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Effect of Prenatal Dexamethasone on Immunoreactive 6-Ketoprostaglandin $F_{1\alpha}$ Levels in Fetal Rat Lungs

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Summary

The effect of prenatal glucocorticoid treatment on levels of immunoreactive 6-ketoprostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) (the stable metabolite of prostacyclin) was studied in fetal rat lungs. During late gestation (20–22 days), levels of 6-keto-$PGF_{1\alpha}$ peaked at 21 days in offspring of control mothers. At a maternal dose of 0.2 mg/kg dexamethasone, maximal enhancement of fetal 6-keto-$PGF_{1\alpha}$ levels occurred at 20 days gestation. At a treatment dose of 0.4 mg/kg, however, dexamethasone increased fetal lung 6-keto-$PGF_{1\alpha}$ concentrations throughout late gestation. Because maturation of fetal lung is known to be delayed in males relative to females, we also studied the impact of sex of the fetus on levels of 6-keto-$PGF_{1\alpha}$. Our results showed no statistically significant differences between females and males in any of the treatment groups at any of the gestational ages studied. These results suggest that prenatal dexamethasone enhances endogenous levels of 6-keto-$PGF_{1\alpha}$ in fetal rat lungs. Since prostacyclin may play important roles in fetal lung maturation and neonatal lung function, the effectiveness of prenatal glucocorticoid therapy for accelerating functional maturity of the fetal lung may in part be due to stimulation of prostacyclin synthesis.

Abbreviations

RDS, respiratory distress syndrome
PG, prostaglandin
RIA, radioimmunoassay
TLC, thin layer chromatography
HPLC, high performance liquid chromatography

In recent years, prenatal glucocorticoid therapy has gained increasing acceptance as a means for accelerating fetal lung maturation and reducing the risk of RDS in prematurely born infants (3, 10, 21). Studies on possible adverse effects of glucocorticoid therapy, such as its potential for reducing the weight of lung, brain, and other organs, have been reported (8, 9). Glucocorticoids are also known to inhibit PG synthesis by inhibiting phospholipase A_2, an enzyme which makes substrate available by releasing arachidonic acid from phospholipids (7). In fetal lung, the major prostaglandins are PGI_2 and PGE_2 (20, 26, 27), both of which are vasoactive. In particular, PGI_2 is a potent vasodilator and bronchodilator, and its presence in an adequate amount is needed to reduce pulmonary vascular resistance (11, 12).

Inhibition of prostaglandin synthesis in general and PGI_2 synthesis in particular, therefore, may cause vasoconstriction which could predispose prematurely born infants to RDS. In addition, diminished PGI_2 synthesis could complicate or retard the course of recovery of infants with RDS. A previous study in our laboratory, however, showed that prenatal dexamethasone treatment stimulated conversion of [^{14}C]arachidonic acid to 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) in lung homogenates of 20-day fetal rats (26). This may indicate that dexamethasone induced the enzymes of PGI_2 synthesis in fetal lung. However, since dexamethasone can inhibit prostaglandin synthesis by inhibiting phospholipase A_2, isotopic studies of arachidonic acid conversion to prostaglandins in lung homogenates cannot assess the overall effect of dexamethasone on fetal lung prostaglandin metabolism. In the present study, we determined the immunoreactive 6-keto-$PGF_{1\alpha}$ concentrations in lungs by radioimmunoassay in rat fetuses of mothers which were treated with dexamethasone.

MATERIALS AND METHODS

Animals. Animals were housed, bred, and treated as previously described (24, 27). Female Sprague-Dawley rats (200–250 g; Holtzman, Madison, WI) were housed overnight with breeder males, the timing of pregnancy known within 12 h. Timed pregnancy rats were also obtained from Holtzman. The dosage of dexamethasone was varied. Separate rats were treated with 0.9% saline as a control or with dexamethasone in 0.9% saline (Organon, Inc., West Orange, NJ), 0.2 mg/kg, or 0.4 mg/kg body weight.

For 2 days before each gestational age studied, the pregnant rats received subcutaneous injections of dexamethasone or saline at 12-h intervals. Four injections were given, with the last injection 12 h before sacrifice of the mother.

