Stimulation of Surfactant Production by Oxytocin-Induced Labor in the Rabbit

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ABSTRACT The respiratory distress syndrome is believed to be due to insufficient surfactant. It is known that there is a greater incidence of the respiratory distress syndrome among infants delivered by cesarean section before labor than among those delivered after labor at the same gestational age. The purpose of this study was to determine the effect of labor on the production of pulmonary surfactant.

We measured the phospholipid content of lung lavage in newborn rabbits delivered by cesarean section before labor at 29, 30, and 31 (full-term) days gestation and after oxytocin-induced labor at 31 days. We also measured the activities of pulmonary cholinephosphate cytidylyltransferase and cholinephosphotransferase, enzymes involved in the de novo synthesis of phosphatidylcholine, the major component of surfactant.

There was a two- to fourfold increase in the amount of lung lavage phospholipid during the first 6 h after birth. This was not dependent upon gestational age at delivery. There was a further two- to fourfold increase in the next 18 h which was, however, dependent upon gestational age. Labor increased the amount of lavage phospholipid from rabbits delivered at full term by 132%, 177%, and 50% at 3, 6, and 24 h after birth, respectively.

There was a postnatal increase in the activity of cholinephosphate cytidylyltransferase. This was almost linear with time during the first 12 h, by which time essentially adult values were attained. Cholinephosphate cytidylyltransferase was not affected by labor. There was also a postnatal increase in the activity of cholinephosphotransferase but this was stimulated 86%, 59%, and 21% by labor at 0, 1, and 24 h after birth, respectively.

These studies suggest that labor stimulates both the synthesis and secretion of surfactant in the immediate postnatal period and thus may be an important factor in the prevention of the respiratory distress syndrome of the newborn.

INTRODUCTION

Pulmonary surfactant is the phospholipid-rich material which is thought to line the alveoli and prevent atelectasis (3). Surfactant is synthesized by the fetal lung towards the end of gestation and seems to be largely stored until birth. Insufficient surfactant is believed to be the principal contributing factor to the respiratory distress syndrome of the newborn, the major cause of morbidity and mortality among premature infants in developed countries (3). The factor(s) which promote surfactant release are not known. We have previously shown that there is a 10-fold increase in the amount of phospholipid in lung

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lavage liquid (largely surfactant) during the first 24 h after natural birth (4). Associated with this there is a postnatal increase in the activities of pulmonary cholinephosphate cytidylyltransferase and cholinephosphotransferase (4), enzymes involved in the synthesis of phosphatidylcholine which is the major surface-active component of surfactant (3). We now report that the initial rate of surfactant release in the first 24 h after birth is markedly stimulated by labor. In addition, labor stimulates pulmonary cholinephosphotransferase activity. These data suggest that labor stimulates both the synthesis and release of surfactant and thus may be an important factor in the production of surfactant and in the prevention of the respiratory distress syndrome in the newborn.

METHODS

Animals. Timed pregnant rabbits (New Zealand white) were purchased from Glochester Rabbitry, Chepachet, R. I. At 29, 30, or 31 (full term) days gestation the doe was sacrificed with an i.v. injection of sodium pentobarbital (80 mg/kg) and the fetuses were immediately delivered by cesarean section. When labor was to be induced, the doe was administered an i.m. injection of 1.5 U (U. S. Pharmacopeia) of oxytocin (Pitocin; Parke-Davis & Co., Detroit, Mich.) at 31 days. Delivery took place 5–15 min later. Animals that did not deliver in this period of time did not deliver for at least a further 24 h and were excluded from the study. After vaginal delivery of the first newborn, labor was assumed to have taken place, the doe was administered sodium pentobarbital, and the remaining fetuses were delivered by cesarean section.

The newborns were killed with an i.p. injection of sodium pentobarbital (30 mg) either before breathing, while still in their amniotic sacs, or after breathing for various periods of time during which they were kept in an incubator at constant temperature (31°C) and humidity. All animals survived equally well during this period. There was no evidence of respiratory distress.

Lung lavage phospholipid analysis. After sacrifice of the newborn the trachea was immediately cannulated and the lungs were lavaged in situ with 0.9% NaCl (4). The lavage liquid was centrifuged at 1,000 *g* for 15 min, to remove cellular material, and lyophilized. Lipids were extracted from the lyophilized material with chloroform-methanol-water and the phospholipids were quantitated by phosphorus assay as described previously (4). The lavaged lung tissue was also lyophilized and weighed. Phosphatidylcholine was quantitated by phosphorus assay after separation by thin layer chromatography on silica gel in chloroform-methanol-7 M NH_4OH (60:35:5, by vol).

