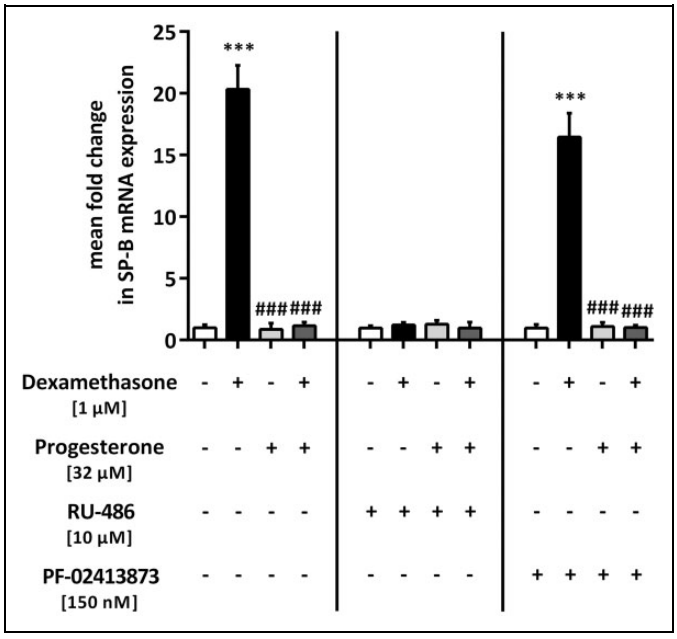

Figure 5. The glucocorticoid and PR antagonist RU-486, but not the specific PR antagonist PF-02413873, inhibits DXM-induced SP-B expression in lung epithelial cells. H441 cells were incubated with DXM (1 μM), the glucocorticoid and PR antagonist RU-486 (10 μM), or the specific PR antagonists PF-02413873 (150 nM) for 12 hours as indicated. Real-time PCR of SP-B mRNA was performed and relative mRNA levels of SP-B were calculated by normalizing signals to detected GAPDH mRNA. Differences compared to untreated cells were calculated. Means (SD) of at least n = 3 independent experiments are shown. $^{***}P < .001$ compared to control cells, $^{\#\#\#}P < .001$ compared to cells treated with DXM or DXM plus PF-02413873, respectively. DXM indicates dexamethasone; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PR, progesterone receptor; mRNA, messenger RNA; SD, standard deviation; SP, surfactant protein; TFG-β1, tissue growth factor β1.

levels of progesterone were found in newborns suffering from RDS.[31] In another study involving premature infants with and without RDS, concentrations of progesterone were similar in maternal venous blood and the umbilical vein and artery.[32]

We found a dose-dependent inhibitory effect of progesterone on DXM-induced SP-B expression on the transcriptional and translational level in lung epithelial cells. To gain further insight into the mechanism of this effect, we also investigated the involvement of TGF-β1 and an activation of Smad signaling, but our results demonstrated that neither TGF-β1 mRNA expression was altered nor Smad signaling was activated. A negative regulation of TGF-β1 on SP-B expression was already described before in H441 cells[18,19] and would have been a possible explanation for the observed inhibitory effect of progesterone on DXM-induced SP-B expression.

Progesterone may further be able to modulate DXM-induced SP-B expression via its own PRs (PR-A and/or PR-B) or by an interaction with the GC receptor.[33] Based on the similar structure of the steroid receptors, the same hormone may simultaneously bind to different types of receptors leading to different types of activation.[34-36] In contrast to the unspecific PR antagonist RU-486, the highly specific, nonsteroidal PR antagonist PF-02413873 is not able to inhibit the GC receptor.[24] As we found that PF-02413873 did not modify the inhibitory action of progesterone on DXM-induced SP-B expression, an involvement of PRs in the observed effect can be excluded.

