Airway Ion Transport on the First Postnatal Day in Infants Delivered Vaginally or by Elective Cesarean Section

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ABSTRACT

To determine airway ion transport in term infants on the first day of postnatal life, and to test the hypothesis that infants born without labor have reduced sodium absorption, we measured nasal potential difference using a modified perfusion protocol suitable for newborn infants. We examined maximal stable baseline potential difference, the change after perfusion with 10^{-4} M amiloride (Δ amil), and the change after perfusion with a zero-chloride solution (Δ zero Cl^-) in infants born after elective cesarean section ($n = 21$) or normal labor ($n = 20$). Maximal stable baseline potential difference was not different in the two cohorts (-24.0 mV, range -9 to -64 mV versus -25.5 mV, range -6 to -44 mV). The majority of infants in both cohorts

showed a substantial fall in potential difference after amiloride perfusion, and there was little capacity for chloride secretion. These results demonstrate a fluid absorptive pattern in the airways on the first postnatal day. In these well infants, the ion transport phenotype was not dependent on the presence or absence of labor. (*Pediatr Res* 54: 58–63, 2003)

Abbreviations

PD, potential difference
ENaC, epithelial sodium channel
RDS, respiratory distress syndrome

The fetal lung is filled with fluid, secreted by pulmonary epithelial cells through active Cl^- transport into the bronchial lumen with water moving along the osmotic gradient (1). Lung fluid production slows in late gestation (2). At the time of birth, changes in airway ion transport facilitate the adaptation to postnatal life and air breathing (3). Chloride ion secretion slows and Na/K ATPases in the basal membrane of the pulmonary epithelial cell actively transport Na^+ out of the cell, creating a transcellular gradient for Na^+ absorption, mediated through the apical ENaC (4).

The ENaC is composed of three homologous subunits, α , β , and γ , that together form a functional ion channel (5). In the human lung, ENaC expression has been found along the whole of the respiratory tract, from the nasal epithelium to the alveoli (6, 7).

Several important findings point to a critical role for the ENaC in the removal of lung fluid during the later stages of

gestation and after birth. Expression of ENaC, especially the α subunit, increases in the respiratory tract with advancing gestational age in humans (8). In the animal model, ENaC expression is further increased by administration of antenatal steroids (9). Transgenic knockout mice with the α ENaC gene disrupted die soon after birth from an inability to remove fluid from their lungs (10). ENaC can be effectively blocked by the drug amiloride, which acts as a competitive antagonist to Na^+ absorption (11). Instillation of amiloride into the lungs of guinea pigs just before their birth results in severe respiratory distress after delivery (12). Animal studies have demonstrated the importance of lung fluid removal on postnatal adaptation (13). Lambs born immediately after removal of half their lung fluid had significantly improved oxygenation and carbon dioxide clearance in the first few hours after birth compared with a control group.

The processes that determine the change in ion transport pattern at birth are not fully determined. It has been suggested that catecholamines play an important role (14, 15). Adrenaline infusion in chronically catheterized fetal lambs results in a switch from lung liquid secretion to absorption (16), and studies of lung liquid flow during normal labor has demon-

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strated a switch to fluid absorption in parallel with a rise in plasma adrenaline (3).

The aim of this study was to examine airway ion transport in infants born at term by either elective cesarean section (without labor) or vaginally after normal labor. The study was designed to test the hypothesis that infants born without normal labor have reduced ENaC-mediated Na^+ transport in their airways on the first day of postnatal life. The nasal PD measurement was used to measure airway ion transport.

A PD is generated across airway epithelia through the active transport of charged ions, and the change in PD after perfusion with various solutions gives a relatively noninvasive insight into the various components of airway ion transport. In particular the topical perfusion of amiloride allows characterization of the degree of ENaC-mediated Na^+ absorption (17, 18). In adults nasal PD correlates well with the PD measured in the trachea and distal bronchi (19, 20).

A previous nasal PD study of term infants suggested altered airway ion transport in infants born without labor (an increased baseline PD and reduced reduction in PD magnitude after perfusion with amiloride) (21). We used a modified nasal PD protocol, validated for use in infants (18), to further examine the airway ion transport phenotype of well infants on the first day of postnatal life.