During the entire breeding and treatment period, mothers were given free access to food and water, while housed under controlled conditions of temperature, humidity, and light. At the various gestational ages, mothers were lightly anesthetized with ether, then sacrificed by cervical dislocation. The fetuses (usually 10–12/litter) were removed from the uterus, decapitated, and weighed. Fetal lungs were then rapidly dissected free of bronchi and vessels and quickly frozen on dry ice. All lungs were maintained at −70°C until assayed for 6-keto-$PGF_{1\alpha}$. Sex of the fetuses was determined by viewing the gonads under ×10 magnification. A transverse incision was made from umbilicus to pubic symphysis. The testes appeared as pink oval organs with serpentine drainage veins along their lateral border. The ovaries were viewed

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after removal of the intestines and were just inferior to the kidneys; they were characterized by fallopian tubes along their lateral edges.

Radioimmunoassay. Frozen lung tissue was weighed and immediately placed in a chilled, silanized homogenizer (Arthur H. Thomas Co., Philadelphia, PA), to which was added indomethacin (0.1 mg/g tissue) and 4 to 10 volumes homogenization buffer (0.05 N sodium phosphate, pH 6.8). Homogenization was accomplished with 10 strokes of a smooth Teflon pestle motor driven at speed 6 (Tri-R Stir-R, Tri-R Instrument, Rockville Center, NY) while cooling the homogenizer in an ice bath. The homogenate was transferred to plastic centrifuge tubes, and centrifuged at 1500 × *g* for 10 min in a refrigerated centrifuge (Beckman TJ-6). The supernatant was decanted and further diluted with homogenization buffer.

Initially, homogenate in a range of dilutions (4:1 to 150:1, buffer/tissue, v/w) was assayed to ascertain the effect of dilution and optimal assay conditions. At dilutions of greater than 25:1, interference by the tissue with RIA diminished to negligible levels. Recovery of added 6-keto-$PGF_{1\alpha}$ (50–200 pg/mg tissue) at 50:1, 100:1, and 150:1 dilutions was 108 ± 15% ($\bar{x}$ ± SD).

Following dilution, RIA was performed with a [^{3}H]6-keto-prostaglandin $F_{1\alpha}$ RIA kit (New England Nuclear, Boston, MA) which used specific rabbit antibodies to 6-keto-$PGF_{1\alpha}$ and a tritiated tracer.

Solvent extraction of homogenate. Two extraction schemes as well as further purification of 6-keto-$PGF_{1\alpha}$ by column, thin layer, and high performance liquid chromatography were explored to determine whether such pretreatments of tissue homogenates would improve quantitation of 6-keto-$PGF_{1\alpha}$ by RIA.

The first extraction involved acidification of the homogenate with citric acid and extraction into ethyl acetate (4). The second extraction involved acidification of the homogenate with HCl and extraction into diethyl ether (27). Following each of these extractions, the fraction containing 6-keto-$PGF_{1\alpha}$ was dried under nitrogen, the residue then being redissolved and subjected to RIA. The recovery of added [^{3}H]6-keto-$PGF_{1\alpha}$ was 75.0 ± 5.6% for ethyl acetate extraction, and 68.5 ± 4.0% for ether extraction.

Chromatography: Sep-pak. Sep-pak C_{18} cartridges (Waters Associates, Milford, MA) were used with minor modifications to the method of Powell (18). Briefly, frozen tissue was homogenized in 5 volumes of homogenization buffer (0.05 M Tris-HCl, pH 6.8) and then diluted with 16 volumes of H_20. After vortexing and centrifuging at 400 × *g* for 10 min, the supernatant was decanted and acidified with 1 N HCl to pH 3.0. Sep-pak cartridges were activated and the acidified supernatant was then applied. The cartridges were then washed sequentially with 20 ml 5% ethanol in distilled water (pH 3.0), 20 ml petroleum ether, and finally 10 ml methyl formate. Aliquots of the methyl formate solution were either dried under a stream of nitrogen and subjected to radioimmunoassay, or saved for TLC purification. The Sep-pak cartridges yielded 65.5 ± 4.6% recovery of added [^{3}H] 6-keto-$PGF_{1\alpha}$. Results of RIA performed on tissue homogenates extracted or purified by any of the three methods described above were essentially identical to, and presented no advantage over, results obtained from sufficiently diluted tissue homogenates, if extraction efficiencies were taken into account.