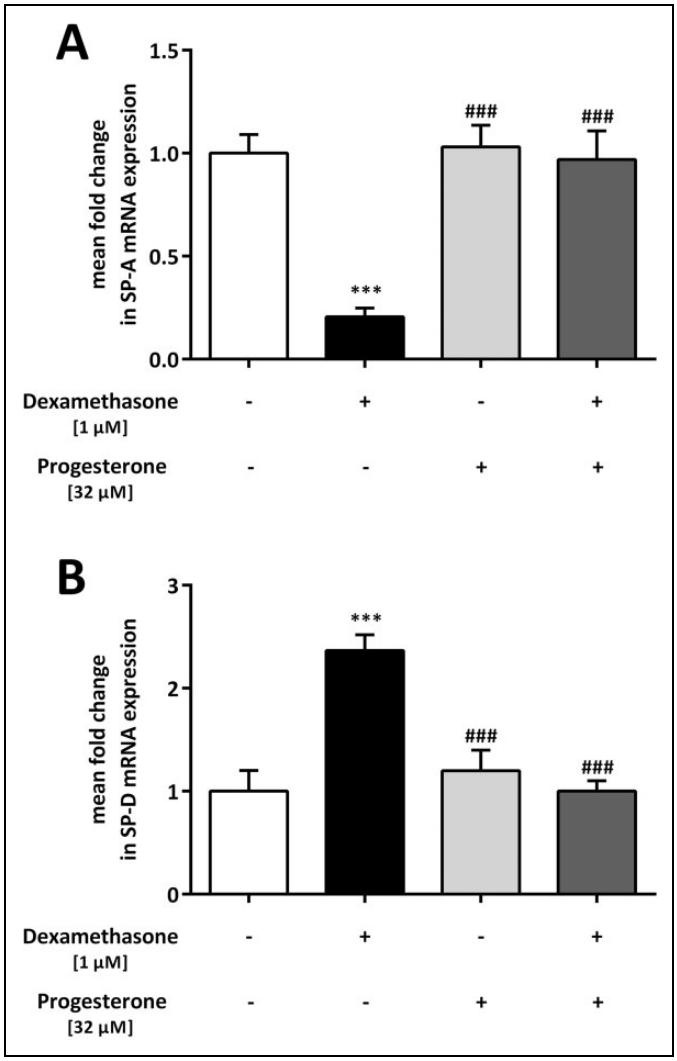

Figure 6. Progesterone antagonizes the DXM-regulated genes SP-A and SP-D in lung epithelial cells. H441 cells were incubated with DXM (1 μM) and/or progesterone (32 μM) for 12 hours. Real-time PCR of SP-A (A) and SP-D mRNA (B) was performed. Relative mRNA levels of SP-A and SP-D were calculated by normalizing signals to detect GAPDH mRNA. Differences compared to untreated cells were calculated. Means (SD) of at least n = 3 independent experiments are shown. ***$P < .001$ compared to control cells, ###$P < .001$ compared to cells treated with DXM. DXM indicates dexamethasone; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; mRNA, messenger RNA; SD, standard deviation; SP, surfactant protein.

While binding of DXM to its receptor leads to transcriptional activation (full agonist), binding of progesterone to the GC receptor is unable to completely activate it (partial agonist).[36] Due to the competition for receptor binding between full and partial agonist, progesterone is able to inhibit the effect of DXM.[36] As the inhibitory potential of progesterone in our experimental system could be attributed to high concentrations, this indicates a competition to replace the high-affinity ligand DXM. The ability of the unspecific GC and progesterone receptor antagonist RU-486 to mimic the effect of progesterone underlines this hypothesis. Glucocorticoid receptor binding studies for the endogenous ligand cortisol (100%) in comparison to other steroids (progesterone [22%], aldosterone [20%], testosterone [1.5%], and estradiol [0.2%]) showed a relatively high binding affinity for progesterone, but no complete agonist activity.[34] A similar inhibitory effect of progesterone could be described for the aromatase induction by GCs in human adipose fibroblasts and for the induction of the epithelial sodium channel by GCs in lung cells.[33,36]

In addition, we could show that the inhibitory ability of progesterone is not specific for SP-B; an upregulation of mRNAs for SP-D[37] and the downregulation of SP-A[38] by DXM could also be antagonized by progesterone in our setting. Thus, the effect of progesterone might be a general phenomenon for DXM-regulated genes in vitro.

