Ventilation-induced release of phosphatidylcholine from neonatal-rat lungs *in vitro*

M. S. NIJJAR*

Department of Pathology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba,
Canada R3E 0W3

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Factors regulating the release of phosphatidylcholine (PC) from neonatal-rat lungs were investigated. The results show that the release of prelabelled PC from the newborn-rat lung was augmented by air ventilation at the onset of breathing. This response was mimicked in lungs of pups delivered 1 day before term and allowed to breathe for different time intervals. Anoxia further augmented the ventilation-enhanced PC release from the newborn-rat lungs. The ventilation-induced release of PC was not abolished by the prior treatment of pups *in utero* or mothers *in vivo* with phenoxybenzamine, propranolol or atropine, suggesting the lack of receptor stimulation in the ventilation-enhanced PC release at birth. The results also show that ventilation stimulated [*methyl*- ^{14}C]choline incorporation into lung PC, presumably to replenish the depleted surfactant stores. The ratio of adenylate cyclase/cyclic AMP phosphodiesterase activities, which reflects cyclic AMP levels in the developing rat lungs, did not change during the 120 min of air ventilation when the release of PC was much enhanced, implying that cyclic AMP may not be involved. This confirms our conclusion that stimulation of β -adrenergic receptor was not involved in the air-ventilation-enhanced release of PC. Since the cell number or size did not change during 120 min of ventilation when the alveolar-cell surface was maximally distended, it is suggested that distension of alveolar wall by air ventilation at the onset of breathing may bring the lamellar bodies containing surfactant close to the luminal surface of alveolar type II cells, thereby enhancing their fusion and extrusion by exocytosis.

It is important to understand the regulation of pulmonary phospholipid metabolism in order to appreciate the biochemical basis of lung functions (Fulmer & Crystal, 1976). The turnover of pulmonary phospholipids has been extensively studied ever since their role in lung physiology has been recognized (Perelman *et al.*, 1981; Van Golde, 1976). In order for surfactant phospholipids to lower effectively the surface tension of alveoli to prevent their collapse during expiration, particularly at the time of birth, surfactant has to be transported from their storage site, i.e., lamellar bodies in the type II cells, to the lumen of alveoli (Massaro & Massaro, 1976). Several investigators have studied this aspect of surfactant metabolism

both *in vitro* and *in vivo*; however, the factors regulating the release of surfactant phospholipids from lungs during the first few hours of extra-uterine life, when surfactant phospholipids play a critical role in pulmonary functions and the survival of the newborn, are not clearly defined.

Previous studies by other investigators have demonstrated that surfactant phospholipids are synthesized at the endoplasmic reticulum, transferred to the Golgi region for packaging and later stored in the lamellar bodies (Massaro & Massaro, 1976). On stimulation, surfactant phospholipids in lamellar bodies are released into the alveolar space by exocytosis (Ryan *et al.*, 1975). Both adrenergic and cholinergic mechanism(s) are seemingly involved in the release of surfactant from lungs (Smith, 1977; Dobbs & Mason, 1979), although recent evidence indicates that the cholinergic effect may be indirect, via stimulation of adrenal medulla

Abbreviation used: PC, phosphatidylcholine.

* Present address: Alvin Buckwold Centre, Department of Pediatrics, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0X0.

and enhanced release of catecholamines, which act through the adrenergic-receptor mechanism (Mas-saro *et al.*, 1982). Secretion of surfactant phospholipids in experimental animals is augmented by a variety of stimuli such as oestrogen, calcium, cyclic AMP and many other agonists (Faridy & Naimark, 1971; Faridy, 1976; Delahunty & Johnston, 1979; Marino & Rooney, 1980, 1981). Whether these stimuli are involved in the secretion of surfactant phospholipids from lungs at the time of birth is not yet known.

Elsewhere we have reported (Nijjar *et al.*, 1983) that secretion of prelabelled phosphatidylcholine from rat lungs was very much enhanced 1 day after birth and that surfactant secretion from lungs of newborn rats of diabetic mothers was suppressed. Insulin treatment of the diabetic pregnant rats for 5 days restored the neonatal upsurge of PC release from the offspring of diabetic mothers to the control values. The present study was undertaken to examine the molecular events that may be involved in the enhanced secretion of PC from the neonatal rat lungs. The results suggest that distension of the alveolar-cell surface by the onset of breathing may be the primary stimulus for the enhanced release of PC from lungs at birth.