Thin layer and high performance liquid chromatography. In early experiments, 6-keto-$PGF_{1\alpha}$ eluted from Sep-pak cartridges was further purified by TLC and HPLC before RIA. Briefly, TLC was carried out with silica gel G plates developed in ethyl acetate:isooctane:acetic acid:H_2O [55:25:10:50 (v/v)] as described by Hassid *et al.* (6).

After chromatography, authentic 6-keto-$PGF_{1\alpha}$ standards were visualized with iodine vapor. Regions of the TLC sample plate, matched against the standard, were scraped into a plastic centrifuge tube and extracted 2 times with 2 ml of acetonitrile:H_20, 90:10, pH 3.0. The combined extracts were pooled; aliquots were removed and dried. Fractions were taken for RIA and HPLC. Fractions for HPLC were redissolved in HPLC elution buffer (acetonitrile/0.015 M phosphoric acid, 67.2:32.8, pH 3.5). HPLC was done according to the method of Terragno *et al.* (23) using a reverse phase column (5-μ, 25-cm Nucleosil C_{18}, Supelco, Bellefonte, PA). The flow rate was 1.7 ml/min with the elution buffer described above. Fractions corresponding to the retention time of [^{3}H]6-keto-$PGF_{1\alpha}$, or 6-keto-$PGF_{1\alpha}$ as determined by absorbance at 192.5 mm, were pooled, dried under nitrogen, and subjected to RIA.

Use of TLC and HPLC purifications did not result in improved RIA quantitation in our hands. The consideration of all of these techniques led us to choose diluted tissue homogenate as the most reproducible preparation of sample prior to RIA. RIA of all samples was done in triplicate. Lungs of two male and two female fetuses were assayed from each litter. Protein of lung homogenate was determined by the method of Lowry *et al.* (13).

Statistics. Data are expressed as the mean ± the standard error of the mean. Student's *t* test for unpaired samples was used for testing statistical significance between treatment groups.

RESULTS

Concentrations of 6-keto-$PGF_{1\alpha}$ were measured in lungs of fetal rats at 20, 21, and 22 days gestational age with or without prenatal dexamethasone treatment (Table 1). Treatment at 0.2 mg/kg dexamethasone slightly increased the 6-keto-$PGF_{1\alpha}$ concentration in 20-day fetal lungs, but had no effect at 21 and 22 days gestational age. Treatment with 0.4 mg/kg dexamethasone significantly increased the 6-keto-$PGF_{1\alpha}$ concentration at 21 days (52% above age-matched control) and 22 days (73% above age-matched control) gestational age. There were no significant differences in pulmonary 6-keto-$F_{1\alpha}$ concentrations between female and male fetuses from control or treated litters, though male fetuses appeared more consistently responsive to 0.4 mg/kg treatment at 21 and 22 days gestation ($p < 0.05$ and <0.001 for males *versus* NS and <0.01 for females at 21 and 22 days, respectively; Table 2).

DISCUSSION

Clinical studies (3, 10, 21) have shown that prenatal glucocor ticoid treatment can reduce the risk of respiratory distress syndrome in premature infants. The generally accepted mechanism for accelerated fetal lung maturation by glucocorticoid is through stimulation of surfactant phospholipid synthesis. Our own work (24, 25) with a rat model of pregnancy has shown enhanced incorporation of radioactive precursors into phosphatidylglycerol and disaturated phosphatidylcholine after prenatal dexamethasone treatment. In addition, Beck *et al.* (2) have demonstrated that glucocorticoids accelerate the differentiation of fetal lung connective tissue. The action of glucocorticoid may be partially explained by the ability of the steroid to raise the level of lung cyclic AMP (1), which in turn has been shown to stimulate the synthesis and secretion of surfactant phospholipids (5).

