Perinatal changes in expression of aquaporin-4 and other water and ion transporters in rat lung

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1. At birth, rapid removal of lung liquid from potential airspaces is required to establish pulmonary gas exchange. To investigate the role for water channels, aquaporins (AQP) and ion transporters in this process, the mRNA expression of AQP, Na^+,K^+-ATPase and the amiloride-sensitive Na^+ channel (ENaC) were studied in the fetal and postnatal rat lung.

2. The mRNA expression of all transporters studied increased postnatally.

3. The following water channels were expressed in the lung, AQP1, 4 and 5. The most specific perinatal induction pattern was observed for AQP4. A sharp and transient increase of AQP4 mRNA occurred just after birth coinciding with the time course for clearance of lung liquid. This transient induction of AQP4 mRNA at birth was lung-tissue specific. Around birth there was a moderate increase in AQP1 mRNA, which was not transient. AQP5 increased continuously until adulthood.

4. Fetal lung AQP4 mRNA was induced by both β-adrenergic agonists and glucocorticoid hormone, which are factors that have been suggested to accelerate the clearance of lung liquid.

5. Immunocytochemistry revealed that AQP4 was located in the basolateral membranes of bronchial epithelia in newborn rats, consistent with the view that this is the major site for perinatal lung liquid absorption.

6. The Na^+,K^+-ATPase $\alpha 1$ subunit and ENaC α-subunit mRNA also increased around birth, suggesting that they co-operatively facilitate lung liquid clearance at birth.

7. These data indicate that removal of lung liquid at birth is associated with pronounced and well-synchronized changes in the expression of AQP and the ion transporters studied. The transient perinatal induction of AQP4, which could be prenatally induced by β-adrenergic agonists, and the localization of this water channel strongly suggest that it plays a critical role for removal of lung liquid at the time of birth.

During fetal life, fluid is secreted into the lung lumen, and the fetal lungs are filled with liquid (Bland & Nielson, 1992). At birth, rapid removal of liquid from potential airspaces is required to establish a timely switch from placental to pulmonary gas exchange. Delay of this switch may contribute to respiratory distress syndrome (RDS) in very premature infants, infants from diabetic mothers and infants delivered by Caesarean section without prior labour (Bland, Bressack & McMillan, 1979). The triggers and mechanisms for this switch are incompletely defined. Hormones, including adrenaline, have been suggested to accelerate the clearance of lung liquid (Barker, Markiewicz, Parker, Walters & Strang, 1990). Both *in vivo* and *in vitro* studies support the view that β-adrenergic activation, via a cAMP-dependent mechanism, may trigger absorption of luminal liquid (Chapman, Carlton, Cummings, Poulain & Bland, 1991), although the target molecules for this effect are still unknown.

For many years it was thought that hydrostatic and protein osmotic pressures accounted for luminal liquid clearance, but in the past decade, several studies have provided evidence that sodium transport across the pulmonary

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epithelium drives removal of luminal liquid. There is accumulating evidence that the amiloride-sensitive epithelial sodium channel (ENaC) and Na^+,K^+-ATPase (NKA) are important for this process. There is an increase in the abundance and activity of NKA in late gestation (Ingbar *et al.* 1996). ENaC-α mRNA also increases in late gestation (O'Brodovich, Canessa, Ueda, Rafii, Rossier & Edelson, 1993; Tchepichev, Ueda, Canessa, Rossier & O'Brodovich, 1995), and it was recently reported that ENaC-deficient mice died just after birth from failure to clear their lungs of liquid, indicating that functioning ENaCs are necessary for lung liquid clearance at birth (Hummler *et al.* 1996). This transcellular sodium transport across the lung epithelium, via ENaC and NKA, produces an osmotic gradient that might contribute to absorption of liquid from the lung lumen during and after birth.

The rate of fluid absorption will also depend on the water permeability of the pulmonary epithelium. The recent molecular cloning of aquaporins (AQP) made it possible to study the molecular mechanism behind water transport (Agre, Brown & Nielsen, 1995; King & Agre, 1996). Channel-mediated water transport across the membrane provides a high osmotic water permeability. It was recently shown that the distal airway had a relatively high osmotic water permeability with low activation energy, providing functional evidence for transcellular movement of water through AQP (Folkesson, Matthay, Frigeri & Verkman, 1996). Among the mammalian AQP, AQP1, 4 and 5 are expressed in adult lung (Folkesson, Matthay, Hasegawa, Kheradmand & Verkman, 1994; Raina, Preston, Guggino & Agre, 1995; Folkesson, *et al.* 1996; King, Nielsen & Agre, 1996). It was reported that the level of AQP1 increased from the last gestational day to the first postnatal day and persisted at high levels into adulthood (King *et al.* 1996). Interestingly, AQP1 is not expressed in airway epithelium but is expressed in peribronchial vessels and visceral pleura, indicating the existence of other aquaporins in the airway epithelium.

The present study compares the developmental expression of AQP and ion transporters in the perinatal rat lung. There was a very pronounced and transient increase of AQP4 mRNA immediately after birth. AQP4 mRNA levels in embryonic rat lung were induced in response to a β-adrenergic agonist and glucocorticoid hormone (GC) treatment. Immunocytochemical studies showed that the AQP4 was present in the plasma membranes of bronchial epithelial cells. The mRNA levels of ENaC-α, NKA-α1 and AQP1 increased just before birth. Taken together, these observations indicate that there is a well-synchronized development of AQP and ion transporters that co-operatively facilitate lung liquid clearance at birth and that AQP4 plays an important role for removal of lung liquid at birth.

METHODS

Materials

Polyclonal, affinity-purified rabbit antibodies to AQP4 were previously described (Terris, Ecelbarger, Marples, Knepper & Nielsen, 1995; Nielsen, Nagelhus, Amiry-Moghaddam, Bourque, Agre & Ottersen, 1997). A full length of rat NKA-α1 subunit cDNA was the kind gift of Dr Jerry B. Lingrel (Cincinnati, OH, USA). Hybridization reagents and [α-^{32}P]-dCTP for Northern blotting were from Amersham. Reverse transcription polymerase chain reaction (RT-PCR) reagents were from Promega (Madison, WI, USA). Terbutalines were from Draco (Lund, Sweden). Betamethasones were from Glaxo Wellcome.

