Lung Edema Clearance: 20 Years of Progress
Invited Review: Clearance of lung liquid during the perinatal period

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Barker, Pierre M., and Richard E. Olver. Invited Review: Clearance of lung liquid during the perinatal period. J Appl Physiol 93: 1542–1548, 2002; 10.1152/japplphysiol.00092.2002.—At birth, the distal lung epithelium undergoes a profound phenotypic switch from secretion to absorption in the course of adaptation to air breathing. In this review, we describe the developmental regulation of key membrane transport proteins and the way in which epinephrine, oxygen, glucocorticoids, and thyroid hormones interact to bring about this crucial change in function. Evidence from molecular, transgenic, cell culture, and whole lung studies is presented, and the clinical consequences of the failure of the physiological mechanisms that underlie perinatal lung liquid absorption are discussed.

lung liquid; sodium absorption; chloride secretion; epithelial sodium channel; cystic fibrosis transmembrane conductance regulator

THE MECHANISMS RESPONSIBLE for lung liquid clearance during the neonatal period develop gradually during the latter part of the third trimester of pregnancy, but the phenotypic switch of the lung epithelium from net secretion to net absorption, triggered by events at birth, is sudden. Although lung liquid absorption at birth is “a performance without rehearsal,” the lung may be called on for an encore in later life when these same mechanisms are activated to clear accumulated edema liquid.

Many of the early studies of perinatal lung liquid clearance, which were mostly undertaken on the intact lungs of rabbits and sheep in the 1970s and 1980s, provided information in vivo on the time course and regulation of lung liquid clearance in a fully integrated physiological context. These data have been supported and augmented in the past decade by cloning of genes that regulate transepithelial ion transport [particularly the sodium pump (Na\(^+\)-K\(^+\)-ATPase) and the epithelial Na\(^+\) channel (ENaC)] and the use of molecular approaches that have uncovered information at a subcellular level.

TIME COURSE OF LUNG LIQUID CLEARANCE
AND THE ROLE OF LABOR

The study of neonatal lung liquid clearance can be said to have started with Faure-Fremiet and Dragiou in 1923 (28), who noted that lung water content in fetal lambs held fairly steady at 87–89% of fresh weight until birth, at which point it fell to 76–78%. However, it was not for another 40 years that any progress was made in understanding the mechanisms underlying this observation. Measuring lung water serially in newborn rabbit pups, Aherne and Dawkins (2) concluded that the clearance of lung liquid began immediately after birth and was virtually complete within 2 h. The finding that delivery by cesarean section slowed lung liquid clearance (1), particularly if undertaken before the onset of labor (16), indicated that labor was in some way important to the process.

Birth was known to be associated with a surge in fetal catecholamine secretion (42), and the critical link between \(\beta\)-adrenergic stimulation and lung liquid clearance was made in 1978 by Walters and Olver (68), who discovered that epinephrine and isoproterenol, but not norepinephrine, infused intravenously into chronically catheterized mature fetal lambs, caused the rapid absorption of lung liquid (Fig. 1) and that the
than that of epinephrine, and its role is uncertain. It was serially by use of an impermeant tracer (131I albumin). Positive values for liquid flow (Jv) indicate secretion, negative values absorption. Epinephrine, norepinephrine, and isoproterenol were infused at equimolar rates. Figure is from Walters and Olver (68); reprinted with permission.

Fig. 1. Effect of intravenous catecholamines on fetal lung liquid secretion in a chronically catheterized fetal lamb at 133 days gestation (term = 147 days). Volume of lung liquid was determined serially by use of an impermeant tracer (131I albumin). Positive values for liquid flow (Jv) indicate secretion, negative values absorption. Epinephrine, norepinephrine, and isoproterenol were infused at equimolar rates. Figure is from Walters and Olver (68); reprinted with permission.

The absorptive response could be inhibited by prior treatment with propranolol. Chapman and co-workers (23) later showed that lung lymphatics drained only a small fraction of the lung liquid cleared at birth, with most liquid passing directly into the pulmonary circulation.