In this context, a vice versa situation, the inhibition of the progesterone effect by GCs, has been described in sheep.[4,39,40] In these experiments, an application of exogenous GCs during pregnancy led to a GC-induced decrease of the efficacy of progesterone ("progesterone withdrawal") leading to precipitating labor and delivery.[4,40] In humans, however, antenatal DXM therapy seems to have no influence on the plasma concentration or the physiological function of progesterone.[41]

The concentrations of progesterone used in this study appear to be above the physiological plasma levels found during pregnancy.[42] However, it is described that under in vitro conditions, higher steroid levels are necessary to yield cellular effects.[42] In earlier in vitro studies, these high concentrations were administered to generate physiological effects.[43,44] Another interesting aspect in this connection may be that tissues are intrinsically able to produce steroid hormones which may, in combination with plasma steroids, contribute to higher local tissue concentrations.[42] In rodent hippocampal tissue, local estrogen production yields tissue steroid levels at approximately 10^{-9} M, which is above those found in plasma.[45] Treatment of pregnant women with exogenous progesterone is likely to further elevate fetal serum progesterone concentrations particularly during late pregnancy, for which data are, however, still lacking.[46] Therefore, the high doses of progesterone used during this study may be clinically relevant.[46]

Besides a direct effect mediated by the receptors for progesterone or GCs, receptor-independent activities may be involved, especially because only high concentrations of progesterone were inhibiting DXM-induced SP-B expression. It has been described that steroid hormones can posttranslationally modify kinase activation states, demonstrating rapid nongenomic activities.[47,48]

Direct interactions with activations of calcium channels, cyclic adenosine monophosphate/protein kinase A, mitogen-activated protein kinases, G proteins, caspases, and transcription factors have been shown.[47-49] In addition, steroid hormones can display nonmonotonic response patterns which differ from those following conventional concentration dependence.[47,48] This has been already described for progesterone.[50] Possible clinical consequences of our in vitro observation have to be examined using fetal type II cells and animal models in future studies.

Authors' Note

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Declaration of Conflicting Interests