Materials and methods

Most of the biochemicals used were of analytical grade and purchased from either Sigma or Fisher Scientific. [*methyl*- ^{14}C]Choline was purchased from New England Nuclear.

Sprague-Dawley timed-pregnant rats were purchased from the Charles River Breeding Farms (Montreal, PQ, Canada), kept in our animal quarters with a 12h cycle of light and dark and provided with pellet food and water *ad libitum*. Diabetes was induced chemically by an intravenous injection of streptozotocin (50mg/kg) via the tail vein on day 10 of pregnancy and confirmed by the daily analysis of glucose in urine with an enzymic test strip (TES TAPE; Eli Lilly). On day 15 of gestation, diabetic pregnant rats were subdivided into two groups. One group of diabetic rats was treated with insulin (10i.u./day), whereas the diabetic control group received an equal volume of the vehicle. At least two pregnant rats from the control, diabetic and insulin-treated diabetic groups were used at each time interval. The mothers were killed 1 day before term, pups delivered, allowed to breathe for different time intervals and then killed. The control pups were decapitated *in utero* to prevent air breathing. Lungs were removed from pups, cleaned of extraneous tissues, and slices of uniform thickness (1mm squares) were prepared from the peripheral portion of lung containing predominantly the alveolar

tissue. Pups were allowed to breathe for different time intervals after normal delivery, killed, their lungs removed and treated similarly. Lung slices from the control pups and those breathing for different time intervals after their operative or normal delivery were placed in a medium containing Krebs-Ringer bicarbonate buffer, pH7.4 (Umbreit *et al.*, 1964), glucose (2mg/ml), bovine serum albumin (30mg/ml) and $1\mu\text{Ci}$ of [*methyl*- ^{14}C]choline/tube (sp. radioactivity 50mCi/mmol) and incubated for 60min in a Dubnoff metabolic shaker at 37°C under a gas phase of O_2/CO_2 (19:1). Carrier-free [*methyl*- ^{14}C]choline was used to radiolabel lung PC as highly as possible within short time of incubation to avoid any adverse effects on the viability of the lung tissue during secretion experiments. A 2ml portion of ice-cold saline (0.9% NaCl) was added to terminate the incorporation, medium withdrawn carefully, avoiding its contamination with the tissue fragments, and slices washed three times with 2ml of ice-cold saline each time. In experiments where effects of calcium on PC secretion were examined, lung slices after the first incubation were washed first with saline, placed in 200 μM -EGTA solution, pH7.4, for 10min at room temperature, followed by another wash with ice-cold saline. To measure synthesis of PC, 8vol. of a mixture containing chloroform, methanol and conc. HCl (500:500:3, by vol.) were added to the washed lung slices to extract lipids (Wells & Dittmer, 1965). The lipid extract was evaporated to dryness under a stream of N_2 gas, redissolved in a small volume of chloroform/methanol (2:1, v/v), applied to a silica-gel G (0.5mm) t.l.c. plate that had been pre-activated for 60min at 110°C, and subjected to one-dimensional t.l.c. with a solvent system containing chloroform, methanol and water (14:6:1, by vol.) (Nijjar, 1972). The t.l.c. plate was air-dried and exposed to I_2 vapour to localize different species of lipids, which were identified on the basis of their R_F values as compared with those of the authentic lipid standards run simultaneously under identical conditions. A Kodak No-Screen X-ray film was placed on the t.l.c. plate and exposed for at least 72h before being developed. Silica gel containing radioactive lipids was scraped into scintillation vials, mixed with 15ml of Scintiverse (Fisher Scientific) and counted for radioactivity in a Searle Mark III liquid-scintillation counter, which was programmed to correct for variable quenching to give d.p.m. values. For secretion experiments, lung slices prelabelled with [*methyl*- ^{14}C]choline for 60min at 37°C as described above and washed three times with ice-cold saline or once each with saline, EGTA and saline were placed in the fresh medium containing Krebs-Ringer bicarbonate buffer, pH7.4, glucose (2mg/ml), bovine serum