Animal protocols

Studies were performed on embryonic and postnatal Sprague–Dawley rats (ALAB, Sollentuna, Sweden). All animal studies were undertaken with protocols approved by the ethical review committee on animal experiment for Northern Stockholm. To determine the fetal expression of AQP and ion transporters, timed gestation pregnant rats were anaesthetized by an intraperitoneal injection of thiobutabarbitone (8 mg (100 g body weight)$^{-1}$) at 18–21 days gestation (term, 22 days) and killed by decapitation. Fetuses were immediately decapitated. Lungs of fetuses from the same litter were rapidly removed and pooled. Young rat pups were maintained with their dams (6–8 pups per dam), which were fed a standard rat chow (Ewos, Södertälje, Sweden) and received tap water *ad libitum*. Rat pups were anaesthetized by an intraperitoneal injection of thiobutabarbitone (8 mg (100 g body weight)$^{-1}$) and decapitated at 2, 10, 14, 18 and 20 days after birth, and the lungs were rapidly removed. Adult rats were also given a standard rat chow (Ewos) and tap water *ad libitum*, and were anaesthetized by an intraperitoneal injection of thiobutabarbitone (8 mg (100 g body weight)$^{-1}$), killed and the lungs rapidly removed.

To determine whether β-agonist or GC treatment could accelerate the mRNA expression of ion transporters and aquaporins, three groups of pregnant rats were injected intraperitoneally with betamethasone, terbutaline or 0·9% NaCl at the 20th day of gestation. Group 1 received a single injection of betamethasone (60 μg (100 g body weight)$^{-1}$) and were killed after 24 h. Group 2 received terbutaline (10 μg (100 g body weight)$^{-1}$), which was injected 3 times within 24 h since terbutaline has a short half-life. The rats were anaesthetized by an intraperitoneal injection of thiobutabarbitone (8 mg (100 g body weight)$^{-1}$) and killed 24 h after the first injection. Group 3 rats (control) received a single injection of 0·3 ml 0·9% NaCl and were killed 24 h after injection. The fetuses were removed and fetal lungs were rapidly removed.

Northern blotting

Total RNA was extracted from freshly dissected tissue as previously described (Yasui *et al.* 1996). Total RNA (20 μg) was then separated on a 1% agarose gel containing 2% formaldehyde, and the RNA was electrophoretically transferred and cross-linked to Hybond+ nylon filters (Amersham). Prehybridization (20 min) and hybridization (3–4h) was performed at 65 °C in Amersham rapid hybridization buffer. The probes were random-primed (Multiprime DNA labelling system; Amersham) with [α-^{32}P]-dCTP (Amersham). NKA-α1 subunit probe is a full length rat cDNA. ENaC-α subunit probe, AQP1, AQP2, AQP3, AQP4 and AQP5 probes were synthesized from rat lung or kidney mRNA by RT-PCR according to the published sequences. Each RT-PCR product

Table 1. RT-PCR primers list

Gene	Primers	GC (%)	T_m (°C)	Length (bp)	Product size (bp)
1. NAK α-1	5′-ATGAT TGACCCTCCTCGAGCTGCT-3′	54·2	59·0	24	816
	5′-GGCCTGGATCATACCGATCTGT-3′	54·5	56·6	22	
2. ENaC α	5′-TGGACCACACCAGAGCCCCTGA-3′	54·5	56·6	22	223
	5′-AAT TCA ATCAGTGCCTCCTCCTCC-3′	50·0	57·3	24	
3. AQP1	5′-CTGTGGTGGCTGAGT TCCTG-3′	60·0	64·7	20	344
	5′-AT T TCGGCCA AGTGAGT TCT-3′	45·0	63·1	20	
4. AQP2	5′-AGTGCTGGCTGAGT TCT TGG-3′	55·0	53·7	20	345
	5′-GCTGTGGCGT TGT TGTGGAG-3′	60·0	52·9	20	
5. AQP3	5′-GAGATGCTCCACATCCGCTAC-3′	57·1	56·2	21	485
	5′-CACACA ATA AGGGCTGCTGTGC-3′	54·5	56·6	22	
6. AQP4	5′-TCCCTCTGCT T TGGACTCAGCAT TG-3′	52·0	62·2	25	576
	5′-GGCT TCCT T TAGGCGACGT T TGAG-3′	54·2	62·1	24	
7. AQP5	5′-CCATCT TGTGGGGATCTACT TCAC-3′	50·0	57·3	24	435
	5′-T TATGGGCT TCTGCTCCTGTCC-3′	54·5	56·6	22	
8. β-Actin	5′-AAGATCCTGACCGAGCGTGG-3′	60·0	55·8	20	429
	5′-GCTAGGAGCCAGGGCAGTA-3′	63·2	55·3	19	

T_m, melting temperature; GC, GC content.

was subjected to restriction enzyme analysis to verify the specificity (data not shown). Autoradiographs were obtained by exposure to hyperfilm (Amersham) with intensifying screens at −80 °C for 1–2 days. Autoradiogram bands were quantified with an LKB Ultrascan XL laser densitometer interfaced to an IBM PC. Peaks were integrated using LKB software. Data were corrected by the intensity of P40 transcripts to which an arbitrary value of 1 is given.

