Pressure-Volume Relationship and Fluid Content in Fetal Rabbit Lung after β -Receptor-Stimulating Drugs

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Summary

A considerable interest has been focused on the effects of various drugs on fetal and neonatal pulmonary maturation and adaptation. In the present study, we have investigated the effects of the selective β_1 - and β_2 -receptor-stimulating agents prenalterol and terbutaline on the pressure-volume relationship and fluid content in fetal rabbit lung at 28 days of gestation. Pressure-volume recordings during deflation showed significantly increased lung volumes at equivalent transpulmonary pressures in terbutaline-treated fetuses as compared to controls. No such effect was noted after prenalterol. In the control animals, wet lung weight/body weight ratio decreased to a steady state level 60 min after birth, indicating a rapid dehydration of the lungs. This dehydration was present at delivery after terbutaline and prenalterol treatment. The amount of fluid collected from the airways was also reduced after terbutaline and prenalterol treatment. The present results indicate facilitated neonatal respiratory adaptation after especially terbutaline treatment. Possible mechanisms behind these effects are discussed.

From studies on the pharmacologic action of sympathomimetic agents, Lands *et al.* (20) postulated the presence of two distinct types of β -receptors, β_1 and β_2 . Either of these receptors was considered to be present in different organs, *e.g.*, the β_1 -type in the heart and the β_2 -type in the bronchioles and uterus. However, more recent studies (9, 10) have demonstrated that both types of receptors are present in the heart and lung tissue. In accordance with these findings, the net effect after β -receptor stimulation depends on the local relationship between the two types of receptors. The presence of β -receptors in the fetus has been demonstrated in various species at an early gestational age (22).

Antenatal corticosteroid treatment is for the time being the usual therapeutic regimen for acceleration of fetal lung maturation. However, there is now both clinical and experimental evidence that lung adaptation in the preterm neonate can be enhanced by alternative pharmacologic methods, such as β -mimetic agents (3, 5, 7, 16). Studies on the fetal rabbit lung using inflation-deflation recordings have shown that the deflation curve correlates particularly well with other parameters as an indicator of lung distensibility. Wyszogrodski *et al.* (25) have shown that administration of the relatively unselective β -mimetic, isoxuprine, causes enhanced retention of air in the rabbit lung during the deflation phase.

The aim of the present investigation was to study the effects of two selective β_1 - and β_2 -receptor-stimulating agents, prenalterol and terbutaline, on fluid content and pressure-volume relationship in the fetal rabbit lung, with particular emphasis on the deflation phase of the recordings.

MATERIALS AND METHODS

Twenty-six white and black rabbits of Danish rural breed were mated with two bucks under direct observation. Copulation was

considered as zero time of gestation. The does were kept in separate cages in the department and fed on standard pellets and water *ad libitum*. Three days before term, at 28 days of gestation, the does were anesthetized with ether administered by open mask. The uterus was exposed by a midline abdominal incision. Terbutaline (0.1 mg in 0.1 ml normal saline), prenalterol (0.1 mg in 0.1 ml normal saline), or normal saline were alternatively injected IM into the fetuses through the intact uterine wall. The abdominal incision was closed with a continuous suture, and the does were allowed to recover. After 3 hr, the abdomen was reopened, and the fetal rabbits were delivered by hysterotomy.

The fetuses used in the pressure-volume measurements and those used for fetal pulmonary fluid (FPF) collection were allowed to remain within the amniotic sac to prevent air breathing, whereas the other fetuses were removed and allowed to breathe for periods ranging from 15 to 120 min.

After weighing the newborn carcass, the trachea was exposed, and a polyethylene tubing (Portex pp 90) was inserted. The tube was attached through a Y-connection to a narrow-bore (3 mm) water manometer and a calibrated gas-tight syringe containing 5.0 ml of air. The FPF was evacuated by applying a negative pressure of 5 cm H₂O for 5 min. The tube including FPF, both with known weight per cm, was then removed and weighed or left *in situ* and measured. Before the pressure-volume recordings were performed, the FPF was restored into the bronchial tree by increasing the pressure to 0 cm H₂O. The lungs were inflated and deflated with air in pressure steps of 5 cm H₂O, and the volume readings of the syringe were made after 2 min of equilibration at each pressure interval. Correction was made for the volume displaced into the manometer and for the compression of air. The volume of air in the lungs at 35 cm H₂O was defined as total lung capacity (TLC). The occasional presence of a pneumothorax was indicated by failure of equilibrium at maximal inflation and confirmed by opening the abdominal cavity to inspect the diaphragms. Recordings were performed in all animals within 3 hr after death, an interval reported not to change the pressure-volume curves (18).

