Alveolar fluid clearance in late-gestational guinea pigs after labor induction: mechanisms and regulation

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Norlin, Andreas, and Hans G. Folkesson. Alveolar fluid clearance in late-gestational guinea pigs after labor induction: mechanisms and regulation. Am J Physiol Lung Cell Mol Physiol 280: L606–L616, 2001.—We tested the hypothesis that labor-induced epinephrine release would stimulate alveolar fluid clearance in preterm fetuses. Preterm fetuses were obtained by cesarean section from timed-pregnant guinea pigs at 61–69 days postconception. Fetal guinea pigs were euthanized and placed on continuous positive airway pressure oxygenation, and an isosmolar 5% albumin solution was instilled. Alveolar fluid clearance was measured over 1 h. The fetal lung began to absorb fluid at 64–66 days postconception, and at birth, alveolar fluid clearance quadrupled. Baseline alveolar fluid clearance when present was sensitive to propranolol inhibition and depended on β-adrenergic stimulation. Measurements of plasma epinephrine in fetal animals confirmed high epinephrine levels in 66- to 69-day postconception fetuses. Prenatal alveolar fluid clearance when present was highly amiloride sensitive, suggesting that amiloride-sensitive Na+ channels were critical. Oxytocin-induced labor initiated an amiloride- and propranolol-sensitive net alveolar fluid clearance in 61-day-gestation animals. Moreover, oxytocin induced significant epinephrine release in all fetuses. These results have clinical implications for infants delivered by cesarean section before the onset of labor. Use of pharmacological agents to induce labor may reduce the occurrence and severity of perinatal respiratory distress.

alveolar epithelium; β-adrenergic stimulation; infant respiratory distress syndrome; prenatal development; epinephrine; sodium transport

NORMAL ALVEOLAR DEVELOPMENT requires fluid-filled fetal lungs, and gas exchange occurs through the placental cord (30). The lung fluid is produced and secreted by the pulmonary epithelium by Na+-coupled Cl− secretion (41). This fluid must be removed to allow gas exchange across the alveolar epithelial-endothelial barrier at the transition from placental to pulmonary gas exchange. Absorption of alveolar fluid is driven by Na+ absorption in newborn (16) and adult (32) guinea pig lungs. Transepithelial Na+ absorption is driven by the basolateral Na+-K+-ATPase (15, 23) and is dependent on apical Na+ channels (17, 22, 28, 29). Studies have suggested that fluid absorption occurs in near-term lamb (6, 40), guinea pig (16, 34), and rabbit (7) lungs. Na+ transport pathways have been demonstrated to be developmentally regulated in other rodent models of lung development (12, 33), whereas no functional physiological studies of fluid transport in small rodents have been carried out. Regulation of lung fluid transport during late prenatal and early postnatal development is poorly understood, but several hormones and other factors are proposed to be involved. Cortisol and triiodothyronine (T3) stimulate lung fluid clearance in lambs (3) and adult rats (18). Epinephrine rapidly increases alveolar fluid clearance in newborn guinea pig (16) and lamb (40) lungs. Little is known about the signals that initiate fluid absorption in fetal lungs. Thus our first aim was to investigate normal late-gestational development of alveolar fluid clearance in preterm guinea pigs. Fetal guinea pigs of gestational ages 61–69 days (term = 69 days) were obtained by cesarean section from timed-pregnant guinea pigs, and alveolar fluid clearance was studied by measuring the change in the concentration of an instilled albumin solution over 1 h. Because epinephrine stimulates alveolar fluid clearance at birth in guinea pigs (16), our second aim was to investigate the time point when alveolar fluid clearance becomes sensitive to endogenous β-adrenergic stimulation as well as to measure plasma epinephrine levels. Because labor releases catecholamines from the adrenal glands (11) and catecholamines are involved in the stimulation of alveolar fluid clearance (16), our third aim was to investigate whether preterm labor induced fluid absorption in fetal lungs. Preterm labor was induced by injecting oxytocin into the timed-pregnant guinea pigs followed by measurement of alveolar fluid clearance in the fetuses. Plasma epinephrine levels were also measured after labor induction. The acute effects on alveolar fluid clearance from oxytocin instilled into the lungs were also investigated. A fourth aim was to study the development of the fractional contribution of amiloride-sensitive transport to alveolar fluid clearance under normal conditions and after premature induction of labor with oxytocin.

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MATERIALS AND METHODS

Animals and Oxytocin Pretreatment

Preterm and newborn Dunkin-Hartley guinea pigs (n = 251 from 60 litters; Sahlins Forsöksdjursfarm, Malmö, Sweden) were used. The timed-pregnant guinea pigs were maintained on 12:12-h day-night rhythm and had free access to food (standard guinea pig chow, Special Diet Services, Witham, UK) and tap water. The Committee on Animal Experiments at Lund University (Lund, Sweden) approved this study.