Semi-quantitative RT-PCR

Semi-quantitative RT-PCR was performed as previously described with some modification (Yasui *et al.* 1996). Reverse transcription was carried out in a 10 μl reaction volume containing 1 μg total RNA. The RT reaction was converted to a 50 μl PCR containing 2·0 U Taq DNA polymerase (Promega), 20 pM specific primer pairs, 200 μM dNTPs, 10 mM Tris–HCl pH 8·8, 1·5 mM $MgCl_2$, 50 mM KCl, 0·1 % Triton X-100, and sequentially cycled 20, 22, 26 and 30 times (60 °C for 45 s, 72 °C for 60 s and 94 °C for 30 s), starting at 96 °C for 2 min and finishing at 72 °C for 5 min. There was linearity of response for at least twenty-six cycles (data not shown). The primers used (Table 1) were designed according to the published sequences. The specific primers were always added with β-actin primers in the same reaction tube. There was no interference between the specific primers and β-actin primers (data not shown). Samples were routinely amplified in the presence of [α-^{32}P]-dCTP (3000 Ci mmol^{-1}; 2 μCi per sample; Amersham). A 20 μl aliquot of the PCR product was separated on a 2% agarose gel and stained with ethidium bromide. Each specific band was cut and counted by scintillation spectrometry. Results are normalized to the amount of amplified β-actin mRNA. When the reverse transcriptase was omitted from the RT-reaction solution, no products appeared, confirming that the products were produced from mRNA and not contaminating genomic DNA. RT-PCRs were carried out at least 3 times with three different RNA preparations. All reaction solutions (except RNA and enzymes) in the RT-PCRs were premixed to eliminate errors during pipetting. DNA molecular weight marker VI (Boehringer Mannheim) was used on all agarose gels.

Immunocytochemistry

For immunocytochemistry, lungs of rat pups at postnatal day 1 were fixed by vascular perfusion via the right ventricle with 4% paraformaldehyde in 0·1 M sodium cacodylate buffer, pH 7·4. Immunocytochemistry was performed as previously described (Yasui *et al.* 1996; King *et al.* 1996). Tissue blocks were postfixed for 2 h, and infiltrated with 2·3 M sucrose–2% paraformaldehyde for 30 min. Blocks were mounted on holders, and rapidly frozen in liquid nitrogen. Thin (0·85 μm) cryosections, cut on a Reichert Ultracut FSC, were incubated with affinity-purified antibodies against AQP4 (LL182AP) characterized previously (Terris *et al.* 1995; Nielsen *et al.* 1997). The labelling was visualized as previously described (Nielsen, Smith, Christensen, Knepper & Agre, 1993; King *et al.* 1996; Yasui *et al.* 1996) using horseradish peroxidase-conjugated secondary antibodies (P448, 1 : 100, DAKO A/S, Glostrup, Denmark) and sections were counterstained using Meier counterstain. Controls where primary antibody was exchanged for pre-immune serum or antiserum absorbed with excess synthetic peptide revealed no labelling. Light microscopy was performed using a Leica Laborlux S microscope.

Statistics

Values are given as means ± S.E.M. The statistical analysis was performed with ANOVA and Student's unpaired *t* tests. Significance was established as $P < 0·05$. For the clarity of presentation, results of the effect of β-adrenergic agonists and GC were normalized as a percentage of control; however, statistical differences were always determined from the primary data.

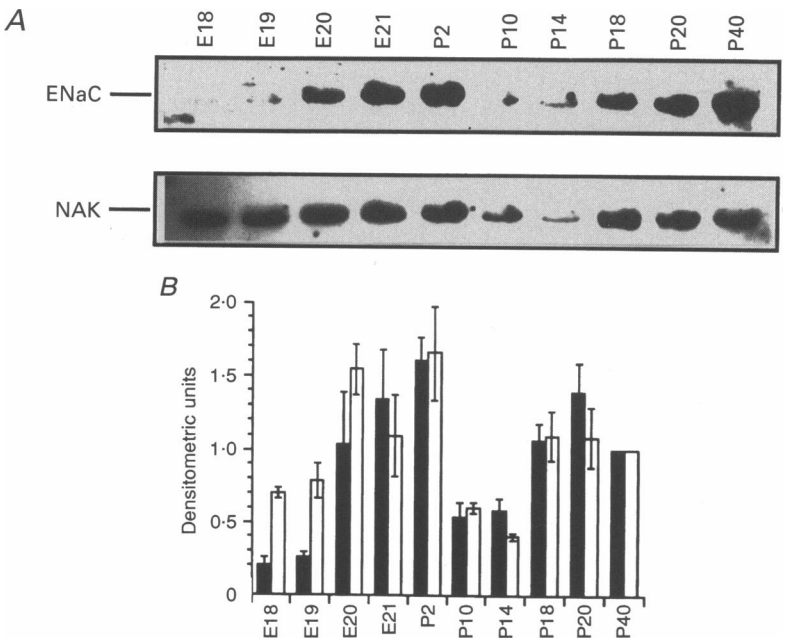

Figure 1. ENaC-α and NKA-α1 Northern analysis

A, whole lung total RNA (20 μg in each lane) were extracted from different gestational ages of fetal (term, 22 days) and postnatal rats. *B*, Northern blots were analysed by densitometry, and the results from three different filters are expressed in arbitrary units as means ± S.E.M. for each age: ENaC, filled bars; NKA, open bars. Arbitrary unit one was given to the transcripts of P40.