In a complementary series of experiments, research groups in Toronto (27) and Boston (43) demonstrated that, in addition to effects on liquid secretion and lung water, β-adrenoceptor agonists caused the release of surfactant; the two physiological processes combined to improve early neonatal lung aeration and gas exchange (14, 29).

These observations were subsequently extended (18) to demonstrate that, at any particular gestation, a log-linear relationship existed between fetal plasma epinephrine concentration and the degree of inhibition of secretion or absorption of liquid, and that the threshold for absorption fell in late gestation to reach a level of 0.12 nmol/ml at term. During spontaneous labor, the relationship between endogenous fetal plasma epinephrine and the rate of liquid absorption was similar to that obtained by epinephrine infusion. Classically, β-adrenoceptor agonists act via the cAMP-protein kinase A signaling pathway, and, as predicted, the addition of a lipid-soluble analog of cAMP, dibuteryl cAMP, could be shown to mimic the effect of epinephrine in the mature fetal lamb (69). The fact that the response was similarly gestation dependent indicated that the rate-limiting step(s) involved post-cAMP regulation of Na⁺ transport, rather than changes at the β-receptor.

Another candidate regulatory hormone, arginine vasopressin, like epinephrine, rises during labor. Although arginine vasopressin can be shown to inhibit lung liquid secretion (54, 55), probably acting via the V₁ receptor and protein kinase C (3), its effect is weaker than that of epinephrine, and its role is uncertain. It has been suggested that it might provide backup for the β-adrenoceptor-dependent pathway and explain why β-adrenoceptor blockade fails to prevent lung liquid absorption during labor (23).

ROLE OF THE β-ADRENOCEPTOR-DEPENDENT PATHWAY IN POSTNATAL LUNG

The lung is absorptive at rest postnatally but can be induced to secrete in the artificially perfused neonatal lamb lung if Na⁺ absorption is blocked by addition of luminal amiloride (60). However, this response is lost after 2 wk of age. Although substantially lower than levels reached during labor, resting postnatal epinephrine levels (19) are above the threshold for absorption in the fetus, and it was naturally assumed (18) that this mechanism was sufficient to account for the postnatal absorptive phenotype. However, more recent work (63) does not support this hypothesis. In newborn lambs (up to 2 wk of age), epinephrine and dibuteryl cAMP both augmented lung liquid absorption, but the β-adrenoceptor blocker, sotalol, only partially inhibited resting absorption. In juveniles (6–12 wk of age), β-adrenergic blockade had no effect on liquid absorption (63). After sotalol, phosphodiesterase inhibition had no effect in newborns but, in juveniles, stimulated lung liquid clearance. Taken together, these data indicate that, although β-receptor activation is the sole source of cAMP in neonates, this mechanism is only partly responsible for resting lung liquid absorption and that beyond the newborn-period cAMP is derived from other sources, at least in part.

IN VIVO EVIDENCE THAT Na⁺ TRANSPORT PROVIDES THE DRIVING FORCE FOR LUNG LIQUID ABSORPTION

The absorptive response to infused epinephrine can be inhibited by mixing the Na⁺-channel blocker amiloride into lung liquid (52), and the inhibitor constant of 4 × 10⁻⁶ µM provides evidence that the effect is mediated by apically located amiloride-sensitive Na⁺ channels. Given that the threshold for absorption in response to infused epinephrine was similar to that resulting from the catecholamine surge in the fetus during spontaneous labor (18) it seemed probable that the epinephrine-induced activation of apical Na⁺ channels and the resulting increase in transepithelial Na⁺ transport were largely responsible for lung liquid clearance at birth. This hypothesis was supported by the observation (48) that amiloride instilled into the trachea of the newborn guinea pig impaired lung water clearance in a dose-dependent manner and was later confirmed by α-ENaC knockout studies (see below).