Preterm labor was induced by subcutaneous oxytocin injections (1 mg/kg body weight; Ferring, Malmö, Sweden) every 15 min for 45 min. The fetuses were delivered by cesarean section (see Surgical Preparation) after 45 min if a normal vaginal delivery did not occur. Timed-pregnant guinea pigs with 1 and 3 days gestation remaining usually delivered their fetuses vaginally within 45 min after oxytocin (68 days gestation, 100% vaginal delivery; 66 days gestation, 98% vaginal delivery), whereas fetuses of timed-pregnant guinea pigs with 5 and 8 days gestation remaining were delivered by cesarean section (64 days gestation, 98% cesarean section).

The oxytocin dose was adapted from the clinical dose for labor induction in humans (9). Separate sets of timed-pregnant guinea pigs (gestational ages 61 and 66 days) were injected with the vehicle (0.9% NaCl) used to dissolve oxytocin to control for effects from the injections per se. Timed-pregnant guinea pigs that were injected with vehicle or were not injected were all delivered by cesarean section at all gestational ages.

Solutions

A 5% albumin solution was prepared by dissolving 50 mg/ml of bovine serum albumin (Sigma, St. Louis, MO) in 0.9% NaCl. In some studies, the Na+ channel inhibitor amiloride (1 mM; Sigma) or the general β-adrenergic receptor antagonist propranolol (0.1 mM; Sigma) was added to the instillate.

Surgical Preparation

The timed-pregnant guinea pigs were anesthetized by intraperitoneal injections of pentobarbital sodium (120 mg/kg body weight; Apoteksbolaget, Umeå, Sweden) and euthanized by intracardiac injections of 60 mg of pentobarbital sodium. A laparotomy was rapidly done, and the fetuses were carefully delivered. The umbilical cord was ligated to prevent bleeding. The fetuses were immediately euthanized with intraperitoneal pentobarbital sodium (12 mg) with 500 IU of heparin (Lövens, Ballerup, Denmark). Newborn guinea pigs were euthanized by intraperitoneal pentobarbital sodium (18 mg) with 500 IU of heparin.

After euthanasia, an endotracheal tube (PE-190, Clay Adams, Becton, Dickinson, Parsippany, NJ) was inserted through a tracheostomy. The animals were immediately connected to a constant oxygen flow (oxygen fraction 1.0; AGA, Lidingö, Sweden), and the lungs were expanded by adjusting the oxygen flow to a constant positive airway pressure (CPAP) of 5 cmH2O. The whole surgical procedure after euthanasia required 5 min. The animals were placed in-between heating pads to maintain body temperature during the experiments. A rectal temperature probe measured body temperature, and heating was adjusted to maintain the temperature at 37–38°C. Airway pressure was continuously monitored by calibrated pressure transducers (UPI model 1050B or TSD104A, BioPac Systems, Goleta, CA) and analog-to-digital converters and amplifiers (UIM100 and MP100, BioPac Systems).

Alveolar Fluid Clearance Experiments

After surgery and connection to the CPAP circuit, the albumin solution (10 ml/kg body weight) was instilled into the lungs through the endotracheal tube as follows. First, the animals were briefly disconnected from the CPAP circuit, and the lungs were deflated by gently aspirating residual air with the instillation syringe. The instillation solution was then instilled into the lungs and withdrawn again. This procedure was repeated four times to allow thorough and adequate mixing of instillate and preexisting fetal lung fluid, and the fluid was finally instilled. Then the animals were reconnected to the CPAP circuit and maintained on CPAP for the 1-h study period. A 0.1-ml sample of the instillation solution-lung fluid mixture (initial solution) remained in the syringe for protein measurement. After 1 h, the lungs and heart were carefully removed en bloc through a midline sternotomy, and a sample of the remaining alveolar fluid was collected. Total protein concentrations in the instilled, initial, and final solutions were determined spectrophotometrically (iEMS Reader MF, Labsystems, Helsinki, Finland) by the Lowry (27) method adapted for microtiter plates.

