Role of γENaC Subunit in Lung Liquid Clearance and Electrolyte Balance in Newborn Mice

Insights into Perinatal Adaptation and Pseudohypoaldosteronism

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Abstract

Genetic evidence supports a critical role for the epithelial sodium channel (ENaC) in both clearance of fetal lung liquid at birth and total body electrolyte homeostasis. Evidence from heterologous expression systems suggests that expression of the αENaC subunit is essential for channel function, whereas residual channel function can be measured in the absence of β or γ subunits. We generated mice without γENaC (γENaC −/−) to test the role of this subunit in neonatal lung liquid clearance and total body electrolyte balance. Relative to controls, γENaC (−/−) pups showed low urinary [K^+] and high urinary [Na^+] and died between 24 and 36 h, probably from hyperkalemia (γENaC −/− 18.3 mEq/l, control littermates 9.7 mEq/l). Newborn γENaC (−/−) mice cleared lung liquid more slowly than control littermates, but lung water at 12 h (wet/dry = 5.5) was nearly normal (wet/dry = 5.3). This study suggests that γENaC facilitates neonatal lung liquid clearance and is critical for renal Na^+ and K^+ transport, and that low level Na^+ transport may be sufficient for perinatal lung liquid absorption but insufficient to maintain electrolyte balance by the distal nephron. The γENaC (−/−) newborn exhibits a phenotype that resembles the clinical manifestations of human neonatal PHA1. (*J. Clin. Invest.* 1998. 102:1634–1640.) Key words: ENaC • lung liquid • sodium • pseudohypoaldosteronism • respiratory distress syndrome

Introduction

The amiloride-sensitive epithelial sodium channel (ENaC)[1] is a heterotetromer composed of three homologous subunits (α, β, and γ), each with intracellular amino and carboxy termini, two transmembrane spanning domains, and a large extracellular loop (1, 2). This heteromeric structure and the amino acid sequence of the individual chains is conserved among mammalian species (1, 2, 4). Amiloride-sensitive electrogenic Na^+ transport mediated by this channel is the rate limiting step for Na^+ absorption by epithelial cells that line the distal renal tubule, distal colon, the ducts of salivary and sweat glands, and lung epithelium.

The kidney is the principal site of total body electrolyte and liquid regulation. ENaC subunits are coexpressed in the distal renal tubule and collecting ducts (5, 6). A role for ENaC in electrolyte and fluid balance is deduced from mutations of ENaC subunits that cause two diseases: pseudohypoaldosteroidism (PHA, Type 1) and Liddle's syndrome. In PHA1, mutations in α, β, or γENaC result in failure of renal distal tubular Na^+ reabsorption leading to salt wasting with hyperkalaemia, metabolic acidosis, intravascular volume depletion, and hyperaldosteronism (7, 8). In Liddle's syndrome, mutations in β- and γENaC raises renal Na^+ reabsorption and cause salt retention and hypertension (9, 10).

Sodium transport is also important for lung liquid balance. At birth, ENaC plays a central role in the transition of the lung at birth from its liquid-filled fetal state to an air-filled organ lined with a thin film of liquid. The increase in the capacity for amiloride-sensitive Na^+ absorption during late gestation in fetal sheep (11) is paralleled by an increase in expression of the genes encoding ENaC, particularly the α- and γENaC subunits in fetal rat lung (12). Amiloride inhibition of liquid absorption by newborn guinea pig lung (13) and waterlogged lungs of postnatal mice deficient in αENaC (14) indicate an impotant role for ENaC, and particularly the αENaC subunit, in clearance of fetal liquid from the newborn lung in these species.

Differences in the onset of expression of the three genes in the fetal lung (15), as well as the finding that the relative levels of mRNA for the subunits can vary significantly in different regions of the lung, kidney, and colon (16–19), suggest that individual subunits may have independent functions. Xenopus oocyte studies predict a greater dependence of amiloride-sensitive Na^+ transport on αENaC than on β- or γENaC (1). Low levels of Na^+ transport (1% of maximal) could be detected in oocytes injected with alpha subunit RNA alone but not in oocytes expressing only the gamma or beta subunits. Coinjection of beta or gamma subunits with alpha ENaC increased Na^+ transport approximately fivefold. Maximal Na^+ current required expression of all three subunits.