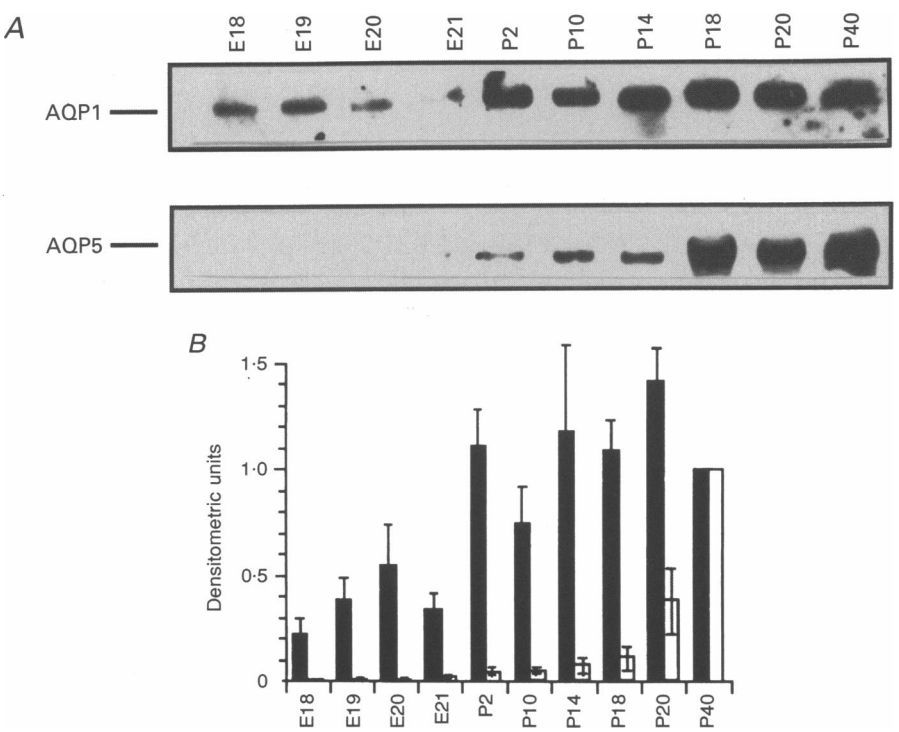

Figure 2. AQP1 and AQP5 Northern analysis

A, representative Northern blots showing AQP1 and AQP5 mRNA expression in perinatal rat lung. *B*, densitometric analysis of Northern blots from three different filters expressed in arbitrary units as means ± S.E.M. with P40 transcripts at one: AQP1, filled bars; AQP5, open bars.

RESULTS

Northern blot analysis was used to study the mRNA expression of ENaC-α, NKA-α1 and AQP1–5 during embryonic (E) and postnatal (P) lung development (Figs 1, 2 and 3*A*). The ENaC-α was not detected in E18, but increased dramatically between E19 and E20 (4·2-fold, $P < 0{\cdot}01$) and showed a very high mRNA expression at P2. A transient decrease in ENaC-α mRNA occurred between P10 and P14, which was followed by a gradual increase from P18 to P40 (Fig. 1). NKA-α1 mRNA was easily detectable in fetal lungs and increased further from E19 to P2 (2·1-fold, $P < 0{\cdot}05$). NKA-α1 mRNA decreased between P10 and P14 and increased again from P18 (Fig. 1).

AQP1 mRNA levels were very low in E18, increased gradually between E19 and E20 and sharply between E21 and P2 (3·3-fold, $P < 0{\cdot}05$). The levels remained high between P2 and P40. AQP1 mRNA decreased somewhat between P10 and P14 and increased again from P18, to level off between P20 and P40 (Fig. 2). AQP5 mRNA appeared around birth and increased slowly and gradually until P40 (Fig. 2). AQP2 and AQP3 mRNA were not detected at any developmental stages (data not shown).

In the study where Northern blotting was used, AQP4 mRNA was not detectable in fetal lung, but was expressed with high intensity at P2. Thereafter AQP4 mRNA decreased and was again very low from P10 to P40 (Fig. 3*A*). The developmental expression of AQP4 mRNA was also examined with semi-quantitative RT-PCR analysis. The reliability of the method was assessed by comparing the results on AQP4 from RT-PCR and Northern blot studies (Fig. 3*A* and *B*, left panel). The relative difference in AQP4 transcript was similar with both methods. To determine if the expression pattern of AQP4 is lung specific, developmental expression of AQP4 mRNA in brain and kidney was also examined (Fig. 3*B*). The very pronounced and transient perinatal increase of AQP4 mRNA appears to be specific for lung tissue. AQP4 in lung increased 3·0-fold ($P < 0{\cdot}05$) between E18 and P1, and decreased from P1 to P10 (Fig. 3*B*, left panel). In brain, AQP4 increased postnatally from P1 to P10 (2·4-fold, Fig. 3*B*, middle panel). In kidney, AQP4 was not detectable in E18 and gradually increased postnatally (Fig. 3*B*, right panel).

The lung distribution of AQP4 protein was assessed by immunocytochemical analysis in newborn (P1) rat lungs