albumin (30mg/ml), but lacking radioactive choline. The mixture was incubated for 90min in a Dubnoff shaking metabolic incubator at 37°C under an O₂/CO₂ (19:1) gas phase. At the end of incubation, medium containing secreted material was carefully withdrawn and extracted for lipids (Wells & Dittmer, 1965). The lipids were resolved into different species by single-dimension t.l.c. (Nijjar, 1972), and analysed as described above. Under these experimental conditions, secretion of prelabelled PC was linear up to 2h of incubation and 65mg of tissue slices.

The lungs from different groups of pups (non-ventilated, ventilated) were homogenized in 9 vol. of 0.27M-sucrose/10mM-Tris/HCl buffer, pH 7.4, and the tissue homogenate was subjected to differential centrifugation to prepare subcellular fractions (Nijjar & Ho, 1980). Activities of adenylate cyclase, 5'-nucleotidase (Michell & Hawthorne, 1974), cyclic AMP phosphodiesterase and phosphorylase were estimated as described previously (Nijjar & Thurlbeck, 1980). Proteins were determined by the method of Lowry *et al.* (1951), with bovine serum albumin (fraction V) as standard. DNA was determined by the method of Schneider (1957). The data were subjected to Student's *t* test to determine the significance of differences between the mean results for control and experimental samples.

Results

We described previously (Nijjar *et al.*, 1983) a marked increase in the release of PC from 1-day-old rat lungs as compared with foetal lungs on different days of gestation. The neonatal upsurge in PC release was markedly reduced in the lungs of newborn rats from diabetic mothers. The factors responsible for the enhanced PC release neonatally are not known. We postulated that the neonatal increase in PC release was caused by air ventilation at the onset of breathing. To examine this hypothesis, we measured PC release from the newborn-rat lungs at different time intervals after birth. The results show that choline incorporation into PC and the release of PC were markedly enhanced after birth up to 90min of breathing, declining thereafter (Fig. 1). The enhanced release and synthesis of PC probably resulted from the air ventilation at birth, since choline incorporation into PC and the release of labelled PC from lungs of those pups that were decapitated *in utero* to prevent breathing were much lower than that in ventilated lungs (Fig. 2).

To substantiate this hypothesis further, rat pups were delivered 1 day before birth by hysterotomy (Caesarian section) from the normal and diabetic mothers and allowed to breathe for different time

Fig. 1. Incorporation of choline into PC and secretion of radiolabelled PC from rat lungs *in vitro*

Lungs were obtained from rat pups killed at different time periods after their natural birth (0min); slices were prepared and incubated with [*methyl*-¹⁴C]choline to study the incorporation of choline into PC and the release of radiolabelled PC from the lung tissue. About 50mg portions of lung slices were incubated in triplicate samples, lipids extracted, separated, and their radioactivity measured as described in the Materials and methods section. To measure the release of PC, medium from the second incubation was extracted for lipids, which were quantified similarly.

intervals and the rate of [*methyl*-¹⁴C]choline incorporation into PC and the release of prelabelled PC from their lungs was measured *in vitro*. The results show that PC secretion from rat lungs of normal mothers increased as a function of ventilation time and reached a maximum level between 1.5 and 2.0h after the onset of respiration (Fig. 2). By contrast, PC release from the lungs of pups of diabetic mothers was very much suppressed, confirming our previous findings of PC release from lungs of 1-day-old rat pups born naturally from the normal and diabetic mothers (Nijjar *et al.*, 1983).

The incorporation of choline into lungs of pups delivered by hysterotomy 1 day before birth and ventilated for different time intervals, was stimulated after the enhanced secretion of PC (Fig. 3).