To test the hypothesis that epithelial Na^+ transport may be less critically dependent on γENaC than on αENaC, we generated a mouse line that encodes only α and βENaC subunits by

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1. *Abbreviations used in this paper:* ENaC, epithelial sodium channel; ES, embryonic stem; PHA, pseudohypoaldosteroidism; PD, potential difference.

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targeting the γENaC gene and compared the phenotype of this model with that of the αENaC knockout mouse. Since expression of α- and βENaC subunits without γENaC projects suboptimal Na^+ transport, this model also allowed us to compare the relative requirements for ENaC function in the newborn lung (liquid clearance) with requirements for ENaC function in the kidney (whole body electrolyte and fluid balance).

Methods

Southern and Northern blotting. DNA obtained from tail biopsies and embryonic stem (ES) cell colonies was prepared by high-salt precipitation (20). DNA was digested with the indicated restriction enzymes, and the digest was separated by electrophoresis and transferred to a nylon membrane (Hybond; Amersham, Buckinghamshire, England). RNA was prepared from tissues by homogenization and acid phenol/chloroform extraction with Rnazol B (Tel-Test Inc., Friendswood, TX) and run on denaturing formaldehyde 1.2% agarose gel and blotted onto a nitrocellulose membrane. ^{32}P-CTP–labeled probe was prepared by random priming, using a commercially available kit (Amersham).

Generation of γENaC (−/−) mouse. A mouse (129SVJ) genomic library was constructed in lambda FIXII (Stratagene, La Jolla, CA) and screened with a full-length rat γENaC cDNA (1). The exonic sequences with the putative start codon were identified by sequence analysis of a 1-kb Nhe 1 fragment and a construct was designed in which this region is replaced by a neomycin gene. Two genomic fragments flanking this exon were cloned into the plasmid vector JNS2 to generate the targeting plasmid (see Fig. 1 *A*). A 2.5-kb Nhe 1 Eco 1 fragment located immediately downstream of this exon was cloned into the multiple cloning site of JNS2 immediately downstream of the PGKneo gene in this vector. A 6.2-kb Nhe 1 fragment located 5′ of the γENaC gene was cloned into the casette immediately upstream of the PGKneo gene. The plasmid was electroporated into E14Tg2a cells. The transfected cells were selected with G418 and gancyclovir as described previously (21). Colonies with plasmid that had integrated by homologous recombination were identified by Southern analysis after digestion of the DNA with Eco RV. Targeted ES cells were introduced into C57Bl/6 mouse blastocysts, and resulting chimeras bred with B6D2 females to identify those capable of transferring the ES cell genome to their offspring. Offspring carrying the mutant allele was intercrossed to obtain litters with animals homozygous for the mutation. Genotype was determined by Southern analysis of tail digests (see Fig. 1 *B*).

Serum and urinary electrolytes and bicarbonate. Serum was obtained by collecting mixed venous blood into serum separator tubes (Microtainer; Becton Dickinson Co., Franklin Lakes, NJ) at the time of decapitation. Tubes were microcentrifuged at 500 *g* for 2 min. Serum was transferred to preweighed tubes and reweighed on a microbalance to determine volume. Urine was collected by manual compression of the abdomen, followed by direct puncture of the bladder, and was transferred and weighed as described for serum. Urine from a few animals was voided before it could be collected, so our ability to compare urine volume between groups was limited. Urine and serum were frozen immediately at the time of collection and then analyzed for Na^+, Cl^-, K^+, and HCO_3^-. Spectrophotometric evaluation of five specimens of serum indicated very low (< 1%) hemoglobin concentration, suggesting that inadvertent release of K^+ by hemolysis during specimen collection did not contribute to serum $[K^+]$ significantly. Regression analysis of serum $[K^+]$ values and specimen size showed that $[K^+]$ was minimally affected when volume exceeded 1 μl. Consequently, we discarded three specimens with volumes < 1 μl. Specimens of newborn serum and urine were diluted in doubly distilled deionized water. Part of each diluted specimen was further diluted into a 0.1-N nitric acid matrix and analyzed for Na^+ and K^+ by flame emission photometry. Bicarbonate in the water-diluted specimen was estimated from the disappearance of NADH absorbance at 340 nm in the coupled enzymic assay system described by Forrester (22). Bicarbonate assay kits were purchased from Sigma Chemical Co. (St. Louis, MO).