Alveolar fluid clearance or alveolar fluid secretion was calculated from the change in protein concentration over 1 h. This is possible because the alveolar epithelium is relatively impermeable to large molecules such as albumin (mol wt 67,000). Therefore, water movement (absorption or secretion) will result in a change in airspace protein concentration. Because the fetal lung is fluid filled in utero (6, 7, 17), we expected that a certain fraction of fluid would still be present in the lungs at the time of experiment. This fluid is virtually free of protein and will not add protein to the instilled albumin concentration. In contrast, it will dilute the protein concentration in the instillates and thereby influence the calculation of alveolar fluid clearance differently depending on the volume of fluid present at the different developmental stages. To control for this factor, we instilled the guinea pig fetuses as described above with 10 ml/kg body weight of the 5% albumin instillate, and the fluid was aspirated and reintroduced four times before a final 0.1-ml sample was taken. The rest was instilled, and the animal was studied for 1 h. The whole procedure required ~1−2 min. During this time, it was unlikely that a significant quantity of protein left or entered the airspaces or that significant volumes of fluid were reabsorbed from or secreted into the airspaces. Therefore, any change in protein concentration would represent dilution by preexisting fluid. The preexisting fluid volume calculated from Eqs. 1 and 2 was used to correct the instilled protein concentrations by the dilution of the instillate that would occur if there was fluid already in the lung before instillation of the 5% albumin solution. The preexisting fluid volume (Vpre) was corrected for body weight of the fetus and is expressed as milligrams per kilogram of body weight in milliliters.

\[
V_{\text{initial}} = (V_{\text{instilled}} \times C_{\text{instilled}})/C_{\text{initial}} \quad \text{(1)}
\]

\[
V_{\text{pre}} = V_{\text{initial}} - V_{\text{instilled}} \quad \text{(2)}
\]

\[
V_{\text{final}} = (V_{\text{initial}} \times C_{\text{initial}})/C_{\text{final}} \quad \text{(3)}
\]

\[
\text{AFC or AFS} = [(V_{\text{initial}} - V_{\text{final}})/V_{\text{initial}}] \times 100 \quad \text{(4)}
\]

where \(V_{\text{instilled}}\), \(V_{\text{initial}}\), and \(V_{\text{final}}\) are the volumes of the instilled, initial, and final solutions, respectively, and
$C_{\text{instilled}}$, $C_{\text{initial}}$, and $C_{\text{final}}$ are the protein concentrations in the corresponding solutions.

To be certain that alveolar fluid clearance proceeded at similar rates in our in situ lungs from the euthanized animals as in the in vivo lungs, we ventilated anesthetized newborn and adult guinea pigs according to previous protocols (16, 32) in parallel to the in situ CPAP studies. Alveolar fluid clearance in the ventilated and the in situ CPAP animals was not significantly different (data not shown).

**Specific Protocols**

Guinea pig fetuses of gestational ages 61, 64, 66, 68, and 69 (term) days were studied. Adult animals were studied for reference. The day of conception was set to the day when the timed-pregnant guinea pigs gave birth to their earlier litter because guinea pigs enter estrus immediately after birth. All groups contained fetuses from at least two litters.

**Control studies.** Preterm guinea pig fetuses and newborn guinea pigs of 61 (n = 8), 64 (n = 7), 66 (n = 7), 68 (n = 8), and 69 (n = 11) days gestation were delivered from the timed-pregnant guinea pigs and surgically prepared as described in *Surgical Preparation*. The 5% albumin solution was instilled, and the fetuses were maintained on 5 cmH$_2$O CPAP for 1 h. The lungs were removed, and a sample of the remaining alveolar fluid was collected. Protein concentrations were measured, and alveolar fluid clearance was calculated. A group of adult animals (n = 3) was studied for reference and to verify the CPAP method.

**Oxytocin studies.** Guinea pig fetuses of 61 (n = 6), 64 (n = 9), 66 (n = 6), and 68 (n = 9) days gestation were delivered from the oxytocin-injected timed-pregnant guinea pigs and were surgically prepared as described in *Surgical Preparation*. Labor was induced by giving the timed-pregnant guinea pigs oxytocin (1.0 mg/kg body weight) every 15 min for 45 min. The fetuses were delivered vaginally or surgically after a maximum of 45 min of oxytocin treatment. The 5% albumin solution containing 1 mM amiloride (Na$^+$ channel inhibitor) was instilled, and the fetuses were maintained on 5 cmH$_2$O CPAP for 1 h. Amiloride at a concentration of 1 mM was used because a large fraction becomes protein bound and another significant fraction rapidly leaves the airspaces due to its low molecular weight (34, 42); therefore, the active concentration in the alveoli was probably lower. Also, the same amiloride concentration has been used in several earlier studies of alveolar fluid clearance (16, 25, 32, 38). The lungs were removed, and a sample of the remaining alveolar fluid was collected. Protein concentrations were measured, and alveolar fluid clearance was calculated.

**Measurement of Plasma Epinephrine**

Plasma was collected from parallel groups of guinea pigs at all gestational ages (n = 4–5 in each experimental group) with and without oxytocin induction of premature labor. The plasma was immediately frozen in liquid nitrogen after centrifugation (3,000 g for 5 min) and then stored at −70°C until analysis. Plasma epinephrine levels were measured with a commercially available radioimmunoassay kit (IBL Gesellschaft für Immunchemie und Immunobiologie, Hamburg, Germany). The assay had a sensitivity of 12 pg/ml and intra- and interassay variabilities of 5 and 12%, respectively.