To examine the possible neural involvement in ventilation-induced release of PC from the newborn-rat lungs, pups were treated *in utero* with atropine (5mg/ml), propranolol (1mg/ml) or phenoxybenzamine (1.5mg/ml) before their delivery and onset of breathing. The control pups received equal volume of saline. Two pups were decapitated *in utero*. Ventilation for 30, 60 or 120min enhanced PC release from control pup

Fig. 2. Secretion of PC from lungs of rat pups delivered 1 day before birth by hysterotomy from the normal (O) and diabetic (Δ) mothers in response to air ventilation for different time periods

The histogram (inset) shows data for the synthesis of PC by lungs of rat pups of normal (□) and diabetic (▨) mothers. Lung slices were prepared and used in these experiments exactly as described in the Materials and methods section.

lungs, as observed previously. Treatment of pups with propranolol and phenoxybenzamine did not affect the ventilation-induced release of PC from rat lungs, whereas treatment with atropine partially blocked the 30min-ventilation-induced PC release (Fig. 4), but had no effect on 120min-ventilation-induced secretion of PC. Similarly, treatment of mothers with these receptor-blocking agents on the previous night and 1 h before they were killed, and delivery of pups had no effect on the release of PC from pup lungs in response to ventilation of different time periods (results not shown).

Since there may be some anoxia during delivery (Chernick & Craig, 1982), we examined the effects of N₂ gas on the ventilation-induced secretion of PC from lungs of pups delivered 1 day before term and allowed to breathe for 120min in an incubator. PC release from the 120min-ventilated pup lungs incubated under an O₂/CO₂ (19:1) gas phase was much higher than that by the non-ventilated pup lungs incubated similarly. However, PC release from the 120min-ventilated pup lungs incubated under 100% N₂ during the second incubation was further augmented when compared with the lungs

Fig. 3. [methyl-¹⁴C]Choline incorporation into PC and the release of radiolabelled PC from neonatal-rat lungs as a function of air-ventilation time

Rat pups were delivered from the normal mothers by hysterotomy 1 day before term, allowed to breathe for different time intervals and then killed (0min ventilation represents decapitation of pups *in utero* before any air breathing occurred). The results are expressed as means ($\pm$ S.D.) for three experiments and each point represents data from lung slices obtained from two or more rat pups.

incubated under an O₂/CO₂ (19:1) gas phase (Fig. 5). These results suggest that anoxia during parturition may also stimulate secretion of PC from the newborn-rat lungs (Lawson *et al.*, 1979).

In another set of experiments, effects of Ca²⁺ in the absence or presence of the calcium ionophore A23187 on the release of PC from rat lungs were examined (Mason *et al.*, 1977). Ca²⁺ had no effect on PC release from the rat lung slices that were washed three times with ice-cold saline after the first incubation. However, when the labelled slices were washed with saline, incubated with 200 μ M-EGTA, pH 7.4, for 10min at room temperature, followed by another wash with cold saline, PC release was stimulated significantly by Ca²⁺ (Table 1) in a concentration-dependent manner (Fig. 6). Addition of the ionophore further augmented PC release from the rat lungs in response to Ca²⁺ (Table 1).

Adenylate cyclase activity in lung membranes (Fig. 7) and cyclic AMP phosphodiesterase in the post-8000g supernatant (Fig. 8) were not altered by

Fig. 4. Effect of different receptor-blocking agents on the air-ventilation-enhanced release of phosphatidylcholine (PC) from neonatal-rat lungs

Three separate experiments were performed. In the first experiment, rat pups were treated with propranolol (1 mg/kg), atropine (5 mg/ml) or phenoxybenzamine (1.5 mg/ml) *in utero*, delivered, and killed after 30 min of air breathing. In the second experiment, the rat pups were treated with propranolol (2.5 mg/kg), allowed to breathe for 45 min, killed, and their lungs processed similarly. In the third experiment, rat pups were treated with atropine (5 mg/kg) but killed after 120 min of air-ventilation. The control pups received equal volumes of saline and breathed air for comparable time as those treated with receptor antagonists. All the other procedures were as described in the Materials and methods section. ■, Saline control; ▨, treated with propranolol (1 mg/kg); ▤, treated with atropine (5 mg/kg), and □, treated with phenoxybenzamine (1.5 mg/kg).