Evidence that labor was also crucial for the activation of the lung epithelial Na⁺-K⁺-ATPase came from measurements of the ouabain-sensitive uptake of rubidium-86 (⁸⁶Rb⁺) in freshly isolated alveolar type II (ATII) cells from rabbit pups. These showed that labor was associated with a three- to fourfold increase in pump activity compared with fetal ATII cells and ATII cells from newborn pups delivered by cesarean section (15, 24).
MOLECULAR EXPRESSION OF KEY TRANSPORT PROTEINS DURING DEVELOPMENT

In vivo and in vitro functional studies identified amiloride-sensitive Na⁺-transport mechanisms that mediate perinatal lung liquid absorption. The genetic identification of the amiloride-sensitive Na⁺ channel ENaC (20) and Na⁺-K⁺-ATPase (40) provided an opportunity for a detailed molecular analysis of this phenotypic switch in lung transepithelial liquid flow.

ENaC mRNA expression has been studied extensively in developing human and rodent lung epithelia. In both rat and mouse whole lung, there is an abrupt increase in α-ENaC mRNA expression in alveolar and airways epithelium around the time of birth. By the end of the first week of life, epithelial expression of α₁- and β₁-subunit mRNA is localized to small airways and the basolateral surface of ATII (25) but not alveolar type I cells (61).

ENaC mRNA expression has been studied extensively in developing human and rodent lung epithelia. In both rat and mouse whole lung, there is an abrupt increase in α-ENaC mRNA expression in late gestation that reaches levels seen in the adult lung shortly after birth (64, 65). In mouse lungs, there is a similar increase in expression of γ-ENaC and a more gradual increase in β-ENaC that peaks in adult lungs. By in situ hybridization, ENaC subunit expression is detected from the 16th gestational day onward. Expression of γ-ENaC parallels that of α-ENaC, with intense expression of subunit mRNA in all regions of the fetal lung, whereas β-ENaC expression in fetal and early postnatal lungs is restricted to the airway epithelium (64). Immunocytochemical studies of human fetal lung surprisingly showed significant α-ENaC protein expression in early midtrimester fetal lung at a time when the fetal lung is not thought to be capable of significant Na⁺ transport (62). Because there is no way of knowing whether the protein detected is functionally competent or appropriately located within the cell, it is possible that there is a discrepancy between developmental ENaC expression in human and rodent lungs.

Studies in guinea pig showed that both α-ENaC mRNA expression and the sensitivity of lung liquid clearance to amiloride and to β-adrenoceptor blockade were highest shortly after birth and declined in parallel with endogenous plasma epinephrine concentration (30), providing further support for the key role of β-adrenoceptor activation of Na⁺ channels at birth. However, because α-ENaC mRNA expression rapidly increases after caesarean section in late gestation and at term in guinea pig lungs (4), it would appear that the upregulation of this particular Na⁺ channel subunit is not related to labor. In fact, it may be a response to the increased lung liquid load at birth after cesarian section.

Other putative amiloride-sensitive Na⁺-permeable channels in fetal lung have been described that, although their molecular identities have not been reported, may contribute to the driving force for lung liquid clearance. These include a number of amiloride-sensitive poorly or nonselective cation channels: G protein regulated (44), β-adrenoceptor agonist or Ca²⁺ activated (45), and cyclic nucleotide gated (39). According to at least one school of thought (38), the β-adrenoceptor-responsive, nonselective channel may be a multimer of α-ENaC subunits, and the role of β and γ subunits may be to confer selectivity on the channel. An additional pathway for Na⁺ absorption is Na⁺-glucose cotransport, which has been reported in fetal sheep (6).