In all animals, the thorax was opened, and the lungs were extracted and weighed. The lungs were desiccated by exposure to 100°C in a drying oven for 24 hr and reweighed to obtain dry lung weight. The ratio of wet lung weight (WLW) to body weight (BW) was calculated from the measurements of BW and WLW including FPF.

Several of the animals not allowed to breathe were used for all measurements (pressure-volume, FPF, and WLW/BW). However, for technical reasons such as preparation errors, animals left more than 3 hr after death, and pneumothorax, some fetuses were used for only one or two of these procedures.

In the statistical analysis of all data, Student's *t* test was used.

RESULTS

The study included 73 controls, 81 terbutaline, and 53 prenalterol-treated animals (Table 1). No differences were noted in mean body weight or dry lung weight between the different groups.

PRESSURE-VOLUME RECORDINGS

The pressure-volume curves at 28 days of gestation expressed as percentage of TLC and as ml air per 100 mg dry lung weight are shown in Figures 1 and 2.

The different transpulmonary pressure levels expressed as a percentage of TLC did not differ between the control, terbutaline, or prenatalerol groups during inflation. At deflation, a significantly higher volume of air was retained in the lungs of the terbutaline-treated fetuses compared to the controls. At low-pressure intervals,

Table 1. Details of material

Treatment	No. of fetuses ¹	BW (g)	Dry lung wt (mg)
Terbutaline	81	35.5 ± 0.7 ² NS	116.5 ± 3.1 NS
Prenalterol	53	34.8 ± 0.7 NS	118.5 ± 3.5 NS
Control	73	35.3 ± 1.1	111.9 ± 3.9

¹ Number of litters = 26.

² Mean ± S.E.

LUNG VOLUME - % OF TLC

Fig. 1. Pressure-volume curves in fetal rabbit lung at 28 days of gestation expressed as a percentage of total lung capacity. Mean ± S.E. *, $P < 0.05$; **, $P < 0.01$.

CORR. VOLUME / 100mg DRY LUNG WEIGHT

Fig. 2. Pressure-volume curves in fetal rabbit lung at 28 days of gestation expressed as ml air per 100 mg dry lung weight. Mean ± S.E. *, $P < 0.05$; **, $P < 0.01$.

the difference was approximately 10%. No such difference was noted between the prenatalerol and the control animals during deflation (Fig. 1).

When the pressure-volume recordings were expressed as ml air per 100 mg dry lung weight, although the differences were not significant, the TLC in the terbutaline group tended to increase compared to the control group, but the opposite tendency was noted in the prenatalerol-treated animals. The pressure-volume curves of the fetuses given injections of terbutaline 3 hr before delivery showed greater retention of air (ml/100 mg dry lung weight) both at inflation and deflation compared to controls. In the prenatalerol-treated group, the inflation curve did not differ from the control recordings, but at deflation, a significantly lower lung volume was noted at 5 and 15 cm H₂O.

PULMONARY FLUID CONTENT

The fluid content in the lungs, expressed as WLW/BW × 1000, decreased rapidly after birth in the control animals. During the first 60 min after birth, WLW/BW × 1000 decreased approximately 25% to a steady state level (0 min versus 60 or 120 min; $P < 0.001$). The lungs of the terbutaline-treated fetuses were at the same steady state level as those of the control animals already at the time of birth. This phenomenon, although not so pronounced, was also noted in the fetuses which had received prenatalerol 3 hr before birth (Fig. 3).

The amount of fluid collected from the bronchioalveolar space at birth from the controls, the terbutaline-, or prenatalerol-treated fetuses is shown in Figure 4. A 65% reduction of FPF was noted after terbutaline treatment as compared to controls. In the fetuses given prenatalerol 3 hr before birth, FPF was reduced by approximately 50%.

Fig. 3. Lung fluid content during the first 2 hr after birth expressed as mg WLW per g BW. Mean ± S.E. *, $P < 0.01$; ***, $P < 0.001$.

FETAL PULMONARY FLUID

Fig. 4. Fetal pulmonary fluid collected from the bronchioalveolar space at birth. Mean ± S.E. Numerals, number of experiments. *, $P < 0.05$; **, $P < 0.01$.

DISCUSSION

The relationship between β_1 - and β_2 -receptors in the organism varies between different organs and different individuals (13). The dominating β -receptor type in the lungs is of the β_2 -type (9, 20). In the present study, the terbutaline-treated animals had an increased TLC compared to the prenatalerol group. A tentative explanation to this finding might be the β_2 -mediated dilatation of the bronchioles. The group of animals treated with prenatalerol tended to decrease in TLC compared to the controls which might be explained by the evidence that this substance seems to have a weak β_2 antagonistic effect (8). Thus prenatalerol might antagonize the surfactant-releasing and bronchodilating effect of endogenously secreted catecholamines which occurs at birth (19).