Enzyme assays. The lungs were immediately excised and homogenized in 0.33 M sucrose-0.01 M Tris HCl-0.001 M EDTA, pH 7.4, in a Potter-Elvehjem Teflon-glass apparatus. Cholinephosphotransferase (EC 2.7.8.2) was assayed in the homogenate as described previously (5) except that the reaction was carried out at pH 8.5 and 19 mM 1,2-dioleolyglycerol and 0.08 mM CDPcholine were used as substrates. Cholinephosphate cytidylyltransferase (EC 2.7.7.15) was assayed in the 105,000-*g* supernate as described previously (4).

Statistics. Statistical analysis was by *t* test for independent variables.

RESULTS

The phospholipid content of lung lavage from newborn rabbits delivered by cesarean section before labor at 29, 30, and 31 days gestation is shown in Table I. In the first 6 h after birth there was a two to fourfold increase in the amount of lavage phospholipid. The differences between the 0- and 6-h values were significant ($P < 0.005$) in all three groups. There was no significant difference between the three groups during this period, with the exception of the newborns delivered at 29 and 31 days which differed at 1 h ($P < 0.001$) and 3 h ($P < 0.025$), indicating that this initial increase was largely independent of gestational age at delivery. There was a further two to fourfold increase during the period 6–24 h after birth but this was dependent upon gestational age. The highest level of lavage phospholipid observed during the 24-h period examined was reached after 18 h by the animals delivered at 31 days and after 24 h by those delivered at 30 days. The newborns delivered at 29 days, on the other hand, had attained only 70% of this level by 24 h.

The phosphatidylcholine content of lung lavage is shown in Table II. Phosphatidylcholine accounted for about 80% of the total phospholipid except before breathing in the newborns delivered at 29 and 30 days.

Labor substantially increased the amount of phospholipid in lung lavage from full-term newborn rabbits (Fig. 1). There was no significant difference between the two groups before breathing. After 3 and 6 h, however, there was two–three times as much phospholipid in the lavage from the newborns de-

TABLE I
The Phospholipid Content of Lung Lavage from Newborn Rabbits during the First 24 h after Birth

Hours after birth	Gestational age at delivery		
	29 days	30 days	31 days
h	*μg phospholipid phosphorus/g lung dry wt*		
0	35±6 (6)	47±8 (7)	49±8 (4)
1	45±3 (5)	71±13 (10)	80±5 (4)
2		69±12 (5)	
3	74±8 (5)	85±13 (11)	127±18 (4)
6	126±21 (4)	111±14 (11)	104±10 (4)
12	160±13 (7)	105±7 (6)	
18	218±29 (8)	193±27 (6)	373±24 (5)
24	273±20 (7)	397±31 (15)	395±18 (4)

The fetuses were delivered by cesarean section before labor as described in Methods. Newborns from six to nine litters were randomized at each gestational age. The lavage from each newborn was analyzed separately. The data are the means±SE from the number of newborns indicated in parentheses.

TABLE II
Phosphatidylcholine Content of Lung Lavage from Newborn Rabbits

Hours after birth	Gestational age at delivery		
	29 days	30 days	31 days
h	*% total phospholipid phosphorus*		
0	65.2±2.5 (3)	72.7±2.3 (3)	83.0±2.0 (2)
3	78.0±2.4 (3)	76.8±0.8 (5)	78.0±1.0 (2)
6	74.6±1.7 (3)	79.4±1.0 (5)	82.3±0.8 (2)
24	76.3±2.3 (3)	80.3±0.8 (5)	83.2±1.1 (2)

The fetuses were delivered by cesarean section before labor as described in Methods. Newborns from two to four litters were randomized at each gestational age. Lavage from 1 to two newborns was pooled for analysis at each time point. The data are the means±SE with the number of samples in parentheses.

livered after labor. These differences were statistically significant ($P < 0.02$). Thus, labor probably stimulates the secretion of surfactant into the alveoli in the immediate postnatal period.

That labor may also stimulate surfactant synthesis was suggested by the fact that there was significantly more ($P < 0.00005$) phospholipid 24 h after birth in the lavage from the animals delivered after labor than in that from those delivered before it. It is probably reasonable to assume that the surfactant stored before birth is essentially all released by 18 h, since there was no significant difference in lavage phospholipid content between 18 and 24 h in the animals delivered at 31 days before labor. Therefore, the further increase at 12, 18, and 24 h in the animals delivered after labor may be due to increased synthesis. Our earlier studies (4) also suggested that synthesis of lung phospholipids may be stimulated in the postnatal period since there was an increase in the activities of cholinephosphate cytidylyltransferase and cholinephosphotransferase between 31 days gestation and 24 h after spontaneous labor and delivery. To determine if labor stimulates these enzymes, we measured their activities in the newborn lung. Fig. 2 shows that there was a postnatal increase in the activity of cholinephosphate cytidylyltransferase. This increase was essentially linear with time during the first 12 h, after which adult activities (4) were reached. Labor had no effect on the activity of cholinephosphate