ENAC TRANSGENIC STUDIES

A number of “knockout” and other transgenic mouse models have been used to explore the role of ion transport in the perinatal regulation of lung liquid flow. Knockout mice for all three ENaC subunits have been reported (11, 36, 46), and additional ENaC mutants have been studied (37, 57). The most striking finding was that mutant newborns lacking the α-ENaC gene were unable to clear lung liquid from the alveolar spaces after birth (Fig. 2A). These mice were readily identifiable compared with their unaffected littermates by increased work of breathing, failure to feed, and inability to clear lung liquid from the alveolar spaces after birth (Fig. 2A). These mice were readily identifiable compared with their unaffected littermates by increased work of breathing, failure to feed, and
failure to move around. Mice with γ-ENaC or β-ENaC null mutations had delayed liquid clearance but had near normal lung water content 12 h after birth (11, 36). All subunit mutations resulted in severe hyperkalemia, which was the most likely cause of death in the early neonatal period. α-ENaC null mice that were “rescued” with an α-ENaC transgene were able to clear lung liquid in the newborn period at near normal rates with ~50% Na+/channel activity (37). These studies indicate that ENaC function is a critical requirement for clearance of neonatal lung liquid in the newborn period and suggest that the α-ENaC subunit is the core, rate-limiting part of the Na+ channel in lung epithelia. Evidently, neonatal and postnatal lung liquid clearance can be sustained with Na+/channel activity that is ~50% normal.

Although several aquaporin-type water channels have been found in different regions of the lung and their expression developmentally regulated, genetic deletions of these proteins in mice suggest that they do not play a significant role in perinatal lung liquid clearance (66).

HORMONAL REGULATION OF MATURATION OF THE EPINEPHRINE RESPONSE

The pivotal role of thyroid and glucocorticoid hormones in the maturation of the absorptive response to epinephrine is evident from a series of experiments by Barker and co-workers, whose initial studies showed a profound blunting of the response to epinephrine (and dibuteryl cAMP) in thyroidectomized fetal lambs (7) that was reversible by infusion of 3,5,3'-triiodothyronine (T3) alone (12). Neither T3 nor hydrocortisone given individually was capable of advancing maturation of the epinephrine response in normal fetuses with intact thyroid glands, but a powerful synergistic effect was observed when T3 and hydrocortisone were given concurrently (8). In thyroidectomized fetal lambs, the response to epinephrine was detectable within 2 h of the start of infusion of the hormone combination, and recovery of function, which could be blocked by the protein synthesis inhibitor cyclohexamide, was lost within 24–48 h of cessation of hormone administration (13). These data, together with the observation (34) that T3 and β-methasone have an additive effect in upregulating fetal rat lung surfactant synthesis in vivo, emphasize the high degree of coordination of development of the lung liquid clearance and surfactant systems for efficient lung adaptation at birth.

The observation (4) that the rise in α-ENaC expression parallels plasma cortisol (but not T3) in the late gestation and newborn guinea pig, and can be blocked by metyrapone after delivery by caesarean section, underscores the pivotal role of this hormone in the developmental regulation of ENaC near term. Tchepichev et al. (65) demonstrated that prenatal steroids, but not thyroid hormones, could advance timing of the increase in α-ENaC mRNA in fetal rat lung, but neither hormone had any effect on expression of β-ENaC or γ-ENaC mRNA expression. Champigny et al. (21) also reported no effect of thyroid hormones on ENaC mRNA expression but did report a potentiation by thyroid hormones of the steroid effect on Na+ currents in rat alveolar epithelial cells. Otulakowski et al. (53) have subsequently identified putative binding sites for thyroid and glucocorticoid receptors in the promoter region of α-ENaC, and, although T3 alone had no effect on reporter gene activity, it potentiated gene stimulation by steroid hormones. These synergistic effects of steroid and thyroid hormones echo their role in the maturation of the epinephrine response and its interaction with oxygen.