Measurement of lung water. A detailed description of measurement of whole lung wet/dry ratio, an index of lung water, has been given previously (23). In brief, the lungs were dissected free from the heart and great vessels, the trachea was separated at the carina, external liquid was removed by blotting, and the lungs placed on a preweighed pan to obtain the wet weight. The lungs were then incubated overnight in a dry atmosphere at 85°C and reweighed to obtain the dry weight.

Explant culture and bioelectric measurements. Tracheas were explanted and were cultured to form cystic preparations as described previously (24). In brief, excised tracheas (one to two explants per trachea) were held in a collagen-salt gel and cultured for 6–8 d in F12 plus 10% fetal bovine serum in air/5%CO_2. The medium was replaced every 48 h.

Transepithelial potential difference across explants was measured as described previously. Explants were impaled with a KCl-containing glass microelectrode that was connected by a Ag-AgCl half cell to a high impedance voltmeter. The microelectrode was referenced by a KBR-agar bridge to the solution bathing the explant. PD was recorded continuously during the experiments. Amiloride (1% of the cyst volume) was microinjected into the cyst to give a final concentration of 10^{-4} M. Then, terbutaline (final concentration 3×10^{-5} M) was added to the bath. Freshly excised colon from newborn pups was studied in Ussing chambers as described previously (25). In brief, tissues were bathed with Krebs Ringer bicarbonate solution and mixed with 95% O_2/5% CO_2. We tested the effect, on basal short-circuit current (ISC), of amiloride (10^{-4} M) in the mucosal bath, and forskolin (10^{-5} M) in the serosal bath.

Results

γENaC (−/−) mice. The γENaC gene was disrupted in the E14Tg2a ES cell line by homologous recombination with a targeting plasmid as shown in Fig. 1 *A*. The targeting plasmid was designed to replace a fragment of the γENaC gene with the neomycin gene. The fragment includes an exon containing a portion of the 5′ untranslated region. We anticipated that homologous integration of the targeting plasmid would result in loss of normal transcription of the γENaC gene and prevent expression and function of γENaC. Northern analysis of whole kidney from γENaC (−/−) newborns showed no expression the wild-type γENaC mRNA that was detected in control (γENaC +/− and γENaC +/+) kidney (Fig. 1 *B*). A smaller mRNA fragment was expressed in γENaC (−/−) and γENaC (+/−) mice.

Survival. The frequency of mice that were homozygous for the mutant allele (20%) was close to that predicted by simple Mendelian distribution and was not different in fetal and postnatal litters. These data suggest that few, if any, homozygous fetuses or newborns died undetected during intrauterine life or in the early newborn period.

109 postnatal mice were studied. 20 newborns from three litters were observed closely to establish the survival pattern for γENaC (−/−) newborns (Fig. 2). Six pups, subsequently determined to be γENaC (−/−), died spontaneously between 24 and 36 h of life. Postmortem examination showed that the major organs were normally developed, and there was no evidence of pulmonary edema that had characterized αENaC (−/−) lungs at the time of death. Two other pups died: one γENaC (+/−) pup died at 24 h of apparent bowel obstruction, and one γENaC (+/+) died at 31 h of no obvious cause. In ad-