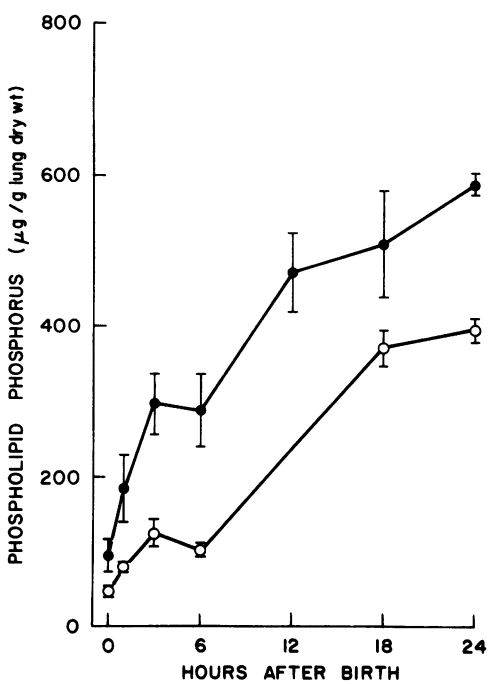

FIGURE 1 The phospholipid content of lung lavage from newborn rabbits delivered at 31 days gestation before labor (○) and after oxytocin-induced labor (●). Newborns from four and seven litters, respectively, were randomized in these experiments. The lavage from each newborn was analyzed separately. Each point is the mean value from four–six newborns. The bar represents±SE.

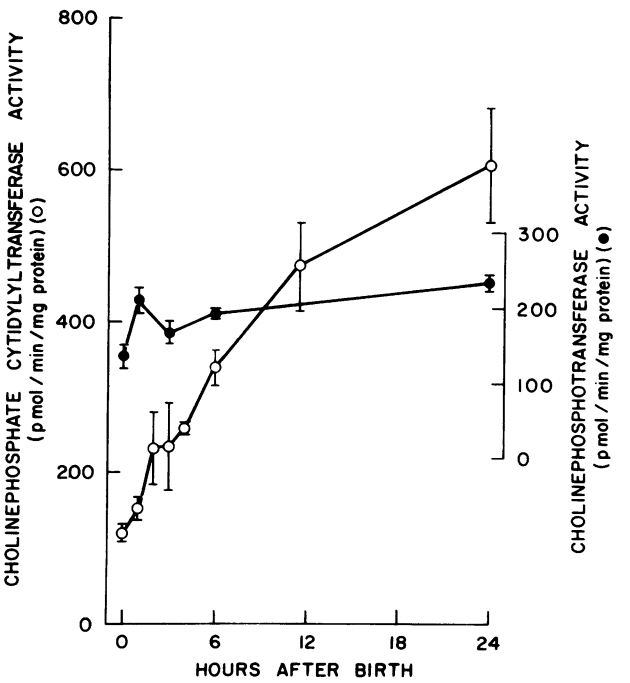

FIGURE 2 Activities of cholinephosphate cytidylyltransferase (○) and cholinephosphotransferase (●) in newborn rabbit lung. The animals were delivered at 31 days gestation before labor. At each time interval cholinephosphate cytidylyltransferase activity was measured in the lungs from two–six groups of five–eight newborns each, obtained from a total of 17 litters, while cholinephosphotransferase activity was measured in the lungs from four–seven individual newborns from 3 litters. Mean values are reported. The bar represents±SE.

cytidylyltransferase, however. Fig. 2 also shows that there was a 75% increase in the activity of cholinephosphotransferase between 0 and 24 h after birth ($P < 0.001$). Cholinephosphotransferase activity was further stimulated by labor (Table III). The effect of labor was greatest before breathing but was also apparent after 24 h.

DISCUSSION

The fetal lung begins to synthesize surfactant towards the end of gestation (6). Most of it, however, appears to be stored, presumably in lamellar inclusion bodies in type II pneumocytes (7), with little released into the alveoli until after birth. Earlier morphological (8) and physiological (9) studies had suggested that there was a massive release of surfactant shortly after birth. Our results show that there is not such an initial release of phospholipid but rather a gradual release which is essentially linear with time during the first 24 h after birth. During this period phosphatidylcholine, the major component of surfactant (3, 6), accounted for about 80% of the lavage phospholipid.

During the first 6 h after birth there was little difference between the newborns delivered at 29, 30, or 31 days with respect to lung lavage phospholipid content, indicating that even the animals delivered at 29 days had sufficient surfactant stored for release during this period. The lack of sufficient surfactant in the premature animals, however, became apparent in the 6–24-h period. These findings in the newborn rabbit are reminiscent of the clinical situation in the human in that symptoms of respiratory distress often do not appear, particularly in the less immature infant, for several hours after birth (10).