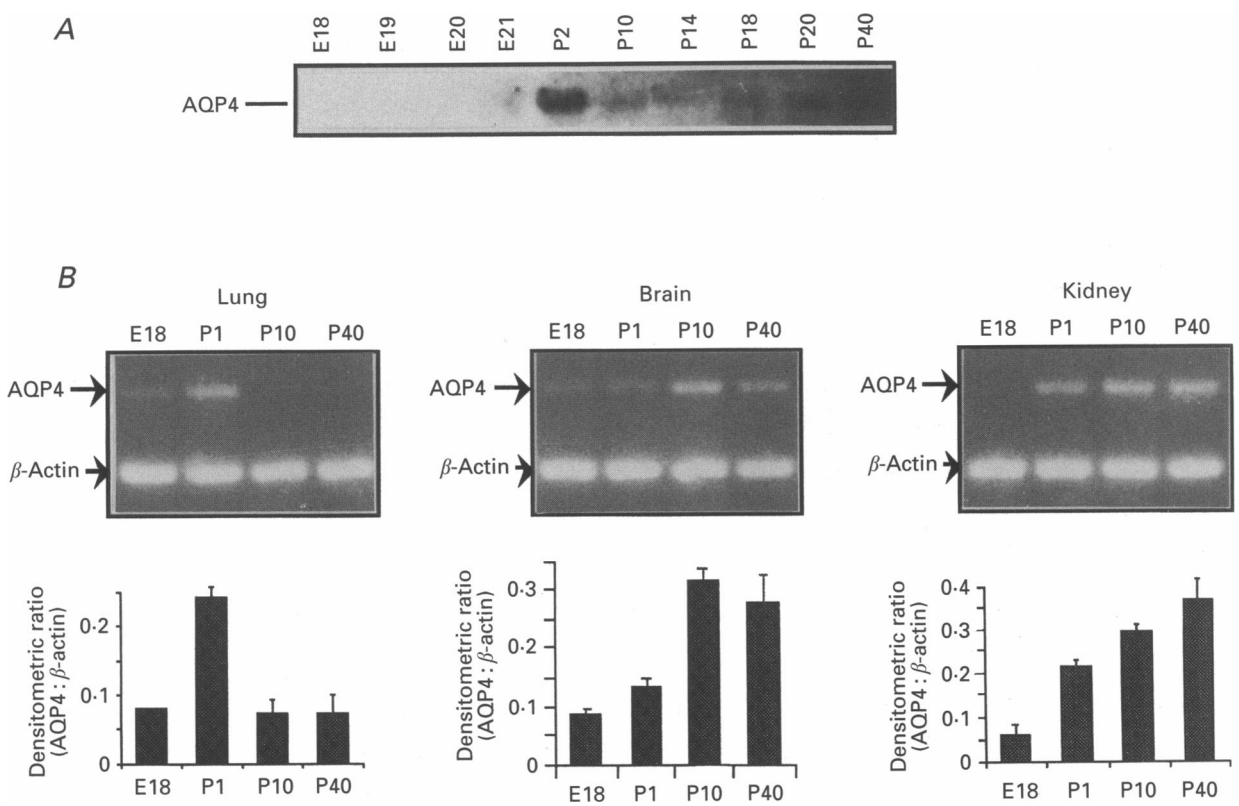

Figure 3. AQP4 mRNA expression in fetal and postnatal lung, brain and kidney

A, representative Northern blot of AQP4 from different gestational ages of fetal and postnatal rats lung. *B*, RT-PCR amplification of AQP4 mRNA. Total RNA (1 μg) was reversed transcribed. After reverse transcription, PCR was carried out for twenty-two cycles at 94 °C for 30 s, 60 °C for 45 s and 72 °C for 60 s with AQP4 and β-actin primers (see Table 1). AQP4 and β-actin were amplified in the same tube in the presence of [α-^{32}P]dCTP. The PCR product was separated on a 2% agarose gel and stained with ethidium bromide. Quantitative results are expressed in absolute values of counts per minute AQP4 fragment/counts per minute β-actin fragment (means ± S.E.M., $n = 3$).

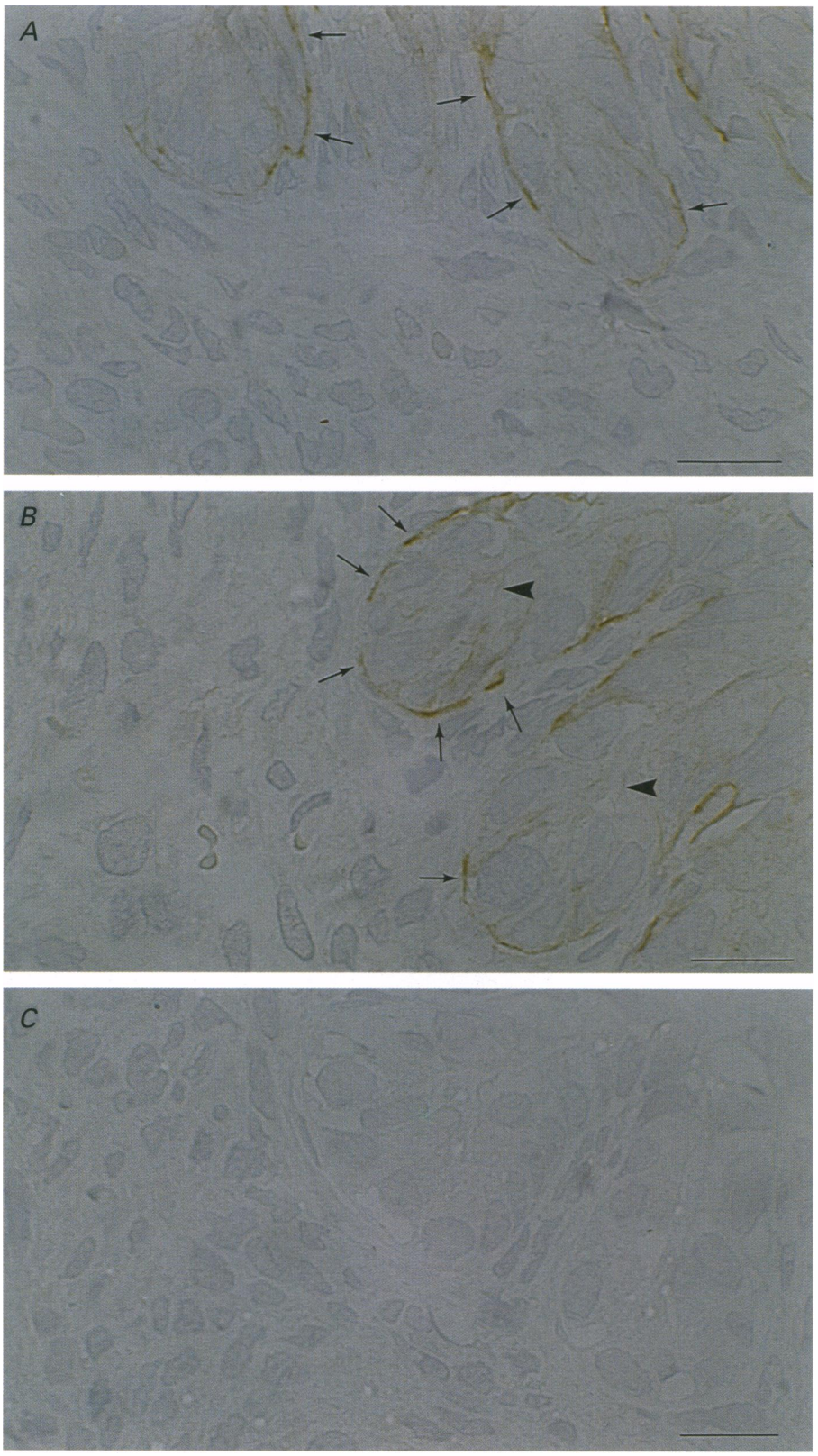

Figure 4. Immunohistochemical localization of AQP4 in neonatal rat lung

Cryosections were incubated with anti-AQP4; immunolabelling control in *C* was incubated with anti-AQP4 preabsorbed with purified AQP4. *A* and *B*, at P1, a significant but weak AQP4 labelling (arrows) was seen in the basal plasma membranes of developing bronchial epithelial cells (*B*). No labelling of apical plasma membrane (*B*, arrowheads) or of other components. Scale bar, 15 μm.

