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Research Article

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Failure to Detect an Effect of Prolactin on Pulmonary Surfactant and Adrenal Steroids in Fetal Sheep and Rabbits

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ABSTRACT Recent reports have indicated an association between low cord prolactin (PRL) and the occurrence of respiratory distress syndrome in premature infants, and it is reported that PRL administration increases the lecithin content of fetal rabbit lung. We administered 1 mg ovine PRL to 32 rabbit fetuses on day 24 of gestation and evaluated lung phospholipid synthesis and content on day 26. Compared with diluent-injected littermates, PRL had no effect on the rate of choline incorporation into lecithin, tissue content of phospholipid and disaturated lecithin, or plasma corticoids. However, both choline incorporation and corticoids were increased in all animals undergoing surgery compared with unoperated controls. We also infused PRL (1 mg/day, i.v.) into three fetal sheep continuously over five periods of 5–8 days. Although supraphysiologic concentrations of PRL were achieved in plasma and amniotic fluid, there was no effect of this treatment on the flux of tracheal fluid surfactant or on plasma concentrations of corticoids or dehydroepiandrosterone sulfate.

Thus, in this study, we failed to detect either a stimulation of the surfactant system or an adrenocorticotrophic effect by PRL as previously postulated. This suggests that the relationship between PRL and respiratory distress syndrome is an indirect association.

INTRODUCTION

Lung surfactant, a mixture of proteins and phospholipids with surface-active properties, stabilizes lung alveoli and allows normal respiration. Surfactant normally appears in fetal lung during the last third of gestation, and its deficiency in premature infants causes respiratory distress syndrome (RDS).¹

Administration of glucocorticoids (1) or thyroid hormone (2) causes precocious appearance of alveolar surfactant in fetal animals, and prenatal corticosteroid therapy reduces the incidence of RDS in premature infants (3). Recent evidence suggests that prolactin (PRL) also may stimulate surfactant synthesis. Serum PRL concentration in the human and sheep fetus increases before the secretion of surfactant (4–6), and low levels of PRL in cord serum are associated with the occurrence of RDS in premature infants (5, 6). In addition, low neonatal levels of estriol, a possible regulator of PRL (7), are reported to be similarly associated with RDS (8). Recently, Hamosh and Hamosh (9) reported that injection of ovine PRL into rabbit fetuses increased the content of phospholipid, lecithin, and dipalmitoyllecithin in the fetal lung, leading them to propose that PRL may regulate surfactant synthesis. It also has been postulated (10) that PRL is adrenocorticotrophic in the fetus, raising the possibility that PRL indirectly affects the lung by stimulating production of adrenal corticosteroids.

In this study we administered PRL to fetal rabbits,

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¹Abbreviations used in this paper: oPRL, ovine prolactin; PRL, prolactin; RDS, respiratory distress syndrome.

using the procedure of Hamosh and Hamosh (9), and to fetal sheep *in utero*. We describe here the effects of PRL treatment on lung phospholipid synthesis and content, lung fluid surfactant, and plasma steroid levels.

METHODS

Rabbits. Five time-dated, pregnant, New Zealand white rabbits were lightly anesthetized with intravenous sodium pentobarbital on day 24 of gestation. The uterus was exteriorized through a midline abdominal incision and all fetuses of one horn were injected in the rump through the intact uterine wall with 1 mg ovine PRL (NIH-P S11) in 0.05 ml of sterile 0.16 M NaHCO₃; fetuses in the other horn received the same volume of diluent. In other experiments (sham surgery), three rabbits were treated identically except that all fetuses received injections of 0.05 ml diluent. 2 days later (day 26), the fetuses were obtained at a second laparotomy; viability, weight, and crown-rump length were determined, and fetal blood was collected after decapitation.

Sheep. Three time-dated pregnant ewes were operated on under general anesthesia at 106, 107, and 108 days gestation (term ~147 days). The maternal abdomen was opened by a midline incision, a small hysterotomy incision was made over the fetal neck, and catheters were placed in the amniotic fluid, trachea, carotid artery, and jugular vein as described previously (11, 12). All tracheal fluid entered the tracheal catheter which was connected outside the ewe's flank to a second catheter leading to a latex reservoir inside the uterus (11, 12). Postoperatively, 1×10^6 U penicillin V and 200 mg kanamycin were given every day into a maternal vein and for 5 days into the amniotic cavity.