**Statistics**

Values are presented as means ± SD. Statistical analysis was carried out with one-way analysis of variance (ANOVA) with Tukey’s test post hoc or Student’s *t*-test when appropriate. Differences were considered significant with $P < 0.05$.

**RESULTS**

**Alveolar Fluid Volume in Developing Fetal Lungs**

Because the lungs are fluid filled in utero, the presence of preexisting fluid in the airspaces of the lungs had to be taken into account in these experiments. This was done by measuring the dilution of the instilled 5% albumin solution after the mixing and immediate retrieval of the instillate-lung fluid mixture. Because the preexisting lung fluid would be protein free (6, 7, 17), any dilution would represent a volume of preexisting alveolar fluid (16). We found, as expected, that the most immature lungs (61 days gestation) contained the highest volume of preexisting lung fluid (Fig. 1). There was no detectable preexisting fluid in the adult lungs. In the subsequent experiments, each individual animal...
served as its own internal control with regard to the preexisting alveolar fluid volume.

**Late-Gestational Development**

Guinea pig fetuses of several gestational ages (61–69 days) were instilled with the 5% albumin solution, and alveolar fluid clearance was studied over 1 h. A fluid sample from the distal airspaces was collected by aspiration of the remaining alveolar fluid at the end of the 1-h experiment, and the alveolar fluid clearance or secretion was calculated from the increase or decrease, respectively, in the alveolar protein concentration. At 61 days from conception, the lungs were secreting fluid into the airspaces (Fig. 2). On gestational day 64, there was no net secretion or net absorption of lung fluid. From gestational day 64 to day 68, the fluid absorption rate slowly increased, and there was a marked increase between the last gestational day and the day of birth.

**Oxytocin-Induced Premature Labor**

Timed-pregnant guinea pigs at different times postconception were obtained, the guinea pig fetuses (61–68 days gestation) from the timed-pregnant guinea pigs were instilled with the 5% albumin solution containing 10^{-4} M oxytocin, and alveolar fluid clearance was studied over 1 h. Instillation of oxytocin in the guinea pig fetuses did not induce alveolar fluid clearance in any age group (Fig. 4). However, in the gestational day 68 and 66 guinea pig fetuses, instillation of oxytocin seemed to reduce the already existing alveolar fluid clearance (Fig. 4).

**Endogenous β-Adrenergic Stimulation**

The effect of endogenous β-adrenergic stimulation on alveolar fluid transport during gestation was studied by the use of propranolol inhibition under control and oxytocin-stimulated conditions. At 66 days gestation, the fetus did not further stimulate alveolar fluid clearance, although there was a tendency for an increase in alveolar fluid clearance. A separate set of timed-pregnant control guinea pigs (gestational ages 61 and 66 days) were injected with the vehicle (0.9% NaCl) for oxytocin alone to study whether the injections per se induced stress hormone release and thereby affected the results from the oxytocin injections. There were no differences in alveolar fluid clearance between the fetuses from vehicle-injected guinea pigs and the fetuses from noninjected control animals (Table 1).

**Acute Effects From Oxytocin Instillation**

Timed-pregnant guinea pigs at different times postconception were obtained, the guinea pig fetuses (61–68 days gestation) from the timed-pregnant guinea pigs were instilled with the 5% albumin solution containing 10^{-4} M oxytocin, and alveolar fluid clearance was studied over 1 h. Instillation of oxytocin in the guinea pig fetuses did not induce alveolar fluid clearance in any age group (Fig. 4). However, in the gestational day 68 and 66 guinea pig fetuses, instillation of oxytocin seemed to reduce the already existing alveolar fluid clearance (Fig. 4).
propranolol blocked alveolar fluid clearance in the control animals completely and even reversed it to fluid secretion (Fig. 5). This inhibition and reversal persisted until 1 day before birth. In newborn guinea pigs (69 days gestation), propranolol inhibited 56 ± 8% of the alveolar fluid clearance. At 64 days gestation, propranolol had no effect, whereas a tendency to inhibit fluid secretion was seen at 61 days gestation.

Alveolar fluid clearance after the oxytocin-induced labor was also investigated by the use of propranolol inhibition. Induction of premature labor at 61 and 64 days gestation with oxytocin induced propranolol-sensitive alveolar fluid clearance in those fetuses (Fig. 6). At both time points, propranolol reversed alveolar fluid clearance to secretion after the oxytocin-induced labor. One day before birth (day 68), propranolol inhibited the oxytocin-induced alveolar fluid clearance by 76 ± 47%, in contrast to control conditions when propranolol reversed alveolar fluid clearance to secretion.