Table 2. Effect of β-agonist and glucocorticoid on ion transporters and aquaporins

	β-agonist	Glucocorticoid
AQP1	1·19	1·25
AQP4	1·65*	1·46*
ENaC	1·10	1·09
NKA	1·23	1·09

An arbitrary value of 1 was given to control values of AQP1, AQP4, ENaC or NKA. $n = 4$, * $P < 0·05$

(Fig. 4). At P1, distinct AQP4 labelling was found in the basolateral plasma membranes of the bronchial epithelium. AQP4 staining was absent at E18 and the labelling was weak but detectable and localized in the bronchial epithelial cells at P40 (data not shown).

The effect of a β-adrenergic agent (terbutaline) and GC (betamethasone) treatment on AQP4 mRNA expression in fetal lung was examined by semi-quantitative RT-PCR (Fig. 5). Pregnant rats (20 days gestation) were given terbutaline intraperitoneally as described in Methods. Terubutaline significantly increased AQP4 mRNA 24 h after treatment (65% increase above control, Fig. 5, lanes 2 and 3, and Fig. 6). A single injection of betamethasone also increased AQP4 mRNA 24 h after treatment (46% increase above control, Fig. 5, lanes 2 and 4). Terubutaline had no significant effects on AQP1, ENaC-α and NKA-α1 mRNA 24 h after treatment (Table 2 and Fig. 6). Since the levels of AQP4 expression was low and its distribution was limited to bronchial epithelium, it was not possible to do quantitative assessment of AQP4 protein expression with immunoblotting.

DISCUSSION

The fetal lungs are filled with liquid that is essential for normal lung growth and development before birth (Bland & Nielson, 1992). At birth, the liquid within the fetal respiratory tract has to be immediately expelled from the lung lumen to establish pulmonary gas exchange. Rapid removal of the lung liquid at birth is followed by continuous absorption of the residual liquid for several days after birth. Adams, Yanagisawa, Kuzela & Martinek (1971) suggested that absorption of fluid from the lung may continue for at least 3 days after birth. In this study, we observed a very narrow period of AQP4 induction just after birth. The transiently increased AQP4 mRNA expression at birth was

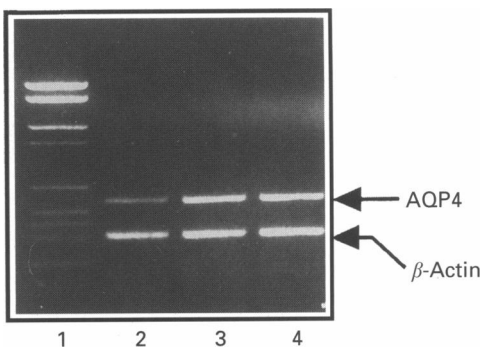

Figure 5. Effect of β-adrenergic agonist and GC on RT-PCR amplification of AQP4 mRNA in fetal rat lung (E21)

Agents (see Methods) were administered to pregnant rats (20 days gestation) for 24 h. The rats were killed at 21 days gestation and then RNA was rapidly extracted from fetal lungs. Total RNA (1 μg) was reversed transcribed. After reverse transcription, PCR was carried out for twenty-two cycles at 94 °C for 30 s, 60 °C for 45 s and 72 °C for 60 s with AQP4 and β-actin primers (see Table 1). AQP4 and β-actin were amplified in the same tube in the presence of [α-^{32}P]dCTP. The PCR product was separated on a 2% agarose gel and stained with ethidium bromide. AQP4 fragment is 576 bp and β-actin fragment is 429 bp. Lane 1, size markers; lane 2, control; lane 3, terbutaline (10 μg (100 g body weight)$^{-1}$); lane 4, betamethasone (60 μg (100 g body weight)$^{-1}$).

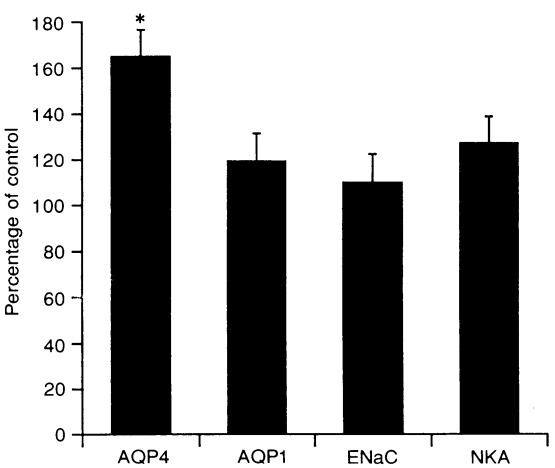

Figure 6. Effect of β-adrenergic agonist on RT-PCR amplification of AQP4, AQP1, ENaC-α, and NKA-α1 mRNA in fetal rat lung (E21)

Results are expressed as a percentage of control values of AQP4, AQP1, ENaC-α, or NKA-α1/counts per minute β-actin (means ± S.E.M., $n = 4$; * $P < 0·05$).

lung specific (Fig. 3) and conincided with the time course for clearance of lung liquid. Moreover, immunocytochemistry has demonstrated that AQP4 is expressed in the bronchial epithelium in newborn rats, consistent with the view that this is the site of perinatal lung liquid absorption (Bland, McMillan, Bressack & Dong, 1980). These observations strongly suggest that AQP4 contributes to the removal of lung liquid at birth. It is noteworthy that the induction of AQP4 was transient and limited to the perinatal period. Very recently, Umenishi, Carter, Yang, Oliver, Matthay & Verkman (1996) examined the mRNA expression of AQP1, 4 and 5 in perinatal rat lung by RNase protection assay. They observed that the expression of mRNA for all water channels studied increased postnatally.