Fig. 5. Effect of anoxic conditions on the release of PC from the neonatal-rat lungs

The rat pups were delivered by hysterotomy 1 day before birth and some killed *in utero* (0 min breathing, NV) and others after 120 min of air breathing (V₁₂₀). Lung slices were prepared and the release of radiolabelled PC was measured, as described in the Materials and methods section, in the presence of either O₂/CO₂ (19:1) or 100% N₂. The results are expressed as means (±s.d.) for three experiments.

Table 1. Effect of Ca²⁺ and calcium ionophore A23187 on PC secretion from rat lung slices prelabelled with [methyl-¹⁴C]-choline and washed with either saline or EGTA solution before incubation for secretion experiment

The results are expressed as mean percentages (±s.d.) of PC secretion from the control lung slices incubated in the Krebs-Ringer medium lacking Ca²⁺ and ionophore.

Additions		Secretion of prelabelled PC (% of control)	
[Ca ²⁺] (mM)	[Ca ²⁺ ionophore A23187] (μM)	Saline wash	EGTA wash
0	0	100 ± 5	100 ± 3
1	0	112 ± 4	178 ± 7*
1	500	140 ± 26	256 ± 10*

*Significantly different from control lacking any addition (*P* < 0.05).

Fig. 6. Influence of Ca^{2+} on the release of prelabeled PC from the neonatal-rat lungs *in vitro*

Lung slices from 5-day-old rats were used to radio-label PC, treated with $200\text{ }\mu\text{M}$ -EGTA, and the release of radiolabeled PC measured, in the absence or presence of Ca^{2+} , as described in the Materials and methods section.

AMP level was not affected in ventilating lungs and thus may not be involved in the ventilation-induced PC release. These findings provide biochemical evidence in support of our conclusion that the β -adrenergic-receptor mechanism does not appear to be involved in the ventilation-induced release of PC from newborn-rat lungs.

In contrast with adenylate cyclase activity, $5'$ -nucleotidase activity in lung membranes was increased as a function of ventilation up to 60 min, declining thereafter (Fig. 7). The differential response of lung adenylate cyclase and $5'$ -nucleotidase to ventilation suggests that either these enzyme activities are present in different cell types of pup lungs or, perhaps more likely, they are oriented or located differently in the membranes of the same lung-cell type. It is known in other tissues that adenylate cyclase is located at the inside surface of cell outer membranes, whereas $5'$ -nucleotidase may be located on the outside of cell outer membranes (Trams & Lauter, 1974).

The phosphorylase *a*/total-phosphorylase activity ratio increased with ventilation of pup lungs (Fig. 8). Total phosphorylase activity was unchanged. These results suggest a conversion of phosphorylase from its inactive (*b* type) into an active (*a* type) form by ventilation of lungs at the

Fig. 7. Effect of air-ventilation on the activities of $5'$ -nucleotidase and adenylate cyclase in a lung particulate fraction prepared from rat pups that were delivered by hysterotomy 1 day before term and killed after air breathing for different time periods

The results represent mean values of enzyme activities from two experiments assayed in duplicate samples as described in the Materials and methods section.

time of birth. Such a transformation in phosphorylase activity implies enhanced glycogen metabolism in the ventilating lungs (Faridy & Naimark, 1971).

Discussion

The present findings, that the release of PC from the newborn rat lungs increased as a function of ventilation time up to 2 h after birth (Fig. 1) and that this response was mimicked in the lungs of pups delivered 1 day before birth and allowed to breathe for different time intervals (Figs. 2 and 3), lend some support to our hypothesis that the neonatal upsurge in the release of PC from the newborn-rat lungs was at least in part due to the onset of breathing (air-ventilation) (Nijjar *et al.*, 1983). The data in Fig. 2 also confirm our previous conclusion that the release of PC from the lungs of rat pups of diabetic mothers was much lower than that from the lungs of pups of control mothers and that [*methyl*- ^{14}C]choline incorporation into their lung PC was not affected by the maternal diabetes.

Fig. 8. Influence of air-ventilation on phosphorylase a/total-phosphorylase activity ratio and the activity of cyclic AMP phosphodiesterase in the supernatant fraction prepared from lungs of rat pups that were delivered by hysterotomy 1 day before term but allowed to breathe air for different time periods

The data represent mean enzyme activities assayed in duplicate samples from two experiments. All the other procedures are given in the Materials and methods section.