OXYGEN AND NEONATAL ION TRANSPORT

Of the other factors that come into play to maintain the absorptive phenotype during postnatal development, oxygen appears to be particularly important. The onset of breathing results in a sharp increase in alveolar PO2 from the fetal level of 23–25 Torr to approaching 100 Torr postnatally. Barker and Gatzy (9) examined the effect of PO2 on liquid production by fetal rat distal lung epithelium. A shift from fetal PO2 (~25 Torr) to room air (~150 Torr) resulted in a reduction in lung water-to-dry weight ratios and in the number and size of liquid filled cysts in late gestation lung explants, whereas there was no effect of PO2 at very early gestations. The effect of oxygen on liquid secretion could be induced in immature explants by coculture in thyroid and steroid hormones. These studies further demonstrated a crucial role of thyroid and steroid hormones in priming the distal lung epithelium to respond to triggers (epinephrine and oxygen) that switch transepithelial liquid flow from secretion to absorption at birth. Studies in rat fetal distal lung epithelial (FDLE) monolayers on the effect of raising PO2 to 150 Torr led to the conclusion that the observed increase in short-circuit current (Isc) was primarily due to increased expression of ENaC (56) and, therefore, by inference, an increase in apical Na+ conductance (GNa). This shift in PO2 was accompanied by a temporary fall in epithelial resistance lasting several hours, which was consistent with the increase in solute permeability noted by Egan et al. (26) at the onset of breathing in vivo and thought to assist passive liquid absorption. Vionna et al. (67) showed, in adult rats, that hypoxia decreased alveolar liquid clearance, an effect that was independent of ENaC and Na+/K+/ATPase subunit expression. These studies indicate that the oxygen-induced augmentation of neonatal alveolar Na+ and liquid transport may be reversible.

Recently, further experiments have been undertaken in mature rat FDLE cells to determine how the effects of shifting PO2 from fetal to neonatal levels and the action of hormones are integrated to control lung liquid transport by using a minimal defined serum-free culture medium (51). These studies established that addition of a combination of dexamethasone and T3 increased basal Isc and GNa irrespective of PO2 and permitted isoprenaline to upregulate these parameters at both fetal and neonatal PO2. Pump activity at neonatal, but not fetal PO2, was stimulated by dexameth-
role in respect of upregulation of G Na by β-adrenergic agonists at fetal and postnatal Po2, whereas a shift to neonatal Po2 is a prerequisite for hormonal upregulation of Na+/K+ -ATPase, with β-adrenergic-medi-
ated control of the pump only seen under hypoxic conditions. Postnatally, steroids and Po2 also have an additive effect in upregulating expression of the highly selective Na+-channel phenotype in ATII cells (37a).

The promoter region of rat α-ENaC contains a consen-
sus nuclear factor (NF)-κB binding element. Further ex-
periments with rat FDLE cells (58) showed that a rise in Po2 to 150 Torr induced this redox-sensitive transcription factor and that blocking NF-κB activation reduced the oxygen-evoked rise in Gnα (35). These findings could be interpreted as indicating that Po2 activation of NF-κB upregulated the expression of ENaC, increasing I sc and Na+ transport at the onset of breathing. However, subsequent experiments using FDLE monolayers (5, 59) demonstrated that a shift from fetal (23 Torr) to postnatal alveolar Po2 (100 Torr), which induces a maximal I sc response, resulted in an immediate increase in NF-κB expression and a detectable increase in Na/K ATPase capacity within 6 h, whereas activation of the α-ENaC promoter was not seen until after 24 h, reaching a maximum (to-
gether with Gnα) at 48 h.

It therefore appears that the early increase in fluid absorptive capacity in response to the rise in alveolar Po2 at birth is primarily the result of an increase in Na+/K+ -ATPase capacity and an increase in Gnα may be secondary to this increase in Na+ transport, not its cause. Both components of the response are enhanced by glucocorticoid and thyroid hormones that are also required for β-adrenergic-mediator control of Gnα. The discrepancy in the time course of activation NF-κB and the rise in α-ENaC expression indicates that activa-
tion of the α-ENaC promoter requires the concerted action of additional transcription factors.

CLINICAL APPLICATIONS

Whereas the role of ion transport in postnatal lung disease (cystic fibrosis, pulmonary edema) is well es-
stablished, the evidence for malfunction of ion transport as a cause of neonatal lung disease is still somewhat circumstantial.