Daily fetal arterial blood samples (2–4 ml) were taken for determination of pH, PCO₂, PO₂, and remaining plasma was frozen for steroid assays. The daily production of tracheal fluid was withdrawn separately from the latex reservoir and, by gentle suction, from the lung. Volumes were measured and portions from each source were frozen separately. A daily amniotic fluid sample (2 ml) was also collected and frozen.

The fetuses were allowed to recover for at least 5 days postoperatively and all had normal arterial blood gases and pH (PO₂ ≥ 18 torr, PCO₂ ≤ 46 torr, pH ≥ 7.35) after surgery. During study periods the ewes remained in a study cage and ovine PRL (oPRL) was infused continuously by Holter pump (model 903; Extracorporeal Medical Specialties, Inc., Culver City, Calif.) into the fetal jugular vein for 5–8 days. The oPRL was a highly purified preparation (Dr. C. H. Li, University of California, San Francisco) which had 1.5 times the potency of NIH-P S11 PRL by both immunoassay (4) and mammary gland receptor assay (13). A fresh infusion solution was prepared every 48 h which consisted of 2 mg oPRL dissolved in 100 ml isotonic saline containing 4 meq NaHCO₃, 2,000 U heparin, and 250 mg ampicillin. The infusion rate was 2 ml/h so that the fetus received 1 mg oPRL daily. In two fetuses, PRL was infused on two occasions (114–122 days and 129–134 days; 113–118 days and 129–135 days gestation) and in the third fetus, from 113 to 121 days gestation only. The timing of the infusions in relation to endogenous concentrations of plasma oPRL (4) and corticoids (12) and to tracheal surfactant production (12) in the normal ovine fetus may be seen in Fig. 1.

Assays. Incorporation of [³H]choline (New England Nuclear, Boston, Mass.) into lecithin by minced fetal rabbit lung was performed as previously described (14). Frozen lung specimens were extracted by the method of Folch et al. (15) and assayed for total phospholipid by the method of Bartlett (16) and for disaturated lecithin by the method of Mason et al. (17). DNA was determined by the procedure of Giles and

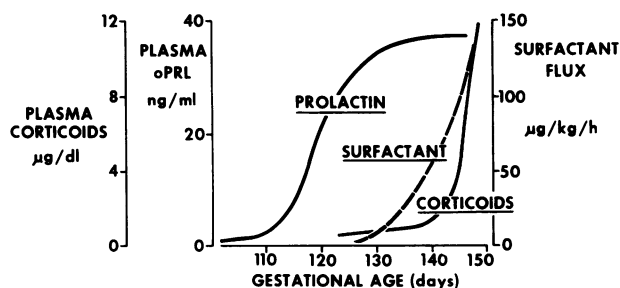


FIGURE 1 Normal developmental pattern of plasma PRL (4), tracheal fluid surfactant (12), and plasma corticoids (12) in fetal sheep.

Meyer (18), and protein was assayed by the method of Lowry et al. (19). oPRL was measured by homologous radioimmunoassay (4) using oPRL (from Dr. C. H. Li) as the standard. This assay showed no significant cross-reaction with ovine growth hormone or ovine chorionic somatomammotropin. Corticoids were determined by the competitive protein-binding assay (20), and corticosteroid-binding capacity was assayed by a charcoal procedure previously described (12). Dehydroepiandrosterone sulphate was measured by radioimmunoassay (21).

Surfactant was assessed by Langmuir-Wilhelmy balance as described previously (11, 12). The production rate was calculated as micrograms of surfactant per kilogram of estimated fetal weight per hour.