METHODS

This study was approved by the local research ethics committee. Informed consent was obtained antenatally from the parents of all infants in this study.

We tested the hypothesis that birth without labor is associated with reduced ENaC-mediated Na^+ absorption, resulting in 1) lower maximal stable baseline PDs and 2) reduced responses to amiloride perfusion. We also investigated the capacity of the infant nasal airway epithelium for Cl^- secretion.

Subjects. We studied 21 infants delivered by elective cesarean section at term (no labor) and 20 infants delivered vaginally (normal labor) at term. Infants in the normal labor group were recruited during a 4-wk period by approaching parents attending the 20-wk antenatal booking clinic (if pregnancy and delivery were expected to be normal). Infants for the elective cesarean section group were recruited consecutively on the antenatal ward when mothers were admitted the day before delivery.

Nasal PD measurement. We performed a nasal PD measurement in the first 24 h after birth. Nasal PD was measured between the nasal epithelium along the floor of the nose of infants and a reference electrode (a sterile butterfly needle with an internal diameter of 0.6 mm (Venisystems, Abbott, Ireland), filled with 0.9% NaCl solution and 3% agar inserted into the umbilical stump and secured to the cord clamp). The exploring electrode consisted of a single-lumen catheter [polyethylene (PE-10) tubing] threaded over a 30-gauge needle. Three perfusion lines (Silastic tubing, inner diameter, 0.51 mm, outer diameter 0.94 mm; Dow Corning Corp, Midland, MI, U.S.A.) were secured together with a saline bridge into the hub of the 30-gauge needle. Both catheters are connected to a high-impedance millivolt meter via saline/agar bridges and half-cell

calomel electrodes (Accumet Miniature Calomel Reference Electrode; Fisher Scientific, Pittsburgh, PA, U.S.A.). Each nostril was sequentially perfused with an isotonic solution at a rate of 0.1 mL min^{-1} , followed by the same solution with amiloride 10^{-4} M added, and finally a zero- Cl^- solution with amiloride (10^{-4} M).

Infants were loosely swaddled, and a skin PD was obtained to check the reference electrode. Nasal PD was measured at 0.25-cm intervals along the floor of the nose. We identified the point of highest stable PD.

From this point three principal measurements were obtained:

1. **Stable maximal baseline PD:** This measurement reflects the sum of all the ion transport processes in the airway epithelium during perfusion with an isotonic solution. In older children and adults the predominant airway ion transport processes (Na^+ absorption and anion secretion) render the PD negative when viewed from the lumen. The median time to achieve a stable maximal PD was 50 s (range, 20–210 s). Occasionally the baseline PD would be drifting up or down, under which circumstance we would not add amiloride until the trace was stable for at least 10 s (median, 20 s; range, 10–60 s).

2. **Δ amil:** This measurement is the response of the PD after addition of amiloride to the isotonic perfusate. As amiloride selectively inhibits ENaC, the reduction in PD magnitude after amiloride perfusion is a direct reflection of the degree of ENaC-mediated Na^+ absorption. In addition to the reduction in millivolts, Δ amil can also be expressed as a percentage of the stable maximal baseline PD.

3. **Δ zero Cl^- :** Subsequent perfusion of an isotonic solution free of Cl^- provides a permissive environment for Cl^- secretion and in older children and adults is associated with an increase (hyperpolarization) of the PD (17), which directly reflects the capacity of the epithelium to secrete Cl^- . The zero- Cl^- solution contains amiloride and is only commenced once the PD has stabilized after amiloride perfusion.

Occasionally the maximal stable baseline PD fluctuated (associated with respiration). The midpoint of the fluctuation was then taken as the maximal stable baseline PD. The median degree of fluctuation was 1 mV (range 0–7 mV). A single investigator (E.A.G.) performed the measurements and, for logistical reasons, was not blinded to the mode of delivery. The nasal PD tracings were analyzed independently by two investigators, one of whom (K.W.S) was unaware of the gestation and clinical condition of the infant at the time of nasal PD measurement.