Transient tachypnea of the newborn. The condition of transient tachypnea of the newborn (TTN) is probably the best described consequence of inadequate neonatal lung liquid clearance. This self-limiting disease, char-
acterized by an increase in respiratory rate, occurs more frequently in infants delivered by elective cesar-
ean section and is thought to result from delayed activa-
tion of liquid clearance in infants not subjected to the stress of labor (47, 50). Gowen and others (32) showed that newborn infants with TTN had a transient de-
crease in amiloride sensitive nasal epithelial Na+ transport compared with normal newborns, supporting the notion that suboptimal clearance of liquid is a cause of TTN. Infants with TTN were found to have low norepinephrine but normal epinephrine levels (33).

Respiratory distress syndrome. The ability to absorb lung liquid, the emergence of amiloride-sensitive Na+ transport, and the increase in ENaC expression are seen only in late gestation. These observations have focused attention on the possibility that infants born at very early gestations may not be able to clear lung liquid adequately and that retention of excessive liquid in the air spaces after birth may contribute to the maladaptation to air breathing seen in many preterm infants. Evidence in support of this proposition comes from studies of Na+ transport in the nasal epithelia of very preterm infants (10). Infants of >30 wk who developed respiratory distress syndrome (RDS) had lower amiloride-sensitive nasal electric potential difference than infants who did not develop RDS. Because this abnormality in nasal electric potential difference was observed within the first 12 h of life, it suggests that the absence of optimal Na+ transport in lung epithelia of very preterm infants may predispose to RDS and ultimately to chronic lung disease. Once lung disease is established, it is possible that inflammatory mediators may interfere with lung liquid clearance (31).

Pseudohypoaldosteronism. The study of human pa-
tients homozygous for mutations in ENaC subunits has yielded important information about the role of ENaC in the lung. A number of mutations in all three ENaC subunits has been described that give rise to the clinical condition of pseudohypoaldosteronism, a salt-
losing state that results in severe electrolyte disturb-
ances. In contrast to the respiratory distress seen in α-ENaC knockout mice, there is an absence of neonatal respiratory symptoms in these patients (41). However, recent studies suggest that oocytes expressing ENaC sunbunit mutants found in pseudohypoaldosteronism may exhibit significantly more Na+ transport than oocytes with an absent ENaC subunit (17, 22). These observations suggest that humans with pseudohypoaldosteronism may have enough residual ENaC activity to avoid perinatal water logging of the lungs and that the requirement for ENaC function in the lungs is considerably less than in the kidney.

SUMMARY

At birth, epinephrine, oxygen, glucocorticoid, and thyroid hormones interact to bring about a permanent change in the distal lung epithelium phenotype as the lung switches from secretion to absorption. Our current understanding, derived from classic integrative physiological studies combined with molecular physi-
ology approaches, indicates the following. 1) Lung liq-
uid clearance is triggered during labor by the surge in fetal catecholamines acting via β-adrenergic receptors lo-
cated in ATII cells. 2) Clearance is driven by active Na+ absorption resulting from an increased apical Gnα and Na+/K+ -ATPase activity. 3) The absorptive phe-
notype is maintained postnatally, at least in part, by

J Appl Physiol • VOL 93 • OCTOBER 2002 • www.jap.org
the rise in alveolar PO₂. 4) Hormone priming of the distal lung epithelium is required for β-adrenoceptor-mediated control of GNa. T₃ and glucocorticoids synergistically upregulate maturation of the absorptive response to epinephrine, principally via control of ENaC expression. 5) A shift to postnatal PO₂ is a prerequisite for upregulation of Na⁺K⁺-ATPase activity by T₃ and glucocorticoid hormones, but β-adrenoceptor agonists upregulate Na⁺K⁺-ATPase only at hyperoxic levels of PO₂. 6) The first discernible effect of the shift from fetal to postnatal PO₂ is activation of the Na⁺ pump. Prolonged elevation of PO₂ increases GNa, coinciding with increased α-ENaC abundance. Neither response to oxygen requires hormone priming. 7) The oxygen-responsive transcription factor NF-κB binds to the α-ENaC promoter, but increased α-ENaC expression in response to oxygen requires the concerted action of additional transcription factors. And 8) lung liquid clearance mechanisms are impaired in TTN and in premature infants with RDS, with the risk of both conditions being greater after delivery by elective cesarean section.

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