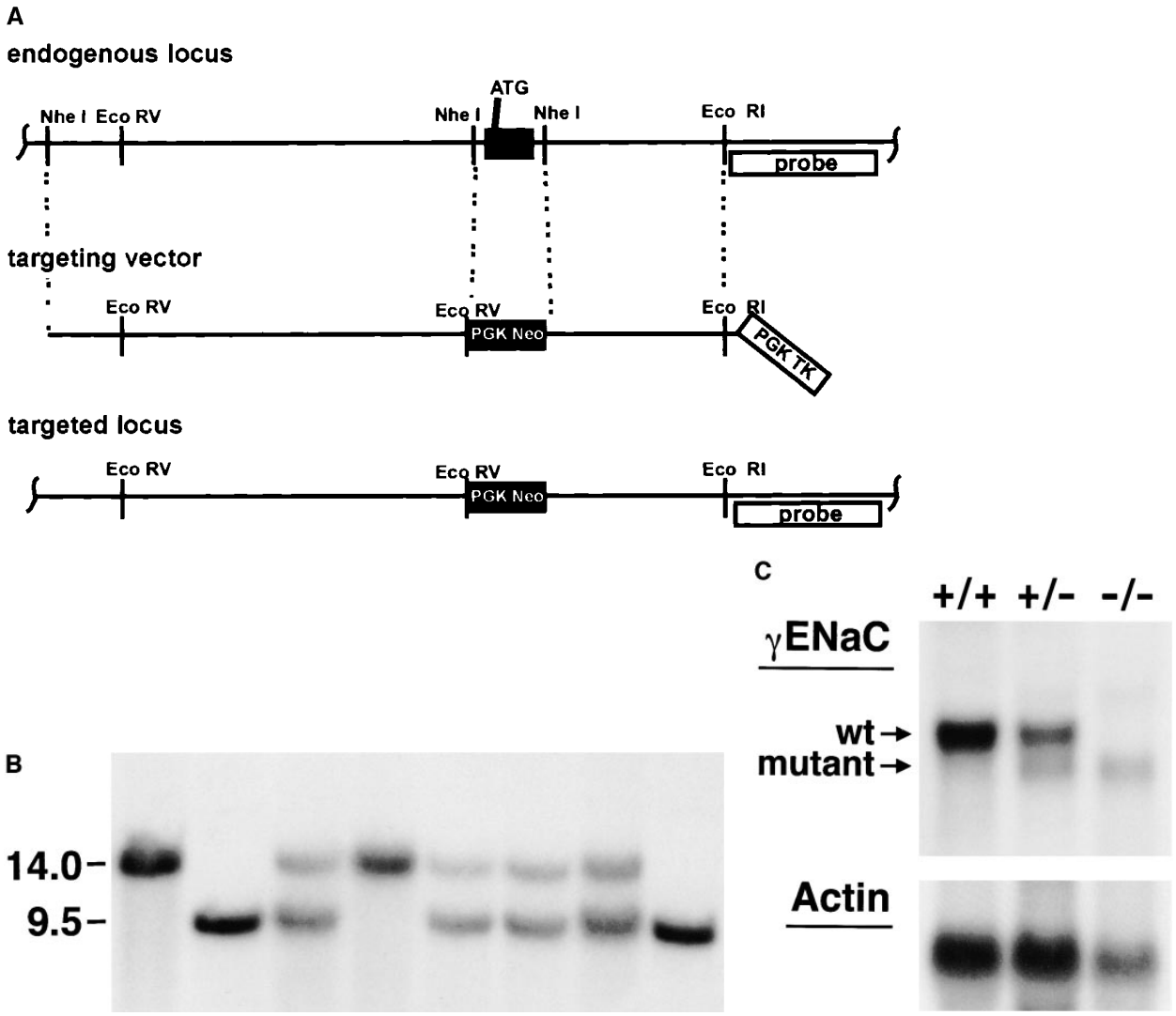

Figure 1. (*A*) Targeted disruption of γENaC locus. Restriction map of the targeting construct and the endogenous and disrupted locus. The γENaC exon is indicated by the filled box, and the PGK-TK and PGK-neo selection casettes are represented by the empty box (PGK, phosphoglycerate kinase; TK, thymidine kinase). The box below the endogenous locus indicates the probes used to detect homologous recombination events by Southern blot analysis. (*B*) Southern blot analysis of murine γENaC. Eco RV digested DNA derived from tail biopsies of progeny from heterozygote breeding pair. Eco RV DNA fragment detected by the probe indicated is reduced from 14 to 9.5 kb because of Eco RV sites in the PGKneo gene. First and fourth lanes are from γENaC (+/+) pups, second and eighth lanes are from γENaC (−/−) pups, and third, fifth, sixth, and seventh lanes are from γENaC (+/−) pups. (*C*) Expression of γENaC RNA in newborn mouse kidney. Upper panel shows kidney RNA probed with full-length murine γENaC probe. Upper band represents wild-type γENaC RNA, and lower band represents mutant γENaC RNA. Lower panel shows specific β-actin hybridization. +/+ is from γENaC (+/+), +/− is from γENaC (+/−), and −/− is from γENaC (−/−).

dition, 89 newborns were observed until tissues were studied at 4, 12, or 24 h. 18 of these were γENaC (−/−) pups. There was one unexpected death of an γENaC (+/−) pup in a litter allocated to the 24-h study. All remaining γENaC (+/−), γENaC (+/+), and γENaC (−/−) newborns in the 4-, 12-, and 24-h study groups survived until the time of tissue analysis.