Statistical analysis. From previous studies (17, 21), we estimated that a total sample size of 40 infants (20 in each group) would be sufficient to detect a clinically relevant difference of 10 mV in the primary outcome measure (maximal stable baseline PD), with 95% power at a 1% significance level. The software program SPSS for Windows 10.0 (SPSS Inc., Chicago, IL, U.S.A.) assisted the analysis. Distribution of the data was analyzed using the Shapiro-Wilk test and by examination of histograms. Comparisons between infants born by cesarean section and vaginal delivery were made using the Mann-Whitney *U* test (histograms for most variables did not show normal distribution). Spearman's rank correlation coef-

ficient was used to test for correlation between the continuous variables, time of measurement and stable maximal baseline PD.

RESULTS

The nasal PD measurement was well tolerated by all infants. The groups were well matched for birth weight and sex; however, gestational age was lower (median, 38 wk *versus* 40 wk), and the time interval between birth and nasal PD measurement was shorter (median, 6 h *versus* 12 h) in the elective cesarean section group (Table 1).

There was no significant correlation between the maximal stable baseline PD and the postnatal age at measurement using Spearman's rank correlation ($r^2 = 0.08$ for the group as a whole, $p = 0.61$; normal labor, $r^2 = -0.08$; $p = 0.74$; no labor, $r^2 = 0.25$; $p = 0.28$).

The maximal skin PD was not different for the two groups. There was no correlation between the maximal skin PD and maximal stable baseline PD. The clinical course for all infants was unremarkable. No umbilical stump became infected.

Comparison of maximal stable baseline PD values between both nostrils was made using the technique described by Bland and Altman (Bland-Altman plot not shown) (22). The SD of the differences between nostrils was 13 mV with limits of agreement of -29.7 and 23.3 mV. There was no statistically significant difference in maximal stable baseline PD values from the first and second nostril (mean difference, -3.7 mV; 95% confidence interval, -8.7 to 1.3 mV). Only data from the first nostril measurement are included (Table 2). Representative tracings are shown in Figures 1 and 2.

Median maximal stable baseline PD was -24.0 mV in the cohort of infants born after elective cesarean section and -25.5 mV in the normal delivery group. After amiloride perfusion, PD magnitude fell by a median of 19.0 mV in the elective cesarean section group compared with 20.5 mV for the normal delivery group. The median change in PD after perfusion with a zero- Cl^- solution was -1.0 mV in the elective cesarean section group ($n = 19$) and -2.5 mV in the normal delivery group ($n = 20$). Two infants in the no labor group became unsettled during perfusion with the zero- Cl^- solution, and we were unable to obtain satisfactory tracings. The zero- Cl^- element of these tracings was not analyzed.

There was no statistically significant difference between the two groups for any ion transport variable studied.

DISCUSSION

The results of this study support an absorptive airway ion transport pattern in healthy term infants on the first day of

Table 2. Variables obtained from perfusion studies*

	No labor	Normal labor	<i>p</i> value
Maximal stable baseline PD (mV)	-24.0 (-9 to -64)	-25.5 (-6 to -44)	0.99
Δ amil (mV)	19.0 (5 to 56)	20.5 (5 to 38)	0.98
Δ amil (%)	-79.0 (-50 to -94)	-83.5 (-36 to -100)	0.59
Residual PD (mV)	-5.0 (-2 to -11)	-5.0 (0 to -20)	0.88
Δ zero Cl^- (mV)	-1.0 (6 to -23)	-2.5 (4 to -22)	0.54
Maximal skin PD (mV)	-31.0 (-13 to -54)	-33.0 (-15 to -57)	0.47

* All values given as median (range). Statistical analysis was performed using Mann-Whitney *U* test.

postnatal life. They do not support the hypothesis that infants born without labor have reduced ENaC-mediated Na^+ transport.

An increased incidence of RDS and transient tachypnea of the newborn has been described in term infants delivered by elective cesarean section before the onset of labor (23). This has been attributed to delayed lung fluid removal in those infants (24). When lung volumes in term infants soon after birth after no labor were compared with those of infants born after normal vaginal delivery, the most striking difference was significantly reduced total lung volumes in the no labor group despite similar thoracic circumference (25). The authors concluded that an increased lung liquid volume with decreased gas component may be the reason for these findings. The respiratory rates between the groups were not different.