Plasma Epinephrine Levels

Plasma epinephrine levels were measured in guinea pigs of all ages with and without maternal oxytocin-induced premature labor. In normal fetuses, plasma epinephrine levels were low at 61 and 64 days gestation and began to increase at 66 days gestation, reaching maximal levels at the time of birth (69 days gestation; Fig. 7A). Concomitantly with the increased plasma epinephrine levels, there was an increase in alveolar fluid clearance (Fig. 7B). Maternal oxytocin treatment increased plasma epinephrine levels significantly in all guinea pig fetuses, although it was most evident in fetuses of 61 and 64 days gestation (Fig. 7A), paralleling the induction of alveolar fluid clearance in these age groups. In the 66- and the 68-day-gestation age groups, maternal oxytocin treatment did not significantly increase alveolar fluid clearance.

Plasma epinephrine levels were also measured in the maternal blood with and without oxytocin injections. The plasma epinephrine levels were low in all control timed-pregnant guinea pigs. Induction of premature labor by oxytocin significantly increased plasma epinephrine levels in the maternal blood at all age groups studied (data not shown).

Fractional Amiloride Inhibition

The fractional amiloride inhibition of alveolar fluid clearance was investigated in the developing fetal guinea pigs. The guinea pig fetuses (61–69 days gestation) from the timed-pregnant guinea pigs were instilled with the 5% albumin solution containing 1 mM amiloride, and alveolar fluid clearance was studied over 1 h. At 61 days gestation, amiloride had no effect on the fluid secretion. However, from gestational days 64 to 68, amiloride completely inhibited the alveolar fluid clearance and even reversed net fluid absorption to net secretion in the fetuses (Fig. 8). In newborn guinea pigs, amiloride sensitivity decreased, and amiloride now inhibited the alveolar fluid clearance by 71 ± 13%.

Fractional amiloride inhibition was also investigated after oxytocin-induced labor in the timed-pregnant guinea pigs. The guinea pig fetuses (61–68 days gestation) from the oxytocin-injected timed-pregnant guinea pigs were instilled with the 5% albumin solution containing 1 mM amiloride, and alveolar fluid clearance was studied over 1 h. Under these conditions, amiloride inhibited the oxytocin-induced alveolar fluid...
clearance in the 61-day fetuses completely (Fig. 9). Amiloride also inhibited alveolar fluid clearance in the fetuses at all other gestational ages after maternal oxytocin-induced labor, in contrast to control fetuses in which amiloride was only effective from 64 days gestation (Fig. 9).

DISCUSSION

We found remarkable differences in the capacity of the alveolar epithelium to remove excess fluid from the airspaces during late-gestational development in guinea pig fetuses. What mechanisms initiate and regulate alveolar fluid clearance near term? In fetal and adult animals, the addition of exogenous β-adrenergic agonists stimulates alveolar fluid reabsorption (4, 17, 19, 29, 32). Plasma catecholamine levels increase during labor and delivery (11, 16) and may be important for stimulating lung fluid clearance at birth. We found the same plasma epinephrine levels in the newborn animals in this study as in the earlier in vivo study on ventilated newborn guinea pigs (16). However, although some data are available, there is a lack of conclusive functional evidence that links endogenous β-adrenergic stimulation and alveolar fluid absorption in the preterm animals. In these studies, we found propranolol-sensitive alveolar fluid clearance in animals of 64 days gestation and older. These results indicated that this level of alveolar fluid clearance was maintained by endogenous β-adrenergic stimulation, probably from epinephrine. This finding was also confirmed by plasma epinephrine measurements where plasma epinephrine levels were clearly detectable in fetuses older than 64 gestational days. Also, plasma epinephrine levels increased sharply toward term and peaked at birth. Could this have been due to an increase in receptor number during late gestation or to increased β-adrenergic stimulation? The small increase in the number of β-adrenergic receptors in developing guinea pig lungs can be explained by the increased alveolar surface area (20), and thus our results

Fig. 4. Alveolar fluid clearance over 1 h in developing fetal guinea pig fetal lungs after oxytocin instillation and in control fetuses. Alveolar fluid clearance < 0% corresponds to net secretion. For no. of animals, see Specific Protocols. There was no induction of alveolar fluid clearance by oxytocin instillation at any developmental stage. At 66 and 68 days gestation, oxytocin instillation tended to decrease the existing fetal alveolar fluid clearance. *P < 0.05 compared with the age-matched control fetuses by t-test.