Prostaglandins can regulate cyclic AMP synthesis by lung which is a major organ of arachidonic acid metabolism. Several reports have also strongly suggested that prostaglandins may play important roles in fetal lung maturation (19, 22, 27). In a previous study (26), we demonstrated that the major metabolite of the cyclooxygenase pathway in fetal lung is PGI_2 (measured as the stable metabolite, 6-keto-$PGF_{1\alpha}$). PGI_2 is a potent vasodilator and therefore may play an important role in the regulation of fetal and neonatal pulmonary circulation. We have also demonstrated that prenatal dexamethasone treatment enhances the ability of fetal lung homogenate to convert exogenously added arachidonic acid to 6-keto-$PGF_{1\alpha}$ (26). In the present study, we focused on measurement of 6-keto-$PGF_{1\alpha}$ by RIA in lungs of offspring from dexamethasone-treated and control rats.

Because males are more susceptible to RDS than females (14) and less responsive to glucocorticoid therapy (3), we also analyzed our data according to sex of the fetus. Our findings demonstrated that prenatal dexamethasone (0.4 mg/kg) increased fetal rat lung 6-keto-$PGF_{1\alpha}$ concentrations as measured by ra-

Table 1. *The effect of prenatal dexamethasone on pulmonary 6-keto-PGF$_{1\alpha}$* levels in fetal rats*

Dexamethasone Treatment	6-Keto-PGF$_{1\alpha}$ (pg/mg protein)					
	20 Days Gestation	*p*	21 Days Gestation	*p*	22 Days Gestation	*p*
Control	217 ± 26		292 ± 49		256 ± 36	
0.2 mg/kg	341 ± 38	0.02†	317 ± 41	NS‡	283 ± 33	NS
0.4 mg/kg	295 ± 52	NS	443 ± 49	0.05†	443 ± 28	0.002†

* Values are mean ± SEM, two female and two male samples from six litters for each gestational age and treatment group.
† Values of *p* refer to treatment *versus* control for each gestational age.
‡ NS, not significantly different ($p > 0.05$).

Table 2. *The effect of sex of the fetus on pulmonary 6-keto-PGF$_{1\alpha}$* levels in offspring of untreated and dexamethasone-treated mothers*

Dexamethasone Treatment	6-Keto-PGF$_{1\alpha}$ (pg/mg protein)								
	20 Days Gestation			21 Days Gestation			22 Days Gestation		
	Male	Female	*p*	Male	Female	*p*	Male	Female	*p*
Control	239 ± 29	195 ± 27	NS†	292 ± 57	292 ± 45	NS	258 ± 37	253 ± 49	NS
0.2 mg/kg	345 ± 34‡	338 ± 51‡	NS	331 ± 42§	304 ± 42§	NS	315 ± 39§	252 ± 36§	NS
0.4 mg/kg	325 ± 71§	266 ± 36§	NS	462 ± 44‡	424 ± 55§	NS	434 ± 40¶	453 ± 37¶	NS

* Values are mean ± SEM for duplicate samples from six litters for each gestational age and treatment group.
† NS, not significantly different between age-matched males and females ($p > 0.05$).
‡ Significantly different from corresponding values of age-matched, sex-matched fetuses from control mothers ($p < 0.05$).
§ Not significantly different from corresponding values of age-matched, sex-matched fetuses from control mothers ($p > 0.05$).
¶ Significantly different from corresponding values of age-matched, sex-matched fetuses from control mothers ($p < 0.01$).

dioimmunoassay at 21 and 22 days gestational ages. At a dose of 0.2 mg/kg, dexamethasone has a slight stimulatory effect on synthesis of PGI_2, although the difference is not statistically significant at 21 and 22 days of gestation. Nielsen and Torday (16) have demonstrated that, in rabbits, lung lavage from female fetuses has a higher lecithin/sphingomyelin ratio and higher disaturated phosphatidylcholine than that from male fetuses. This sex difference in phospholipid synthesis has been demonstrated to be under hormonal control (15). In the present study, our findings indicate that sex of the fetus does not significantly influence concentration of 6-keto-PGF$_{1\alpha}$ in lung at any of the gestational ages or dexamethasone dosages studied. From mothers receiving dexamethasone at 0.4 mg/kg, however, levels of 6-keto-PGF$_{1\alpha}$ in lungs of male fetuses were maximal at 21 days gestation, while in female fetuses, levels were maximal at 22 days.