In rat lung, AQP1, AQP4 and AQP5 are expressed. In accordance with King *et al.* (1996) we found that the expression of AQP1 mRNA increased around birth (Fig. 2), indicating a possible role of AQP1 for removal of lung liquid from the lung lumen at birth. In the study of King *et al.* (1996) the immunolocalization of AQP1 was found in capillary endothelial cells around bronchi but not in pulmonary epithelium. AQP1 might play a role for the removal of lung liquid from interstitial spaces to vascular spaces.

AQP5 was weakly expressed at birth and showed the highest abundance in the adult lung (Fig. 2). AQP2 and AQP3 were not expressed in lung tissue. These observations indicate that AQP2, AQP3 and AQP5 are not involved in lung liquid removal at birth.

The ENaC and NKA appear to be of particular importance for the creation of the sodium gradient in lung epithelial cells. We found that ENaC-α, NKA-α1 and AQP1 mRNA were abundant around the time of birth. It has recently been reported that α-ENaC-knockout mice develop respiratory distress and die within 40 h after birth from failure to clear their lung fluid, indicating that ENaC-dependent sodium absorption is the limiting step for lung fluid clearance at birth (Hummler *et al.* 1996). NKA activity is increased around birth (Ingbar *et al.* 1996). These studies together with our present data support the hypothesis that ENaC and NKA create the osmotic gradient for lung liquid clearance around birth.

Immunocytochemistry revealed that AQP4 was expressed in the basolateral plasma membranes of bronchial epithelial cells in newborn rat lung (P1). It is noteworthy that AQP4 is expressed in bronchial epithelial cells but not in alveoli. Bland *et al.* (1980) observed the shifts of residual lung liquid from potential airspaces into distensible perivascular spaces around large pulmonary blood vessels and bronchi. They hypothesized that accumulation of liquid in these connective tissue spaces, which are distant from sites of respiratory gas exchange, allows time for small blood vessels and lymphatics to remove the displaced liquid with little or no impairment of pulmonary function. The immunolocalization of AQP4 in bronchial epithelial cells is consistent with their hypothesis. AQP1 is expressed in capillary endothelial cells around bronchi but not around alveoli (King *et al.* 1996). Recently, Folkesson *et al.* (1996) demonstrated functional evidence of water channel-mediated water movement across the distal airway epithelium. These observations together with AQP1 and AQP4 immunolocalization in bronchial endothelial and epithelial cells also strongly support the hypothesis that the bronchi are important for absorption of lung liquid around birth.

It is well established that GCs play an important role for the differentiation and functional maturation of the lung. The stimulatory effect of GCs on surfactant phospholipid synthesis is well established (Papageorgiou, Desgranges, Masson, Colle, Shatz & Gelfand, 1979; Ballard, 1989; Schellhase & Shannon, 1991). However, since GCs are known to accelerate the developmental expression of many specialized proteins, it is probable that they have multiple beneficial effects on the immature lung. Here we have observed that maternal GC increased expression of AQP4 mRNA in fetal lung. AQP1 is induced by GCs in rat lung (King *et al.* 1996). Expression of NKA-α1 and ENaC-α are also induced by GCs and the effect is most prominent in the perinatal period (Celsi, Wang, Akusjärvi & Aperia, 1993; Tchepichev *et al.* 1995). Taken together, GCs appear to play an important role for the removal of lung liquid around birth.

β-Adrenergic agonists have been commonly used in clinical practice as tocolytic agents. There is evidence that these agonists act not only in reducing the uterine contractions of the mother but also in enhancing the removal of fetal lung liquid (Barker *et al.* 1990; Bland, 1990; Bland & Nielson, 1992). Brown, Olver, Ramsden, Strang & Walters (1983) reported a positive correlation between plasma concentrations of adrenaline and absorption of lung liquid during labour in fetal lambs. We observed that the β-adrenergic agonist, terbutaline stimulated AQP4 mRNA expression in fetal lung. This is, to our knowledge, the first demonstration of an effect of a catecholamine on water channel expression. The effect of terbutaline on AQP4 mRNA was specific, since it had no significant effect on mRNA of AQP1, ENaC-α, and NKA-α1. It is noteworthy that the effect of β-agonist on AQP4 expression is age dependent since there was no effect on AQP4 in E18 (data not shown). Interestingly, the concentration of pulmonary β-adrenergic receptors increases in the fetus at term and is upregulated by GC (Cheng, Goldfien, Ballard & Roberts, 1980; Whitsett, Manton, Darovec-Beckerman, Adams & Moore, 1981). Our data are compatible with the hypothesis that prenatal combination therapy of β-adrenergic agent with GC facilitates the lung liquid absorption.

ADAMS, F., YANAGISAWA, M., KUZELA, D. & MARTINEK, H. (1971). The disappearance of fetal lung fluid following birth. *Journal of Pediatrics* **78**, 837–843.

AGRE, P., BROWN, D. & NIELSEN, S. (1995). Aquaporin water channels: unanswered questions and unresolved controversies. *Current Opinion in Cell Biology* **7**, 472–483.

BALLARD, P. L. (1989). Hormonal regulation of pulmonary surfactant. *Endocrine* **10**, 165–181.

BARKER, P. M., MARKIEWICZ, M., PARKER, K. A., WALTERS, D. V. & STRANG, L. B. (1990). Synergistic action of triiodothyronine and hydrocortisone on epinephrine-induced reabsorption of fetal lung liquid. *Pediatric Research* **27**, 588–591.

BLAND, R. D. (1990). Lung epithelial ion transport and fluid movement during the perinatal period. *American Journal of Physiology* **259**, L30–37.

BLAND, R. D., BRESSACK, M. A. & MCMILLAN, D. D. (1979). Labor decreases the lung water content of newborn rabbits. *American Journal of Obstetrics and Gynecology* **135**, 364–367.