Fig. 5. Alveolar fluid clearance over 1 h in developing fetal guinea pig lungs after β-adrenergic inhibition with propranolol and in control fetuses. Alveolar fluid clearance < 0% corresponds to net secretion. For no. of animals, see Specific Protocols. Propranolol switched alveolar fluid clearance to net secretion in the 66- and 68-day-gestation fetuses. In the newborn animals (69 days gestation), propranolol inhibited alveolar fluid clearance by 55 ± 8% compared with the control value. *P < 0.05 compared with the age-matched control fetuses by t-test.
cannot solely be explained by changes in receptor number. Instead, it is more likely that alveolar fluid clearance was elevated by increased receptor stimulation. Can preterm endogenous epinephrine release and β-adrenergic stimulation induce the conversion from chloride and fluid secretion to sodium and fluid absorption? Enhorning et al. (14) discovered that injection of β-adrenergic agonists into pregnant rabbits reduced fetal lung water. Intravenous epinephrine decreased lung fluid secretion in late-gestational fetal sheep, an effect that was inhibited by β-adrenergic antagonists (40). A positive correlation between plasma epinephrine levels and reabsorption of fetal lung fluid has been demonstrated in the near-term lamb (10) and the newborn guinea pig (16). In this study, endogenous β-adrenergic stimulation increased as term approached simultaneously with the appearance of amiloride-sensitive alveolar fluid clearance. Paralleling the appearance of β-adrenergic stimulation of alveolar fluid clearance, plasma epinephrine levels increased, suggesting that this catecholamine was important for the conversion of the alveolar epithelium from secretion to absorption in the late-gestational lungs. The Na⁺ channel inhibitor amiloride blocked a large fraction of the epinephrine-stimulated alveolar fluid reabsorption in the fetal lungs in both these studies and other investigations (16, 36), an inhibition that was reduced later in life (16, 32). This result suggested that epinephrine stimulated Na⁺ transport via amiloride-sensitive Na⁺ channels primarily in the near-term fetal but also in the newborn lungs.

Oxytocin-induced premature labor initiated alveolar fluid clearance in preterm lungs at gestational ages in which secretion dominated. In these studies, the oxytocin-induced alveolar fluid clearance was completely inhibited by the addition of the β-adrenergic inhibitor propranolol, providing evidence that the oxytocin-induced alveolar fluid clearance occurred by endogenous β-receptor stimulation. The measurements of plasma epinephrine in the fetuses confirmed and extended the interpretation that endogenous plasma epinephrine was the main mechanism after oxytocin induction of alveolar fluid clearance. However, oxytocin induction of labor had no effect on alveolar fluid clearance at gestational days 66 and 68. At that time, endogenous plasma epinephrine levels already had started to increase and stimulate fluid absorption, and a further increase in epinephrine plasma concentration appeared to have little effect on the epinephrine-stimulated alveolar fluid clearance in these animals. Similar results were observed in the earlier study by Finley et al. (16) on newborn guinea pigs where exogenous epinephrine failed to stimulate alveolar fluid clearance when there was endogenous epinephrine stimulation. Another possible explanation is that other stress hormones, i.e., cortisol, were released at these gestational ages and contributed to the endogenous stimulation of fluid absorption in those animals. Plasma cortisol levels have been shown to begin to rise 3 days before term in the guinea pig (1). Because labor releases epinephrine (11) and rabbits born after the onset of labor had less lung water than rabbits delivered by cesarean section before the onset of labor (5), it is likely that endogenous epinephrine released during the induced premature labor mediated the induction of alveolar fluid clearance at gestational ages when fluid secretion predominated. Secretion of fluid into the trachea of fetal lambs and guinea pigs decreases before birth (13, 26, 37) simultaneously with the appearance of endogenous catecholamines and stress hormones.

During labor, not only is epinephrine released, but other hormones are released that could have potential effects on fluid movements across the alveolar epithelium. These hormones include cortisol, thyroid hormones, and prostaglandins. It has been shown that simultaneous pretreatment with thyroid hormones and glucocorticoid hormones synergistically promotes maturation of epinephrine-induced stimulation of amiloride-sensitive fluid absorption in the near-term fetal sheep lung (2). In the adult rat lung, pretreatment
with dexamethasone and thyroid hormones upregulates alveolar fluid clearance via additive pathways (18). Also, in the adult guinea pig, endogenous cortisol maintains normal alveolar fluid clearance (31), and it was suggested that late-gestational expression of the epithelial Na⁺ channel was controlled by endogenous cortisol (1). These hormones might thus be involved in the normal maturation of fluid absorption as gestation proceeds to term. However, most of the effects from cortisol are mediated through protein synthesis, and

the time frames of induction or stimulation (45 min) of alveolar fluid clearance in this study after oxytocin-induced labor are too short to be explained by regulation with cortisol alone.