These data further support the notion that defective secretion of surfactant from the newborn-rat lungs of diabetic mothers may be partly responsible for the higher incidence of respiratory-distress syndrome among the offspring of diabetic mothers (Farrell & Avery, 1975). However, the molecular events involved in the ventilation-induced release of surfactant from lungs at the time of birth are not yet defined, nor do we know which of these events are most affected by the maternal diabetes.

In an attempt to elucidate the receptor mechanism(s) and the possible neural control of ventilation-induced secretion of PC from rat lungs, the pups were treated *in utero* before the onset of breathing, and pregnant rats *in vivo*, with phenoxybenzamine, propranolol or atropine, and their effects on ventilation-induced PC release from pup lungs were examined. The results show that the treatment of rat pups with phenoxybenzamine or propranolol did not prevent the enhanced release of PC in response to air ventilation (Fig. 4). The

treatment of pups with atropine appears to have marginally reduced the 30 min-ventilation-induced release of PC, but had no effect on the 120 min-ventilation-induced release of PC (Fig. 4). The reason for this variable effect of atropine on ventilation-induced PC release is not obvious, but the transient inhibitory effect of atropine may be related to its rapid breakdown by atropinesterase activity (atropine acylhydrolase, EC 3.1.1.10) (Sawin & Glick, 1943). Oyarzun & Clements (1978) have reported previously that treatment of rabbit pups with atropine before their delivery did not affect the release of surfactant from normally breathing pups, but it abolished further increase in the release of surfactant in response to hyperventilation. Other investigators have also reported lack of atropine effect on surfactant release (Dobbs & Mason, 1979; Marino & Rooney, 1980, 1981; Brown & Longmore, 1981). Studies of Brown & Longmore (1981) on isolated perfused rat lungs showed that stimulation of cholinergic- and adrenergic-receptor mechanisms with appropriate agonists markedly enhanced the release of labelled disaturated PC. However, in the isolated type II cells in culture, only β -adrenergic receptor agonists were effective in promoting the surfactant secretion, and α -adrenergic- and cholinergic-receptor agonists had no effect on the surfactant release. These findings were consistent with those obtained with the isolated type II cells in culture by Dobbs & Mason (1979), who found that only β -adrenergic agonists caused an increase in the release of ^{14}C -labelled disaturated PC, but not the cholinergic agonists. Since then, Massaro *et al.* (1982) have reported data indicating that the cholinergic stimulation of disaturated-PC release from rat lungs was due to an indirect effect through stimulation of adrenal medulla to secrete catecholamines. Therefore it would appear that the cholinergic-receptor-mediated effect on surfactant secretion may not be significant in the air-ventilation-induced release of PC from the newborn-rat lungs (Fig. 4).

The present finding, that propranolol did not inhibit the air-ventilation-enhanced release of PC, is contrary to the observations by other investigators that show blockade of enhanced surfactant release by propranolol in response to various β -adrenergic agonists (Oyarzun & Clements, 1978; Dobbs & Mason, 1979; Brown & Longmore, 1981) and hyperventilation (Oyarzun & Clements, 1977, 1978). The factors regulating the release of PC in response to air-breathing at the time of birth may be quite different from those stimulating the release of surfactant later in life. The differential sensitivity of surfactant release to different receptor-blocking agents neonatally and during adult life may have something to do with the development of

appropriate receptors on lung cells (Faridy, 1976). Whitsett *et al.* (1981) have shown that β -adrenergic receptors were scarce in neonatal rat lungs, but became abundant later in life. Thus the failure of propranolol to prevent the enhanced release of PC from newborn-rat lungs by the onset of breathing suggests the insignificance of β -adrenergic receptors in ventilation-induced release of PC from newborn-rat lungs at birth. This conclusion was further substantiated by the biochemical evidence in that the adenylate cyclase/cyclic AMP phosphodiesterase ratio was not altered in tissue fractions prepared from lungs that had been ventilated for 120 min (Fig. 8). Under these conditions, the release of PC from the newborn-rat lungs was much enhanced (Figs. 2, 3 and 4). Since the adenylate cyclase/phosphodiesterase activity ratio has been shown to reflect cyclic AMP levels in the developing rat lungs (Nijjar, 1979; Nijjar & Thurlbeck, 1980), these results would imply no change in the levels of cyclic AMP during air-ventilation neonatally. Klass (1978) has shown that hyperventilation-induced release of PC from the isolated rat lungs was not accompanied by any alterations in cyclic AMP levels. However, cyclic