The increased prostaglandin concentration in lung tissue of fetuses from dexamethasone-treated mothers appears to contradict earlier findings (7) that glucocorticoids inhibit phospholipase A_2 activity. Several possible explanations exist for this apparent discrepancy. First, it is possible that much higher doses of dexamethasone than those used in this study may be needed to achieve the required concentration in fetal circulation to inhibit the activity of fetal lung phospholipase A_2. Second, since lung is composed of more than 40 types of cells, several isoenzymes of phospholipase A_2 may exist. In addition, those isoenzymes of phospholipase A_2 (or other phospholipases such as the phosphatidylinositol-specific phospholipase C) that are mainly responsible for releasing of arachidonic acid in fetal lung tissue may be less susceptible to the inhibitory action of dexamethasone. In support of the argument that not all phospholipase A_2 are susceptible to dexamethasone inhibition, Ohuchi *et al.* (17) have recently shown that dexamethasone did not directly inhibit the phospholipase A_2 activity in carrageenin-induced rat inflammatory tissue. In depth studies involving the isolation and characterization of the various isoenzymes of phospholipase in fetal lung tissue in terms of their sensitivities to the direct and protein-mediated effects of dexamethasone inhibition may be needed to resolve this problem. While the exact mechanism through which dexamethasone increases the PGI_2 concentration in fetal lung is not known, the results of this and our previous work (25) tend to support the hypothesis that dexamethasone either induces the synthesis, or increases the activities of the enzymes involved in the production of PGI_2. This is analogous to its effect on fetal lung enzymes involved in phospholipid synthesis. Alternatively, it is possible that dexamethasone decreases the rate of utilization/catabolism of PGI_2.

In conclusion, prenatal dexamethasone treatment does not interfere with the ability of fetal lung to synthesize PGI_2. Rather, dexamethasone treatment increases the capacity of fetal lung to synthesize PGI_2. Since adequate amounts of PGI_2 are needed to reduce pulmonary vascular resistance, part of the beneficial effect of glucocorticoid treatment in reducing the incidence of RDS in premature infants may be due to the stimulation of PGI_2 synthesis.

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A Longitudinal Study of Rhesus Monkey (*Macaca mulatta*) Milk Composition: Trace Elements, Minerals, Protein, Carbohydrate, and Fat

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Summary

The concentrations of iron, copper, zinc, calcium, magnesium, sodium, potassium, protein, carbohydrate, and fat were analyzed in milk from rhesus monkeys (*Macaca mulatta*) during the course of lactation. Concentrations of iron, copper, zinc, magnesium, sodium, potassium, and protein were higher in milk of early lactation (colostrum) than in mature milk, while concentrations of calcium increased with lactation time. Concentrations of zinc in monkey colostrum and mature milk were similar to that of human milk, while iron, copper, calcium, and protein concentrations were higher than in human milk.

There is a paucity of data in the literature regarding the effects of maternal diet on the composition of breast milk, and the resulting impact on the breast-fed infant. In addition, there is fragmentary information on the extent of nutrient utilization from milk by the breast-fed infant. While epidemiological studies can contribute to an understanding of these areas, data obtained from such studies are often difficult to interpret due to the lack of rigid definition of the maternal and infant environment, and the lack of appropriate controls. For ethical reasons, there are several limitations in designing studies in which the investigator manipulates the maternal environment since the diet must always be kept within "normal" and "appropriate" limits; the number of blood samples taken from the infants should be few, and only rarely can justification be made for soft tissue sampling. Furthermore, if the experimental manipulation appears to be causing an insult to the infant, the study should be halted, or changed, in the interest of the child. For these reasons, it is necessary to identify and develop animal models which can be used for the study of maternal-infant nutrition. While several laboratory animals (primarily rodents) have been used to evaluate the effects of maternal dietary environment on milk composition, and the subsequent suckling outcome, the gross composition of milk from most of these species is not well matched to that of the human (12, 13, 17).

In order to evaluate the possibility of using a non-human primate as an animal model for milk composition, and metabolism studies, with special emphasis on trace elements, we have analyzed the nutrient composition of rhesus monkey milk, and noted changes throughout the course of lactation. These data are compared with data on human milk composition.

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MATERIALS AND METHODS

Animals. Milk samples were obtained from healthy rhesus monkeys (*Macaca mulata*) at the California Primate Research Center. The animals were housed in five 4500-m^2 outdoor cor-