Gowen and colleagues (21) reported a significant difference in mean baseline PD (which corresponds to our maximal stable baseline PD) between infants born at term after normal labor (mean, -19.7 mV) compared with infants born after no labor (mean, -27.7 mV) on the first day of postnatal life. This mean baseline PD was higher still in infants with raised respiratory rates (mean, -29.6 mV) and was associated with a reduced reduction in PD magnitude in response to amiloride perfusion. The fall in PD after perfusion with amiloride (10^{-5} M) was less than 40% in all groups on the first postnatal day. The authors speculated that these findings might reflect ongoing chloride secretion or amiloride-insensitive sodium absorption.

Barker *et al.* (26) reported lower maximal PD values in infants of less than 30 wk gestation with RDS compared with non-RDS infants (mean, -16.5 *versus* -22.5 mV). Infants with RDS also had significantly reduced inhibition of amiloride-sensitive ENaC-mediated Na^+ transport compared with non-RDS infants (29.5% inhibition *versus* 38.6%). Again the authors speculate that these results indicate an incomplete switch from a secretory to a fluid-absorbing airway epithelium. The latter study also found higher maximal PDs with increased gestation, suggesting increased ENaC-mediated sodium transport in more mature infants.

In this study, maximal stable baseline PDs in the no labor group are lower (median, -24.0 mV) compared with those reported by Gowen *et al.* (21); however, we found higher PDs in the group experiencing normal labor (median, -25.5 mV).

We also found the range of maximal stable baseline PD values to be wide in both cohorts, suggesting variability in ENaC-mediated Na^+ transport on the first postnatal day. This

Table 1. Clinical characteristics of infants*

	No labor	Normal delivery	<i>p</i> value
<i>n</i>	21	20	
Male (%)	14 (67)	10 (50)	
Birth weight (g)	3700 (2180–4540)	3546 (2768–4300)	0.39
Gestation (wk)	38 (37–40)	40 (38–41)	<0.001
Age at measurement (h)	6 (3–10)	12 (2–22)	<0.001

* All values given as median (range). Mann-Whitney *U* test was used for statistical analysis.

Figure 1. PD tracing from a 38-wk gestation infant, vaginal delivery, 22 h old. After checking zero and skin PD, the maximal stable baseline PD was identified (-22 mV). Amiloride perfusion (0.1 mL/min) was then commenced, and PD fell by 21 mV to -1 mV. There was no change in PD after zero- Cl^- perfusion at 0.2 mL/min. Zero was rechecked at completion of the study.

Figure 2. PD tracing from a 39-wk infant, elective cesarean section, 5 h old. Maximal stable baseline PD was -28 mV. After amiloride perfusion 0.1 mL/min PD fell to -9 mV. There was a marked response to perfusion with zero- Cl^- solution (0.2 mL/min), with $\Delta\text{zero Cl}^-$ of -23 mV.

may reflect more advanced lung fluid clearance in some infants compared with others; however, all these infants were well.

There are a number of methodological differences that may account for the disparity between our results and those of Gowen *et al.* (21). We used a perfusion protocol modified and validated for use in infants with lower perfusion rates (18). Using this method we were able to obtain high-quality recordings. We found the technique was well tolerated; however, infants would occasionally become unsettled during the second nostril measurement, giving less satisfactory recordings. For the analysis we used only results from the first nostril recording. Despite low perfusion rates, fluid could be detected in both nostrils during the first nostril recording, giving rise to the possibility of amiloride contamination in the second nostril. (This was most obvious in cases in which PDs were high). Comparison of maximal stable baseline PD values between the nostrils using the technique described by Bland and Altman (22) confirmed this clinical suspicion, and although the differ-

ence did not reach statistical significance, we decided not to include second nostril data in the analysis.

We have not calculated a mean PD. Maximal stable PD is, in our opinion, a more valid and reproducible measure to describe the level of ENaC activity. In the study by Gowen *et al.* (21) the use of mean PD or mean maximal PD did not alter the conclusions from the study.

Finally, we demonstrated much greater responses to perfusion with amiloride than Gowen *et al.* (21), and this may reflect the higher concentration of amiloride used in our study (10^{-4} M versus 10^{-5} M). Our dose of amiloride was derived from previous work demonstrating a further reduction in PD when changing from 10^{-5} M to 10^{-4} M (18). In addition to the methodological disparities, the infants in our study were all well, and their ion transport phenotypes may reflect this. Indeed our findings are not at odds with the hypothesis that ENaC-mediated Na^+ absorption is primed by catecholamines, as previous studies have demonstrated

similar catecholamine levels in infants born with or without labor (27).