Physical signs. We did not observe any signs that distinguished γENaC (−/−) pups in the first 12 h of life. In particular, we did not see the increased ventilatory effort characteristic of αENaC (−/−) pups (14). However, from 12 h onwards, pups that were determined subsequently to have the γENaC (−/−) genotype became progressively less active. γENaC (−/−) pups also consistently lost weight, whereas pups from other genotypes gained weight (Δ weight from birth to 24 h of life: γENaC (−/−) −11%, γENaC (+/−) +8%, γENaC (+/+) +7%). At 24 h, the γENaC (−/−) pups (8) were noted to be lethargic and pale compared with littermate controls.

Blood and urine chemistry. The volume of urine collected tended to be lower in γENaC (−/−) than in γENaC (+/−) and γENaC (+/+) pups at 12 and 24 h. The mean volume of urine collected from γENaC (+/−) and γENaC (+/+) pups increased from 3.9±0.4 μl at 4 h to 11.9±1.3 μl at 24 h. By contrast, urine volumes collected from γENaC (−/−) pups remained low (5.2±1.3 μl at 4 h, 4.2±0.6 μl at 24 h). We speculate that the volumes collected at 24 h from γENaC (−/−) pups reflect decreased circulating blood volume and hypotension.

There were no differences between any γENaC (+/−) and γENaC (+/+) compositional analyses so data from these geno-

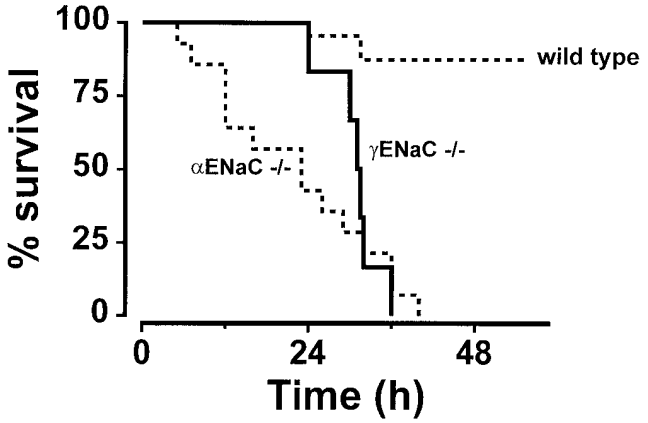

Figure 2. Survival curve of γENaC (−/−), αENaC (−/−), and wild-type (littermate control γENaC +/+) newborn mice. Time of death was estimated as the midpoint between two observation points. Litters were checked every 6 h after birth. The data for αENaC (−/−) mice are given for reference and were reported previously by Hummler et al. (14).

types were pooled. The principal chemical difference between γENaC (−/−) and other genotypes was the serum [K^+]. Serum [K^+] declined between 4 and 24 h in ENaC (+/−) and (+/+) pups from 12.6 to 9.7 mEq/l. By contrast, serum [K^+] in γENaC (−/−) pups tended to increase over this period (15.8 mEq/l at 4 h to 18.3 mEq/l at 24 h) and was significantly different from ENaC (+/−) and (+/+) serum K^+ at 4, 12, and 24 h (Fig. 3 *A*, Table I). Serum Na^+ tended to be lower in γENaC (−/−) pups than in other genotypes at 24 h (Table I).