GMP levels were markedly increased, suggesting that hyperventilation-induced release of PC from the rat lungs may be mediated by cyclic GMP, which is synonymous with cholinergic stimulation of surfactant release. All these data appear to indicate that the regulation of surfactant release from the neonatal rat lungs in response to the onset of normal breathing at birth may be different from that involved in the surfactant release from lungs later in life in response to hyperventilation and other agonists. The discrepancy in results from different laboratories may arise from the fact that different experimental models were used to study the release of surfactant.

In an earlier study, Faridy & Naimark (1971) showed that excised dog lung distended by air ventilation consumed more glycogen, lipid and oxygen than did static lungs. They concluded that mechanical deformation of lung cells may activate some membrane-bound enzymes or change the ionic permeability of lung cells so that their metabolism is enhanced. The results of the present study show an increase in phosphorylase activity without a change in the total phosphorylase activity during air ventilation after birth, sug-

Fig. 9. Schematic representation of possible mechanical distension of alveolar wall at birth resulting in enhanced release of PC from the alveolar type II cells

Abbreviations used: Fb, fibroblast; M, macrophage.

Table 2. *Effect of air ventilation on protein, DNA and protein/DNA ratio of the neonatal-rat lung*

The pups were delivered by hysterotomy 1 day before term and killed at different time intervals after the onset of breathing; their lungs were examined for different biochemical parameters by methods described in the Materials and methods section. The results are expressed as means ($\pm$ S.D.) for three experiments.

Ventilation time (min)	Protein (mg/g of lung)	DNA (mg/g of lung)	Cell size (Protein/DNA)
0	71.7 $\pm$ 3.0	10.97 $\pm$ 1.07	6.54
15	70.8 $\pm$ 11.1	—	—
30	62.8 $\pm$ 3.5	11.40 $\pm$ 0.50	5.51
60	74.3 $\pm$ 2.5	12.10 $\pm$ 0.93	6.14
90	78.2 $\pm$ 7.3	13.57 $\pm$ 1.37	5.76
120	72.2 $\pm$ 5.2	12.10 $\pm$ 0.77	5.97
180	76.1 $\pm$ 4.7	—	—

gesting the conversion of phosphorylase from its inactive (*b*) into an active (*a*) form (Fig. 8). Such a change in the activity of phosphorylase would imply enhanced glycogen breakdown in the ventilating newborn-rat lungs (Faridy & Naimark, 1971).

The present findings that (1) propranolol, phenoxybenzamine or atropine did not block the release of PC from the newborn-rat lungs in response to the onset of breathing (Fig. 4) and that (2) there was neither an increase in cell number nor cell size (Table 2), but a tremendous increase in the alveolar-cell surface due to the inspiration of air (Faridy & Naimark, 1971; Oyarzun & Clements, 1977, 1978; Klass, 1979) suggest that the neonatal upsurge in surfactant release in response to air ventilation is not mediated by either α - and β -adrenergic, or cholinergic-receptor mechanisms, but may result from the mechanical distension of lungs by the first few breaths (Fig. 9). Distension of the alveolar type II cells may flatten the cells in such a way so as to bring the lamellar bodies containing surfactant close to the luminal-cell surface. This could enhance their fusion with each other, thereby allowing the extrusion of lamellar bodies into the alveolar space by exocytosis. The results of the present study reveal that the onset of breathing (air-ventilation) is the primary stimulus for the enhanced release of PC from the newborn-rat lungs, although asphyxia at birth may also contribute to the enhanced release of surfactant (Lawson *et al.*, 1979; Chernick & Craig, 1982).