Could oxytocin itself induce alveolar fluid clearance in the fetal guinea pigs? To answer this question, we carried out experiments where oxytocin was instilled directly into the fetal lungs. We found no induction of alveolar fluid clearance at any developmental stage. In contrast, oxytocin seemed to lower alveolar fluid clearance in the animals at the developmental stages where the lung could already clear alveolar fluid. One way of interpreting these data is that injected oxytocin increases alveolar fluid clearance via epinephrine secretion, whereas, although not studied here, instilled oxytocin has a direct inhibitory effect on clearance. Also, the concentration of oxytocin instilled was likely severalfold higher than that which could be possible if any oxytocin could cross the placenta to the fetuses. Thus it is unlikely that a direct effect of oxytocin is mediating the induction of alveolar fluid clearance seen after oxytocin injections at the gestational ages of 61 and 64 days.

To control for the effect of the injections alone, which may cause stress and discomfort to the animal, we injected control animals with the vehicle (0.9% NaCl) for oxytocin on the same schedule as oxytocin. There were no effects from the injections per se compared with noninjected control animals. Thus the induction of alveolar fluid clearance seen after oxytocin injections must have been due to endogenous epinephrine release caused by oxytocin-induced labor.

There were different degrees of preexisting alveolar fluid in the lungs. The highest preexisting fluid volume was, as expected, observed in the least developed fetuses, i.e., the 61-day fetuses. The fluid volume then significantly decreased between gestational days 61 and 64, likely due to a decreased secretory rate of fluid into the alveolar spaces. Fluid secretion has been shown to decrease before birth in other animal species (26). Then alveolar fluid volume again started to decrease between gestational days 66 and 69 (term), concomitantly with the appearance of amiloride-sensitive Na⁺ transport and fluid absorptive capacities.

What is the driving force for the clearance of fetal lung fluid as term approaches? In adult lungs, Na⁺ transport through amiloride-sensitive and amiloride-insensitive Na⁺ channels is a key mechanism driving alveolar epithelial fluid reabsorption (for reviews, see Refs. 28, 29). In fetal and newborn guinea pig lungs, amiloride impairs reabsorption of fetal lung fluid to different degrees (16, 34, 36). However, few, if any, functional data link the possible differences in amiloride sensitivity to prenatal lung development. We expected that an amiloride-sensitive Na⁺ transport across the alveolar epithelium would be present whenever alveolar fluid clearance was evident in the developing fetal lungs. Our results indicate, in fact, that amiloride-sensitive pathways driving water reabsorption in the developing fetal guinea pig lungs first appeared around 64 days gestation, the time when secretion and

![Fig. 7. A: plasma epinephrine levels in developing guinea pig fetuses with and without maternal oxytocin-induced labor. Plasma epinephrine levels were low in 61-day fetuses (n = 5), detectable at 64 days gestation (n = 4), started to increase at 66 days gestation (n = 5), and continued to increase until term [68 (n = 5) and 69 (n = 4) days gestation] to peak at birth. Oxytocin induction significantly increased plasma epinephrine in 61-day (n = 5) and 64-day (n = 5) fetuses concomitantly with an increased alveolar fluid clearance. In 66-day (n = 5) and 68-day (n = 5) fetuses, oxytocin induction also significantly increased plasma epinephrine levels, although they were less evident. B: correlation between plasma epinephrine levels and alveolar fluid clearance in the developing guinea pig fetuses without maternal oxytocin-induced labor. Plasma epinephrine levels showed a fair correlation to increased alveolar fluid clearance in these fetuses.](http://ajplung.physiology.org/Downloadedfrom)
absorption matched each other, so no net fluid movement across the alveolar epithelium occurred. Amiloride-sensitive fluid transport then increased as term approached.

Amiloride does not inhibit the same fraction of alveolar fluid clearance in adult lungs after β-adrenergic agonist stimulation or stimulation by other factors (19, 32, 39) as that in the fetal and developing newborn guinea pig lungs (16). Thus the increased amiloride sensitivity as term approaches cannot solely be a result of the stimulation of alveolar fluid clearance from the elevated endogenous epinephrine levels but also a result from more Na⁺ transporting pathways in the alveolar epithelial cell membranes and thus an increased amiloride-sensitive Na⁺ transport capacity. An increased expression and/or function of the amiloride-sensitive epithelial Na⁺ channels could be responsible for the elevated alveolar fluid clearance as term approaches. In fact, Finley et al. (16) recently reported that the α-subunit of the epithelial Na⁺ channel (ENaC) mRNA expression was increased in the newborn guinea pig lung. Isolated rat fetal distal lung epithelial cell studies suggested that terbutaline promoted the trafficking of amiloride-sensitive cation channels to the apical cell membranes (24). In a study set up to investigate whether premature delivery by cesarean section would affect expression and function of ENaC, Baines et al. (1) delivered guinea pigs before term and measured α-ENaC and β-ENaC expression postnatally. The expression data were well correlated with the ability of the lung to reabsorb fluid that had been instilled into the distal airspaces of prematurely delivered animals. In conclusion, although terbutaline and other β-adrenergic agonists, e.g., epinephrine, will increase channel activity, the increased amiloride sensitivity in the developing lungs is more likely due to an increased synthesis and insertion of Na⁺ channels into the epithelial cell membranes.