The volume of air present in the lung at low transpulmonary pressures has been shown to correlate well with other parameters of measuring alveolar surface active material (11). The percentage of air retained in the fetal lungs at low transpulmonary pressures at deflation after terbutaline treatment was significantly increased compared to the controls. No such effect was present after prenatalerol administration. This finding is indicative of increased presence of surface active material in the fetal lungs after β_2 -adrenoceptor stimulation. In fact, in a previous study, we have actually found an increased amount of lecithin in alveolar lavage of 28-day-old fetuses whose mothers had received terbutaline (5). Similarly, other authors have found an increased L/S ratio in alveolar lavage fluid in 28-day-old rabbit fetuses treated with unselective β -mimetics, such as isoxuprine (12, 15). The mechanism behind the increased amounts of surfactant phospholipids on the alveolar lining in the 28-day-old fetal rabbit lung is probably due to an enhanced release and possibly also to an enhanced synthesis of surfactants, which might be caused by the β -adrenergic-mediated increase in intracellular content of cyclic adenosine 3':5'-monophosphate (2). The corticosteroid receptors, which appear in the rabbit fetal lungs around day 20 of gestation and increase in number to reach a maximum by day 28, do not increase in number after treatment with β -mimetic agents. The plasma levels of corticosteroids in rabbit fetuses are also unaffected by β -receptor-stimulating agents (6). In the study of Bichler *et al.* (6), β -receptor-stimulating agents had no effect on the dipalmitoyllecithin levels in alveolar lavage or lung homogenate of fetal rabbits. However, this study was performed on the 25th and 30th days of gestation. The lack of effects is not surprising because on the 25th day of gestation the rabbit fetal lung is not yet morphologically mature, and on the 30th day of gestation, biochemical maturation is already completed. Thus, the effect of β -mimetics to increase the release and possibly also the synthesis of surfactants seem to be limited to some few days of gestation. In the rabbit fetus, β -receptor-stimulating agents seem to be effective around the 28th day of gestation, when the lung is morphologically but not biochemically mature (14, 17). This statement is further supported by the findings of Wyszogrodski *et al.* (25) who showed an improved pulmonary adaptation as measured by pressure-volume characteristics after isoxuprine in 28-day-old, but not in 26 or 27-day-old rabbit fetuses. On the 29th day of gestation, the biochemical maturation seems to be completed, and further increase in lung distensibility cannot be achieved (17).

In the rabbit fetus, there is a decrease in the WLW/BW ratio during the first 2 hr after birth, indicating a dehydration of the lungs. Previous studies have shown that adrenergic agonists, such as adrenaline, isoprenaline, and isoxuprine, cause a dehydration of the fetal lung (1, 12, 24). In the present study, we can demonstrate that both β_1 - and β_2 -receptor-stimulating agents can reduce the WLW/BW ratio and the amount of FPF at the time of birth. A reduced WLW/BW ratio after terbutaline and prenatalerol was noted as early as 15 to 30 min of postnatal life. After 60 min, when the control animals had reached a steady state level, no further differences were noted between the prenatalerol- or terbutaline-treated and control animals. Moreover, there is evidence that endogenous catecholamines, and thus β -stimulation, are very high in the blood during labor and at the time of birth (19). By

giving β -mimetics prenatally, we might therefore mimic the increased activity of the adrenal medulla which occurs at birth and thus precede the beneficial effect on the postnatal pulmonary adaptation by naturally occurring catecholamines.

The exact mechanism behind this β -mimetic-mediated response is not fully known. Several tentative explanations are possible, such as changes in pulmonary hemodynamics, direct effects on the respiratory epithelium responsible for secretion of lung liquid, or effects on the postcapillary venular leakage of fluid (12, 21, 23, 24). However, earlier studies by Olver and Strang (21) indicate that changes in fetal pulmonary blood pressure do not influence the rate of production of FPF, and therefore, it seems more likely that β -mimetics have a direct effect on the production of FPF by the respiratory epithelium or disappearance of fluid by the postcapillary venular leakage sites.

In summary, the present study demonstrates that both β_1 - and β_2 -receptor-stimulating agents enhance the pulmonary dehydration in the immediate postnatal period. The β_2 -receptor agonist, terbutaline, also caused an increased pulmonary distensibility, which might be beneficial in early postnatal pulmonary adaptation.

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