TABLE III
The Effect of Labor on Newborn Rabbit Lung Cholinephosphotransferase Activity

Hours after birth	0	1	24
		pmol/min/mg protein	
Delivery before labor	134±15 (7)	209±17 (4)	234±10 (5)
Delivery after induced labor	249±23 (4)	333±15 (4)	282±5 (3)
Percent stimulation by labor	86	59	21
P value	<0.005	<0.002	<0.02

The rabbits were delivered at 31 days gestation either before labor (three litters) or after oxytocin-induced labor (two litters) as described in Methods. Lungs from each newborn were analyzed separately. The data are the means±SE with the number of samples in parentheses.

The amount of surfactant phospholipid required for normal lung function is not known. Despite the differences in phospholipid content of lung lavage, the newborns delivered at the different gestational ages survived equally well for 24 h. No difficulty in breathing was seen. Thus, it is likely that more surfactant than the minimum required is released in the immediate postnatal period.

It has long been recognized by clinicians that delivery by cesarean section before labor results in a greater incidence of the respiratory distress syndrome than vaginal delivery or delivery by cesarean section after labor (3, 10, 11). This is most apparent, in the human, at 37–38 wk gestation and less apparent at 31–33 wk (10), presumably because the risk of respiratory distress per se is greater at the earlier gestational age due to pulmonary immaturity and consequent insufficient storage of surfactant. Despite these clinical observations, the effect of labor on surfactant production had not been examined. Recently, however, two groups (12, 13) reported that both the concentration of phosphatidylcholine and the phosphatidylcholine/sphingomyelin ratio in amniotic fluid, indexes of fetal lung maturation (14), were increased during labor in humans.

The present studies in the rabbit demonstrate that oxytocin-induced labor increases both the initial rate of release of phospholipid into the alveoli as well as the total amount released in the first 24 h after birth. In addition, it stimulates the activity of cholinephosphotransferase, the enzyme which catalyzes the final step in the de novo synthesis of phosphatidylcholine. These findings suggest that labor stimulates both the synthesis and release of surfactant in the immediate postnatal period.

Onset of spontaneous labor in the rabbit is dependent upon a sudden release of oxytocin (15, 16). We, therefore, used oxytocin to induce labor in these experiments. Driscoll and Yen (17) have reported that oxytocin has no effect on the incidence of the respiratory distress syndrome. It has also been reported that spontaneous and induced labor are similar in this respect (11, 17). Craven et al. (12) recently reported that spontaneous and oxytocin-induced labor are similar with respect to changes in amniotic fluid phosphatidylcholine content. It is likely, therefore, that spontaneous labor has the same effect on surfactant production as that induced with oxytocin.

The mechanism by which labor stimulates surfactant production remains to be established. However, since it is known that stress stimulates fetal lung maturation and surfactant production (18, 19), as well as the activity of pulmonary cholinephosphotransferase (18), it is probable that labor and other forms of stress operate via a common mechanism. It is known, for instance, that pharmacological agents which may be

released endogenously in response to stress accelerate fetal lung maturation and surfactant production. These include cortisol (3, 5, 18, 20, 21), thyroxine (22, 23), adenosine 3',5'-monophosphate (24–26), and catecholamines (27–29). It is possible that such agents may have multiple effects on the surfactant system. It is likely that synthesis and secretion, for instance, are affected by different agents. There is evidence that cortisol (5, 18, 30–34) and adenosine 3',5'-monophosphate (24, 25) stimulate surfactant synthesis while thyroxine may stimulate secretion (35). In addition, Massaro (36) has recently reported that secretion of a protein into the surface-active fraction of lung lavage is under neurohumoral control. Further studies are needed to elucidate the effects of the various components of stress on surfactant production.

Factors other than stress may also be involved. The postnatal increase in cholinephosphate cytidylyltransferase activity, for instance, was independent of labor. Similarly, although injection of fetal rabbits with saline resulted in a greater than threefold increase in the activity of pulmonary cholinephosphotransferase, cholinephosphate cytidylyltransferase activity actually decreased (18). It is, therefore, likely that these enzymes are influenced by different factors. Stern et al. (37) recently reported that cholinephosphate cytidylyltransferase from fetal rat lung supernate is stimulated by phosphatidylinositol and phosphatidylserine. These workers also reported increased amounts of these phospholipids in the supernatant fraction during the last 4 days of gestation and 1st day after birth and suggested that this accounts for the postnatal increase in cholinephosphate cytidylyltransferase activity in the rat (37). The postnatal increase in the same enzyme in the rabbit lung may also be due to stimulation by acidic phospholipids in the supernatant fraction. However, we previously reported (4) that there is a decrease in the amount of phosphatidylinositol and phosphatidylserine combined in lavaged rabbit lung during the period 30 days gestation to 2 days after birth. Subcellular distribution of the phospholipids, however, was not examined in that study.

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