Vectorial transport of ions requires an entry step at the apical cell membrane and an exit step at the baso-
lateral cell membrane. In the alveolar epithelium, the entry step is ENaCs and the exit step is basolateral Na\(^+-K^+\)-ATPase. In this study, we focused our attention on the apical amiloride-sensitive ENaCs and their role in driving alveolar fluid clearance in the fetal lung during normal development and after labor induction. It is likely that both the apical entry step (Na\(^+\) channels) and the basolateral exit step (Na\(^+-K^+\)-ATPase) are regulated in parallel to be able to accommodate the observed changes in fluid transport across the alveolar epithelium. Although not studied here, developmental changes in lung epithelial Na\(^+-K^+\)-ATPase levels and activity have been investigated in the chase for the mechanism responsible for clearing the developing airspaces from fluid at birth. Several studies (8, 12, 23) suggest that events associated with labor may stimulate the basolaterally located Na\(^+-K^+\)-ATPase in lung epithelial cells, contributing to the alveolar fluid volume decline seen at birth. The main findings suggest that pump activity increases at birth, whereas the number of Na\(^+-K^+\)-ATPase pumps increases after birth. Those findings go well with our findings of the changes in Na\(^+\) transport and fluid absorption rate in the late-gestation guinea pigs.

Did the induction of premature labor affect amiloride sensitivity? Oxytocin already induced alveolar fluid clearance in fetuses at 61 days gestation when there was net fluid secretion in age-matched control fetuses. This induction of clearance resulted from endogenous β-adrenergic stimulation by labor-induced epinephrine release because the induced alveolar fluid clearance was completely inhibited by propranolol and plasma epinephrine levels were significantly increased. Moreover, the oxytocin-induced alveolar fluid clearance was also completely blocked by amiloride, an inhibition that was not present in normal age-matched control fetuses. Also, to functionally upregulate fluid absorption across the alveolar epithelium, both the Na\(^+\) channel number or activity and the Na\(^+-K^+\)-ATPase number or activity have to be stimulated. Thus oxytocin-induced labor initiated an insertion or an activation or opening of functional Na\(^+\) channels and/or Na\(^+-K^+\)-ATPases in the fetal epithelial cell membranes, giving the fetal alveolar epithelium absorptive characteristics. It is unlikely that significant new protein synthesis took place because the time after oxytocin induction and the alveolar fluid clearance experiments was too short (<90 min). Thus oxytocin induction of labor induced the fetal lung to acquire adult fluid-absorptive characteristics and become better prepared for the air-breathing postnatal life.

The conversion by oxytocin from secretion to absorption in the fetal lungs may have significant clinical implications because babies that are prematurely delivered by cesarean section before the onset of labor may develop respiratory distress (21). This distress may result, in part, from an immature alveolar epithelium with a limited fluid absorption capacity in conjunction with a deficient surfactant secretion (35). In a clinical study of the influence of labor and route of delivery on respiratory morbidity in the neonate, it was demonstrated that a cesarean delivery after labor had started drastically reduced the respiratory morbidity in the neonate (21). Although no direct mechanism was proposed in that study, it is tempting to speculate that factors released during labor matured the lung fluid-absorptive capacity and perhaps surfactant release and production. If this alveolar fluid-absorptive capacity can be induced either permanently or transiently, respiratory distress may be less and discomfort for the newborn less severe. Also, because the alveolar epithelium acquired the capacity of fluid absorption after oxytocin-induced labor, the fetal lung seems to be more mature and ready for the postnatal life. Fetuses born or delivered by cesarean section from the oxytocin-injected timed-pregnant guinea pigs always seemed to be stronger and more alert in their general appearance compared with age-matched control fetuses.

Functional evidence was found for stimulated alveolar fluid clearance in late-gestational guinea pig fetal lungs that rapidly increased during the last gestational day. This elevated alveolar fluid clearance depended on endogenous β-adrenergic stimulation from labor-released epinephrine. The ability to clear fluid from distal lung airspaces correlated with the appearance of amiloride sensitivity. Oxytocin-induced labor resulted in the induction of both propranolol- and amiloride-sensitive fluid absorption in the fetal lungs at gestational ages (61 days) when fluid secretion predominated in the age-matched control fetuses. It was also demonstrated that oxytocin induction of alveolar fluid clearance in the fetal animals relied on endogenous epinephrine release. These results suggest that the induction of premature labor may better prepare the prematurely delivered babies for the air-breathing life and prevent neonatal respiratory distress.

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