Urinary Na^+ wasting and K^+ retention were suggested by measurement of urine electrolyte concentrations. Urinary [Na^+] was higher and [K^+] was lower at all time points in ENaC (−/−) pups than in urine from other genotypes (Table I), and these differences were independent of urine volume. Urinary and plasma Na/K was significantly different in γENaC (−/−) and other pups at all three time points (Fig. 3 *B*, Table I).

Bicarbonate levels in urine also tended to be higher in γENaC (−/−) than in other pups at the time points studied, but differences were not significant (Table I).

Lung water. We assessed perinatal lung liquid clearance by an analysis of total lung wet/dry (W/D) weight for pups at 4, 12, and 24 h after birth. Values obtained from γENaC (−/−) newborns were compared with those of other genotypes from the same litters and with prelabor wet/dry values obtained for 19 d (full-term) fetuses (Fig. 4). The water content of fetal lung was similar for γENaC (−/−), heterozygote, and wild-type mice. 4 h after birth, W/D of γENaC (+/−) and γENaC (+/+) mice (mean 5.3±0.1) decreased to values that were similar to those of 12-h, 24-h, and adult mice. By contrast, lung water clearance in the γENaC (−/−) mice was impaired. W/D measurements indicated that very little lung water was cleared from γENaC (−/−) lungs in the first 4 h of life and that liquid was cleared slowly from γENaC (−/−) lungs between 4 and 24 h after birth. However, lung water content was normal in the five γENaC (−/−) pups that died spontaneously between 24 and 36 h (mean W/D 5.8, range 5.3–6.3).

Bioelectric characteristics. Transepithelial bioelectric properties of tracheal and colonic epithelia were studied. Basal PD, inhibition of PD by apical amiloride (10^{-4} M), and hyperpolarization by basolateral terbutaline were similar for heterozygote and wild-type tracheal explants (Fig. 5 *A*). 17 tracheal explants from 10 γENaC (−/−) mice were characterized by a mean change of basal PD after amiloride (−5±2%) that was less than wild type (−24±2%) and not significantly different from the change in PD after saline injection (−2±3%). PDs of 5 of the 17 γENaC (−/−) explants, however, were inhibited by 8–15% suggesting that there may be low level electrogenic Na^+ transport by γENaC (−/−) tracheal epithelium that cannot be detected reliably with this method. Hyperpolarization of γENaC (−/−) explants by terbutaline was not different from that of heterozygote and wild-type explants.

Short-circuit currents (ISC) across 30 freshly excised postnatal distal colonic preparations were measured, and, of these, 4 were determined subsequently to be γENaC (−/−). In contrast to γENaC (+/−) and γENaC (+/+) preparations, all γENaC (−/−) colonic preparations exhibited low basal ISC and no detectable response to amiloride. Amiloride-sensitive ISC of heterozygote preparations was ~ 50% of that of wild-type preparations (Fig. 5 *B*). ISC changes in response to carbachol (10^{-4} M) and forskolin (10^{-4} M) were similar for all preparations.