RESULTS

Rabbit experiments. Compared to the diluent-injected control littermates, PRL administration did not affect fetal growth or survival. Mean values \pm SE for control ($n = 24$) and PRL-treated ($n = 32$) fetuses, respectively, were body weight, 19.73 ± 0.97 vs. 18.61 ± 0.73 g; crown rump, 7.42 ± 0.13 vs. 7.33 ± 0.10 cm; viability, 77.4 vs. 76.3%; lung weight, 528 ± 32 vs. 541 ± 30 mg; lung DNA, 8.94 ± 0.71 vs. 8.59 ± 0.62 mg/g wet lung; and lung protein, 7.43 ± 0.14 vs. 7.91 ± 0.45 mg/mg DNA. Results for choline incorporation, phospholipid content, and plasma corticoids are presented in Table I. Compared with control littermates (diluent injected) we found no effect of PRL on the rate of choline incorporation into lecithin, lipid content, corticoid levels or plasma corticosteroid-binding capacity (not shown). The amount of phospholipid and disaturated lecithin was similar in unoperated, sham-operated, and both diluent and PRL-treated fetuses. Compared with unoperated controls, however, fetuses exposed to surgery and injection had higher rates of choline incorporation and elevated corticoids.

PRL immunoreactivity in fetal plasma was assayed using antibody to oPRL. The mean concentration of 44.9 ± 6.3 ng/ml (SE) for 20 PRL-injected fetuses was significantly higher ($P < 0.05$) than for diluent-injected littermates (18.8 ± 2.9 ng/ml, $n = 17$) and sham surgery fetuses (28.7 ± 1.8 ng/ml, $n = 17$).

Sheep experiments. Fig. 2 shows daily surfactant

TABLE I
Effect of PRL and Surgery on Choline Incorporation, Phospholipid and Disaturated Lecithin Content, and Corticoids in the Fetal Rabbit

Determination	Unoperated control	Sham surgery control	Diluent injected	PRL injected
Choline incorporation into lecithin, $\mu\text{mol/mg protein/h}$	33.2 \pm 1.2 (47)	52.2 \pm 5.1* (22)	45.6 \pm 2.3* (21)	50.2 \pm 3.1*† (24)
Phospholipid, $\mu\text{mol/g wet lung}$	13.56 \pm 0.52 (4)	14.62 \pm 0.31 (3)	13.8 \pm 0.59 (5)	13.74 \pm 0.95 (5)
Disaturated lecithin, $\mu\text{mol/g wet lung}$	2.48 \pm 0.16 (4)	2.30 \pm 0.22 (3)	2.49 \pm 0.14 (5)	2.23 \pm 0.15 (5)
Plasma corticoids, $\mu\text{g/dl}$	0.86 \pm 0.06 (36)	1.15 \pm 0.07* (20)	1.46 \pm 0.12* (21)	1.35 \pm 0.11*† (26)

Mean \pm SE values are shown with the number of determinations in parentheses. For choline incorporation and corticoids, the numbers in parentheses equal the number of fetuses examined. For lipid analyses, equal amounts of tissue were pooled from at least four fetuses per litter to provide one sample per litter.

* $P < 0.05$ compared with unoperated control (Student's unpaired t test).

† NS compared with diluent-injected and sham surgery control.

production rates and oPRL concentrations in the three fetuses throughout the study. During all five PRL infusions, oPRL rose rapidly to concentrations of 52–160 ng/ml (mean 101.7 \pm 5.5 ng/ml [SE]) and fell to normal concentrations within 24 h of stopping the infusion. In the normal fetus at term, the endogenous PRL concentration is 35.8 \pm 2.3 ng/ml (4). Surfactant was first detected at 126, 129, and 133 days gestation. In all three fetuses both the appearance and production rate of surfactant were within normal limits (12), and no stimulation of surfactant flux by oPRL infusion was detected. Arterial blood gases and pH were always within normal limits in fetuses 3804 and 3749. In fetus 3645, a period of unexplained hypoxia (PO_2 11–17 torr) was observed between 119 and 124 days but arterial gases were normal during both infusions. Fetuses 3645 and 3749 died in labor at 138 days gestation and fetus 3804 died at 131 days, probably secondary to fetal infection.

Fig. 2 also shows the concentrations of amniotic fluid PRL and fetal plasma steroids. Amniotic PRL rose in relationship to the infusions. We observed no changes in plasma concentrations of corticoids, dehydroepiandrosterone sulphate, or corticosteroid-binding globulin (not shown) related to the PRL infusions. PRL was not detected at any time in tracheal fluid, and the infusions did not alter the rate of tracheal fluid production (data not shown).