BLAND, R. D., MCMILLAN, D. D., BRESSACK, M. A. & DONG, L. (1980). Clearance of liquid from lungs of newborn rabbits. *Journal of Applied Physiology* **49**, 171–177.

BLAND, R. D. & NIELSON, D. W. (1992). Developmental changes in lung epithelial ion transport and liquid movement. *Annual Review of Physiology* **54**, 373–394.

BROWN, M. J., OLVER, R. E., RAMSDEN, C. A., STRANG, L. B. & WALTERS, D. V. (1983). Effects of adrenaline and of spontaneous labour on the secretion and absorption of lung liquid in the fetal lamb. *Journal of Physiology* **344**, 137–152.

CELSI, G., WANG, Z.-M., AKUSJÄRVI, G. & APERIA, A. (1993). Sensitive periods for glucocorticoids' regulation of Na,K-ATPase mRNA in the developing lung and kidney. *Pediatric Research* **33**, 5–9.

CHAPMAN, D. L., CARLTON, D. P., CUMMINGS, J. J., POULAIN, F. R. & BLAND, R. D. (1991). Intrapulmonary terbutaline and aminophylline decrease lung liquid in fetal lambs. *Pediatric Research* **29**, 357–361.

CHENG, J. B., GOLDFIEN, A., BALLARD, P. L. & ROBERTS, J. M. (1980). Glucocorticoids increase pulmonary β-adrenergic receptors in fetal rabbit. *Endocrinology* **107**, 1646–1648.

FOLKESSON, H. G., MATTHAY, M. A., FRIGERI, A. & VERKMAN, A. S. (1996). Transepithelial water permeability in microperfused distal airways – evidence for channel-mediated water transport. *Journal of Clinical Investigation* **97**, 664–671.

FOLKESSON, H. G., MATTHAY, M. A., HASEGAWA, H., KHERADMAND, F. & VERKMAN, A. S. (1994). Transcellular water transport in lung alveolar epithelium through mercury-sensitive water channels. *Proceedings of the National Academy of Sciences of the USA* **91**, 4970–4974.

HUMMLER, E., BARKER, P., GATZY, J., BEERMANN, F., VERDUMO, C., SCHMIDT, A., BOUCHER, R. & ROSSLER, B. C. (1996). Early death due to defective neonatal lung liquid clearance in alpha-ENaC-deficient mice. *Nature Genetics* **12**, 325–328.

INGBAR, D. H., WEEKS, C. B., GILMOREHEBERT, M., JACOBSEN, E., DUVICK, S., DOWIN, R., SAVIK, S. K. & JAMIESON, J. D. (1996). Developmental regulation of Na,K-ATPase in rat lung. *American Journal of Physiology* **14**, L619–629.

KING, L. S. & AGRE, P. (1996). Pathophysiology of the aquaporin water channels. *Annual Review of Physiology* **58**, 619–648.

KING, L. S., NIELSEN, S. & AGRE, P. (1996). Aquaporin-1 water channel protein in lung – ontogeny, steroid-induced expression, and distribution in rat. *Journal of Clinical Investigation* **97**, 2183–2191.

NIELSEN, S., NAGELHUS, E. A., AMIRY-MOGHADDAM, M., BOURQUE, C., AGRE, P. & OTTERSEN, O. P. (1997). Glial cells contain specialized membrane domains for water flux: high resolution immunogold cytochemistry of aquaporin-4 in rat brain. *Journal of Neuroscience* **17**, 171–180.

NIELSEN, S., SMITH, B. L., CHRISTENSEN, E. I., KNEPPER, M. A. & AGRE, P. (1993). CHIP28 water channels are localized in constitutively water-permeable segments of the nephron. *Journal of Cell Biology* **120**, 371–383.

O'BRODOVICH, H., CANESSA, C., UEDA, J., RAFII, B., ROSSIER, B. C. & EDELSON, J. (1993). Expression of the epithelial Na^+ channel in the developing rat lung. *American Journal of Physiology* **265**, C491–496.

PAPAGEORGIOU, A. N., DESGRANGES, M. F., MASSON, M., COLLE, E., SHATZ, R. & GELFAND, M. M. (1979). The antenatal use of betamethasone in the prevention of respiratory distress syndrome: a controlled double-blind study. *Pediatrics* **63**, 73–79.

RAINA, S., PRESTON, G. M., GUGGINO, W. B. & AGRE, P. (1995). Molecular cloning and characterization of an aquaporin cDNA from salivary, lacrimal, and respiratory tissues. *Journal of Biological Chemistry* **270**, 1908–1912.

SCHELLHASE, D. E. & SHANNON, J. M. (1991). Effects of maternal dexamethasone on expression of SP-A, SP-B, and SP-C in the fetal rat lung. *American Journal of Respiratory Cell and Molecular Biology* **4**, 304–312.

TCHEPICHEV, S., UEDA, J., CANESSA, C., ROSSIER, B. C. & O'BRODOVICH, H. (1995). Lung epithelial Na channel subunits are differentially regulated during development and by steroids. *American Journal of Physiology* **269**, C805–812.

TERRIS, J., ECELBARGER, C. A., MARPLES, D., KNEPPER, M. A. & NIELSEN, S. (1995). Distribution of aquaporin-4 water channel expression within rat kidney. *American Journal of Physiology* **269**, F775–785.

UMENISHI, F., CARTER, E. P., YANG, B., OLIVER, B., MATTHAY, M. A. & VERKMAN, A. S. (1996). Sharp increase in rat lung water channel expression in the perinatal period. *American Journal of Cell and Molecular Biology* **15**, 673–679.

WHITSETT, J. A., MANTON, M. A., DAROVEC-BECKERMAN, C., ADAMS, K. G. & MOORE, J. J. (1981). β-Adrenergic receptors in the developing rabbit lung. *American Journal of Physiology* **240**, E351–357.

YASUI, M., MARPLES, D., BELUSA, R., EKLOF, A. C., CELSI, G., NIELSEN, S. & APERIA, A. (1996). Development of urinary concentrating capacity – role of Aquaporin-2. *American Journal of Physiology* **40**, F461–468.

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