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References

1. Eichenwald EC, Stark AR. Management and outcomes of very low birth weight. *N Engl J Med*. 2008;358(16):1700-1711.
2. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. *Science*. 2014;345(6198):760-765.
3. Iams JD.Clinical practice. Prevention of preterm parturition. *N Engl J Med*. 2014;370(3):254-261.
4. Hallman M. The story of antenatal steroid therapy before preterm birth. *Neonatology*. 2015;107(4):352-357.
5. Malloy MH. Antenatal steroid use and neonatal outcome: United States 2007. *J Perinatol*. 2012;32(9):722-727.
6. Roberts D, Brown J, Medley N, Dalziel SR. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database Syst Rev*. 2017;21(3).
7. Goodnight W. Clinical application of progesterone for the prevention of preterm birth, 2016. *Am J Perinatol*. 2016;33(3):253-257.
8. Romero R, Nicolaides K, Conde-Agudelo A, et al. Vaginal progesterone in women with an asymptomatic sonographic short cervix in the midtrimester decreases preterm delivery and neonatal morbidity: a systematic review and metaanalysis of individual patient data. *Am J Obstet Gynecol*. 2012;206(2):11.
9. Trotter A, Maier L, Grill HJ, Kohn T, Heckmann M, Pohlandt F. Effects of postnatal estradiol and progesterone replacement in extremely preterm infants. *J Clin Endocrinol Metab*. 1999;84(12):4531-4535.
10. Trotter A, Maier L, Kron M, Pohlandt F. Effect of oestradiol and progesterone replacement on bronchopulmonary dysplasia in extremely preterm infants. *Arch Dis Child Fetal Neonatal Ed*. 2007;92(2):F94-F98.
11. Perez-Gil J, Weaver TE. Pulmonary surfactant pathophysiology: current models and open questions. *Physiology*. 2010;25(3):132-141.
12. Rausch F, Schicht M, Brauer L, Paulsen F, Brandt W. Protein modeling and molecular dynamics simulation of the two novel surfactant proteins SP-G and SP-H. *J Mol Model*. 2014;20(11):2513.
13. Bersani I, Speer CP, Kunzmann S. Surfactant proteins A and D in pulmonary diseases of preterm infants. *Expert Rev Anti Infect Ther*. 2012;10(5):573-584.
14. Weaver TE, Conkright JJ. Function of surfactant proteins B and C. *Annu Rev Physiol*. 2001;63:555-578.
15. Whitsett JA, Weaver TE. Hydrophobic surfactant proteins in lung function and disease. *N Engl J Med*. 2002;347(26):2141-2148.
16. Speer CP, Robertson B, Curstedt T, et al. Randomized European multicenter trial of surfactant replacement therapy for severe neonatal respiratory distress syndrome: single versus multiple doses of Curosurf. *Pediatrics*. 1992;89(1):13-20.
17. Fehrholz M, Hutten M, Kramer BW, Speer CP, Kunzmann S. Amplification of steroid-mediated SP-B expression by physiological levels of caffeine. *Am J Physiol Lung Cell Mol Physiol*. 2014;306(1):25.
18. Beers MF, Solarin KO, Guttentag SH, et al. TGF-beta1 inhibits surfactant component expression and epithelial cell maturation in cultured human fetal lung. *Am J Physiol*. 1998;275(5 pt 1):L950-L960.
19. Kumar AS, Gonzales LW, Ballard PL. Transforming growth factor-beta(1) regulation of surfactant protein B gene expression is mediated by protein kinase-dependent intracellular translocation of thyroid transcription factor-1 and hepatocyte nuclear factor 3. *Biochim Biophys Acta*. 2000;1492(1):45-55.
20. Kunzmann S, Wright JR, Steinhilber W, et al. TGF-beta1 in SP-A preparations influence immune suppressive properties of SP-A on human CD4+ T lymphocytes. *Am J Physiol Lung Cell Mol Physiol*. 2006;291(4):28.
21. Grotendorst GR, Okochi H, Hayashi N. A novel transforming growth factor beta response element controls the expression of the connective tissue growth factor gene. *Cell Growth Differ*. 1996;7(4):469-480.
22. Dammeier J, Beer HD, Brauchle M, Werner S. Dexamethasone is a novel potent inducer of connective tissue growth factor expression. Implications for glucocorticoid therapy. *J Biol Chem*. 1998;273(29):18185-18190.
23. Fehrholz M, Glaser K, Speer CP, Seidenspinner S, Ottensmeier B, Kunzmann S. Caffeine modulates glucocorticoid-induced expression of CTGF in lung epithelial cells and fibroblasts. *Respir Res*. 2017;18(1):51.
24. Bungay PJ, Tweedy S, Howe DC, Gibson KR, Jones HM, Mount NM. Preclinical and clinical pharmacokinetics of PF-02413873, a nonsteroidal progesterone receptor antagonist. *Drug Metab Dispos*. 2011;39(8):1396-1405.
25. Brower M, Carney DN, Oie HK, Gazdar AF, Minna JD. Growth of cell lines and clinical specimens of human non-small cell lung cancer in a serum-free defined medium. *Cancer Res*. 1986;46(2):798-806.
26. Dennler S, Itoh S, Vivien D, ten Dijke P, Huet S, Gauthier JM. Direct binding of Smad3 and Smad4 to critical TGF beta-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene. *EMBO J*. 1998;17(11):3091-3100.