There were significant differences between our two cohorts with respect to gestation and time of measurement, related to logistical considerations (all elective cesarean sections were performed at 38 wk and their measurements were performed in the afternoon after delivery in the morning). These are potentially important confounding variables. There is evidence for a gestation-dependent increase in ENaC; however, this relates to subunit (8) or subunit-encoding mRNA levels (9) rather than functional studies. There are to our knowledge no reported data from clinical studies on ENaC-mediated Na^+ transport in moderately preterm infants greater than 30 wk gestation. When comparing our data to PDs reported by Gowen *et al.* (21), the difference in baseline PD in the no labor cohorts is relatively small. We did, however, find higher maximal stable baseline PDs in the cohort of infants who experienced normal labor.

Timing of the nasal PD measurement may also have influenced our results. There was, however, no correlation between maximal stable baseline PD and postnatal age in hours; these data are consistent with the findings of Gowen *et al.* (21).

This is the first report of nasal PD measurements assessing the capacity for Cl^- secretion in infants. The median response of PD to perfusion with zero- Cl^- solution was low in both cohorts of infants. Nasal PD protocols designed for older children and adults use a further zero- Cl^- solution with the addition of a β -adrenergic agonist such as isoproterenol. This results in further cAMP-mediated Cl^- secretion. Isoproterenol was omitted from our protocol because of concerns of systemic effects in infants. Perfusion with a zero- Cl^- solution is associated with sustained hyperpolarization of PD, generally with a change in PD of greater than -10 mV. Knowles *et al.* (17) in 72 studies involving subjects with an age range from 1 to 70 y found a mean change in PD after zero- Cl^- solution of -18.0 mV (range, 0 to -40 mV).

Some infants in our study did demonstrate a capacity to secrete Cl^- ; however, the median response of -1.0 (no labor) and -2.5 mV (normal labor), respectively, suggests that Cl^- secretion is not a significant ion transport process in infants shortly after birth. As Cl^- channel expression is not reduced at birth, this finding possibly reflects active down-regulation of Cl^- transport on the first postnatal day.

In contrast, maximal stable baseline PDs were often high, and Δamil was large [median % inhibition in PD magnitude, 83.5% (no labor) and 79.0% (normal labor)]. Indeed, one infant fulfilled predetermined criteria to screen for cystic fibrosis (a condition characterized by airway ion transport abnormalities; namely Na^+ hyperabsorption and lack of Cl^- secretory capacity). The infant did not have clinical features suggestive of cystic fibrosis, and subsequent sweat electrolyte analysis was normal (28).

CONCLUSIONS

In summary, this study does convincingly demonstrate that, on the first day of postnatal life, well term infants have an ion transport profile that is predominantly absorptive, with well-developed ENaC-mediated Na^+ absorption and a reduced ca-

capacity for Cl^- secretion. This study does not support the hypothesis that infants born without labor have reduced ENaC-mediated Na^+ transport on the first day of postnatal life if they are well.

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Erratum

In the article, “Effects of Dietary Sphingomyelin on Central Nervous System Myelination in Developing Rats” (*Pediatr Res* 53:589–593), a few corrections were not made to the final proof. They are listed below. The publisher regrets the error.

1. Last sentence in the “ABSTRACT” should read

These findings suggest that dietary SM contributes to CNS myelination in developing rats with experimental inhibition of SPT activity.

2. Correspondence:

Kyoichi Oshida, Ph.D.

3. “Study design.” in the “METHODS” (right column, line 11)

s.c. injection of LCS (100 mg/kg; Sigma-Aldrich Japan K.K., Tokyo, Japan)

4. “Morphometric analysis of optic nerve myelination.” in the “METHODS” just above Equation 1

The axon area was obtained from the digitized axon perimeter using NIH Image v.1.61 software

5. “Morphometric analysis of optic nerve myelination.” in the “METHODS” just after Equation 1

The fiber area was obtained using NIH Image software to detect the digitized outermost major dense line of the myelin sheath...

6. Last footnote in Table 2

For the LCS group, values in parenthesis indicate the percent decreases relative to the non-LCS group.