DISCUSSION

The rabbit experiments reported here were carried out according to the protocol of Hamosh and Hamosh (9),

and we used the same methods for determination of total phospholipid and disaturated lecithin. The levels of pulmonary phospholipid (13.8 $\mu\text{mol/g}$) and disaturated lecithin (2.49 $\mu\text{mol/g}$) found in our diluent-injected control animals are similar to their control values (12.16 and 2.3 $\mu\text{mol/g}$, respectively). In contrast to their results, however, we did not observe any effect of PRL treatment. We used the same dose and preparation of oPRL, and found it to be active by both immuno- and receptor assays. In addition, plasma PRL immunoreactivity 48 h after injection was elevated in treated animals, but not in control littermates, indicating that the hormone was absorbed. We are unable to explain the discrepancy between our results and those of Hamosh and Hamosh (9).

In the normal sheep fetus plasma PRL increases from low concentrations (<5 ng/ml) before 110 days gestation to concentrations >30 ng/ml after 130 days gestation (4). This rise precedes the increase in fetal pulmonary surfactant production (Fig. 1.) During the experiments reported in the present study, high plasma concentrations of PRL were achieved prematurely in the ovine fetus. However, no change in surfactant production was seen. Dexamethasone treatment of the ovine fetus using the identical experimental model stimulates surfactant production as early as 108 days gestation (11). The PRL infusions did result in elevated PRL concentrations in amniotic fluid, indicating that PRL can enter amniotic fluid from the fetal circulation.

It has been postulated (10) that PRL might be adreno-corticotrophic in the fetus. However, in the present study

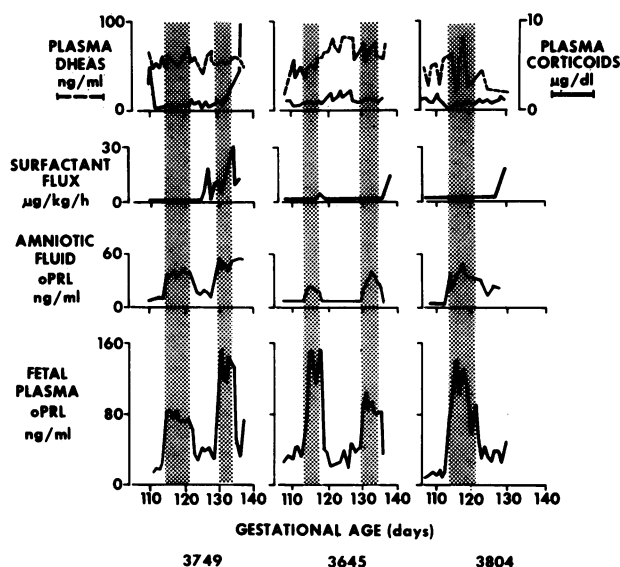


FIGURE 2 Effect of PRL infusion in the fetal sheep on tracheal fluid surfactant, oPRL levels in plasma and amniotic fluid, and plasma concentrations of corticoids and dehydroepiandrosterone sulphate (DHEAS). Daily determinations were carried out as described in Methods. The infusions of 1 mg/day of oPRL in three fetuses are shown by the shaded areas. The highest rate of surfactant production in fetus 3749 (30 $\mu\text{g/kg per h}$) is similar to the mean value for control animals at 135 days (Fig. 1). The rise in plasma corticoids observed in fetus 3749 between 134 and 138 days is most likely a preparturient surge.

there was no evidence that supraphysiological concentrations of PRL stimulated fetal adrenal steroid production in either the rabbit or sheep. Lowe et al. (22) also report no effect of PRL infusion on cortisol concentrations in fetal sheep. Plasma corticoid levels were elevated in rabbit fetuses exposed to surgery and injection; this increase may account in part for the greater rate of choline incorporation into lung lecithin in these animals (14).

Thus, in this study, we were unable to demonstrate any effect of PRL on either the amount of phospholipids in lung tissue or the secretion of surfactant into tracheal fluid. Moreover, we found no effect of PRL on plasma levels of adrenal steroids or corticosteroid-binding capacity. We conclude that PRL is neither a stimulator of the surfactant system nor adrenocorticotrophic in fetal rabbits and sheep at the gestational ages studied. This suggests that the observed relationship between PRL and RDS in the human (5, 6) is an indirect association. It is possible that this association reflects the influence of estrogen on both PRL secretion (7) and lung maturation (23).

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