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References

- Brown, L. A. S. & Longmore, W. J. (1981) *J. Biol. Chem.* **256**, 66–72
- Chernick, V. & Craig, R. J. (1982) *Science* **216**, 1252–1253
- Delahunty, T. J. & Johnston, J. M. (1979) *Lung* **157**, 45–51
- Dobbs, L. G. & Mason, R. J. (1979) *J. Clin. Invest.* **63**, 378–387
- Faridy, E. E. (1976) *Resp. Physiol.* **27**, 99–114
- Faridy, E. E. & Naimark, A. (1971) *J. Appl. Physiol.* **31**, 31–37
- Farrell, P. M. & Avery, M. A. (1975) *Am. Rev. Resp. Dis.* **111**, 657–688
- Fulmer, J. D. & Crystal, R. G. (1976) in *Biochemical Basis of Pulmonary Functions* (Crystal, R. G., ed.), pp. 419–466, Marcel Dekker, New York
- Klass, D. J. (1978) *J. Appl. Physiol.* **45**, 587–594
- Klass, D. J. (1979) *J. Appl. Physiol.* **47**, 285–289
- Lawson, E. E., Bordwell, R. L., Huang, P. S. & Tausch, J. H. W. (1979) *Pediatr. Res.* **13**, 611–614
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Marino, P. A. & Rooney, S. A. (1980) *Biochem. Biophys. Acta* **620**, 509–519
- Marino, P. A. & Rooney, S. A. (1981) *Biochem. Biophys. Acta* **664**, 389–396
- Mason, R. J., Dobbs, L. G., Greenleaf, R. D. & Williams, M. C. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 2692–2702
- Massaro, D. & Massaro, G. D. (1976) in *Biochemical Basis of Pulmonary Functions* (Crystal, R. G., ed.), pp. 389–416, Marcel Dekker, New York
- Massaro, D., Clerch, L. & Massaro, G. D. (1982) *J. Appl. Physiol.* **243**, C39–C48
- Michell, R. H. & Hawthorne, J. N. (1974) *Biochem. Biophys. Res. Commun.* **21**, 333–338
- Nijjar, M. S. (1972) Ph.D. Thesis, University of Manitoba
- Nijjar, M. S. (1979) *Biochim. Biophys. Acta* **586**, 464–472
- Nijjar, M. S. & Ho, J. C. H. (1980) *Biochim. Biophys. Acta* **600**, 238–243
- Nijjar, M. S. & Thurlbeck, W. M. (1980) *Eur. J. Biochem.* **105**, 403–407
- Nijjar, M. S., Khangura, B. S. & Juravsky, L. T. (1983) *Diabetologia* in the press

- Oyarzun, M. J. & Clements, J. A. (1977) *J. Appl. Physiol.* **43**, 39–45
- Oyarzun, M. J. & Clements, J. A. (1978) *Am. Rev. Resp. Dis.* **117**, 879–891
- Perelman, R. H., Engle, M. J. & Farrell, P. M. (1981) *Lung* **159**, 53–80
- Ryan, U. S., Ryan, J. W. & Smith, D. S. (1975) *Tissue Cell* **7**, 587–599
- Sawin, P. B. & Glick, D. (1943) *Proc. Natl. Acad. Sci. U.S.A.* **29**, 55–59
- Schneider, W. C. (1957) *Methods Enzymol.* **3**, 680–684
- Smith, B. T. (1977) *Am. Rev. Resp. Dis.* **115**, 285–293
- Trams, E. G. & Lauter, C. J. (1974) *Biochim. Biophys. Acta* **345**, 180–197
- Umbreit, W. W., Burns, R. H. & Stauffer, J. F. (1964) *Manometric Techniques and Tissue Metabolism*, 4th edn., p. 132, Burgess Publishing Co., Minneapolis
- Van Golde, L. M. G. (1976) *Am. Rev. Resp. Dis.* **114**, 977–1000
- Wells, M. A. & Dittmer, J. C. (1965) *Biochemistry* **4**, 2459–2467
- Whitsett, J. A., Manton, M. A., Darovec-Beckerman, C., Adams, K. G. & Moore, J. J. (1981) *Am. J. Physiol.* **240**, E351–E357