27. Fehrholz M, Bersani I, Kramer BW, Speer CP, Kunzmann S. Synergistic effect of caffeine and glucocorticoids on expression of surfactant protein B (SP-B) mRNA. *PLoS One*. 2012;7(12): e51575.
28. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001;25(4):402-408.
29. Joensuu H, Kero P, Pulkkinen MO. Lung tissue progesterone in the human fetus and neonate. *Biol Neonate*. 1981;40(3-4):183-186.
30. Pulkkinen MO, Kero P. Effect of progesterone on the initiation of respiration of the newborn. *Biol Neonate*. 1977;32(3-4):218-221.
31. Kero PO, Pulkkinen MO. Plasma progesterone in the respiratory distress syndrome. *Eur J Pediatr*. 1979;132(1):7-10.
32. Hercz P, Varady E, Ungar L, Siklos P, Buky B. Respiratory distress syndrome: steroid and peptide hormone levels in maternal venous blood and the umbilical vein and artery. *Acta Paediatr Hung*. 1987;28(1):17-21.
33. Schmidt M, Renner C, Loffler G. Progesterone inhibits glucocorticoid-dependent aromatase induction in human adipose fibroblasts. *J Endocrinol*. 1998;158(3):401-407.
34. von Langen J, Fritzemeier KH, Diekmann S, Hillisch A. Molecular basis of the interaction specificity between the human glucocorticoid receptor and its endogenous steroid ligand cortisol. *Chembiochem*. 2005;6(6):1110-1118.
35. Pearce D, Yamamoto KR. Mineralocorticoid and glucocorticoid receptor activities distinguished by nonreceptor factors at a composite response element. *Science*. 1993;259(5098):1161-1165.
36. Schmidt C, Klammt J, Thome UH, Laube M. The interaction of glucocorticoids and progesterone distinctively affects epithelial sodium transport. *Lung*. 2014;192(6):935-946.
37. Deterding RR, Shimizu H, Fisher JH, Shannon JM. Regulation of surfactant protein D expression by glucocorticoids in vitro and in vivo. *Am J Respir Cell Mol Biol*. 1994;10(1):30-37.
38. O'Reilly MA, Gazdar AF, Morris RE, Whitsett JA. Differential effects of glucocorticoid on expression of surfactant proteins in a human lung adenocarcinoma cell line. *Biochim Biophys Acta*. 1988;970(2):194-204.
39. Liggins GC. Premature parturition after infusion of corticotrophin or cortisol into foetal lambs. *J Endocrinol*. 1968;42(2):323-329.
40. Liggins GC. Premature delivery of foetal lambs infused with glucocorticoids. *J Endocrinol*. 1969;45(4):515-523.
41. Ogueh O, Jones J, Mitchell H, Alaghband-Zadeh J, Johnson MR. Effect of antenatal dexamethasone therapy on maternal plasma human chorionic gonadotrophin, oestradiol and progesterone. *Human Reproduct*. 1999;14(2):303-306.
42. Trotter A, Kipp M, Schrader RM, Beyer C. Combined application of 17beta-estradiol and progesterone enhance vascular endothelial growth factor and surfactant protein expression in cultured embryonic lung cells of mice. *Int J Pediatr*. 2009;2009: 170491.
43. Bosse R, Rivest R, Di Paolo T. Ovariectomy and estradiol treatment affect the dopamine transporter and its gene expression in the rat brain. *Brain Res Mol Brain Res*. 1997;46(1-2):343-346.
44. Watson CS, Alyea RA, Hawkins BE, Thomas ML, Cunningham KA, Jakubas AA. Estradiol effects on the dopamine transporter—protein levels, subcellular location, and function. *J Mol Signal*. 2006;1:5.
45. Ishii H, Tsurugizawa T, Ogiue-Ikeda M, et al. Local production of sex hormones and their modulation of hippocampal synaptic plasticity. *Neuroscientist*. 2007;13(4):323-334.
46. Shields AD, Wright J, Paonessa DJ, et al. Progesterone modulation of inflammatory cytokine production in a fetoplacental artery explant model. *Am J Obstet Gynecol*. 2005;193(3 pt 2): 1144-1148.
47. Vandenberg LN, Colborn T, Hayes TB, et al. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr Rev*. 2012;33(3):378-455.
48. Watson CS, Hu G, Paulucci-Holthauzen AA. Rapid actions of xenoestrogens disrupt normal estrogenic signaling. *Steroids*. 2014;81:36-42.
49. Kirk CJ, Bottomley L, Minican N, et al. Environmental endocrine disrupters dysregulate estrogen metabolism and Ca2+ homeostasis in fish and mammals via receptor-independent mechanisms. *Comp Biochem Physiol A Mol Integr Physiol*. 2003;135(1):1-8.
50. Sonnenschein C, Olea N, Pasanen ME, Soto AM. Negative controls of cell proliferation: human prostate cancer cells and androgens. *Cancer Res*. 1989;49(13):3474-3481.