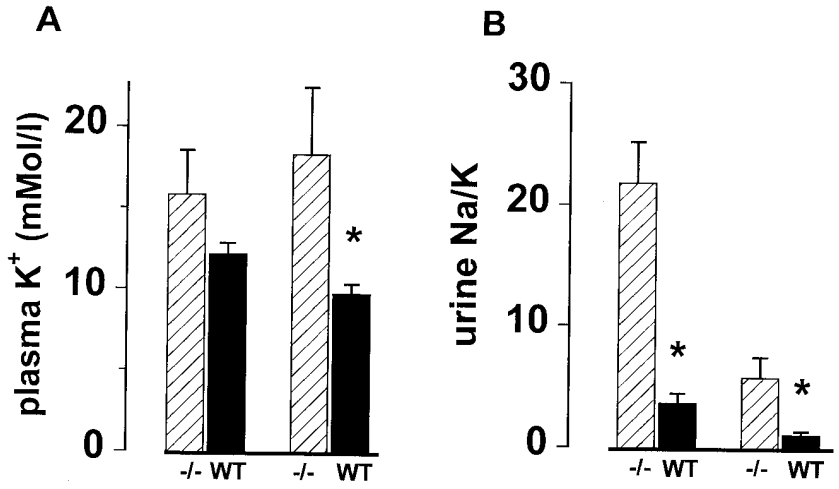

Figure 3. Plasma and urinary electrolyte values from γENaC (−/−) mice (−/−) and γENAc (+/−) and γENaC (+/+) (*WT*) mice at 4 and 12 h after birth (*left-hand pair of columns and right-hand pair of columns per panel*). Asterisks denote electrolyte values in γENaC (−/−) were significantly different from wild-type at the time samples were obtained.

Table I.

		Volume (ml)	[K^+] (meq/l)	[Na^+] (meq/l)	Na/K	[bicarb] (meq/l)
Serum						
4 h	−/−	5.2 (1.1)	15.8 (2.7)	143.1 (17.1)	9.5 (1.0)*	23.9 (5.9)
	Control	3.9 (0.3)	12.1 (0.7)	144.8 (6.8)	12.2 (0.6)	35.3 (3.2)
24 h	−/−	4.2 (0.5)*	18.3 (4.0)*	106.0 (4.9)	6.4 (1.0)*	18.5 (5.3)
	Control	11.9 (1.1)	9.7 (0.6)	121.3 (5.4)	12.8 (0.7)	22.8 (0.7)
Urine						
4 h	−/−	11.5 (4.0)	7.1 (0.8)	147.9 (5.3)	21.8 (3.4)*	8.2 (3.0)
	Control	9.9 (1.6)	39.3 (7.1)	95.8 (11.2)	3.6 (0.8)	2.6 (0.6)
24 h	−/−	18.2 (3.1)*	17.8 (5.0)*	75.6 (7.7)	5.7 (1.7)*	7.3 (5.1)
	Control	25.5 (5.5)	53.5 (4.9)	44.9 (10.2)	1.0 (0.3)	1.4 (0.5)

Discussion

We have generated a novel mouse model by targeted deletion of γENaC that resulted in a lethal neonatal phenotype. In this model, deletion of γENaC resulted in a slowing of fetal lung liquid clearance and a severe total body electrolyte disturbance. The most likely cause of death of γENaC (−/−) mice was hyperkalemia. Serum [K^+] of γENaC (−/−), γENaC (+/−), and wild-type newborns was similar at 4 h, but between 4 and 24 h, [K^+] in γENaC (+/−) and wild-type mice decreased. By contrast, 24 h after birth, serum [K^+] in γENaC (−/−) mice had risen to values that were twofold higher than those of heterozygote and wild-type mice.

Serum [K^+] of wild-type and heterozygote mice is slightly higher than values for older mice reported by others (26, 27) and reflects, compared with humans, the higher serum [K^+] of laboratory rodents. Moreover, serum [K^+] of wild-type and heterozygote newborns follows the well-described trend in rodents and humans for neonatal values to be significantly greater than adult values (e.g., human newborn 5.0–7.7 mEq/l, adult 3.5–5.0 mEq/l; John Chapman, Ph.D., McLendon Laboratories, University of North Carolina, personal communication). We know of no reports of serum or plasma [K^+] for newborn (< 5 d) rodents with which our values can be compared. Whilst it is possible that the methods we used to sample serum from small (∼ 1 g) newborn mice may have influenced absolute serum [K^+] in some undetermined way, samples from all populations would have been equally affected, and methodologic nuances would not account for the twofold difference in [K^+] values of γENaC and wild-type mice.

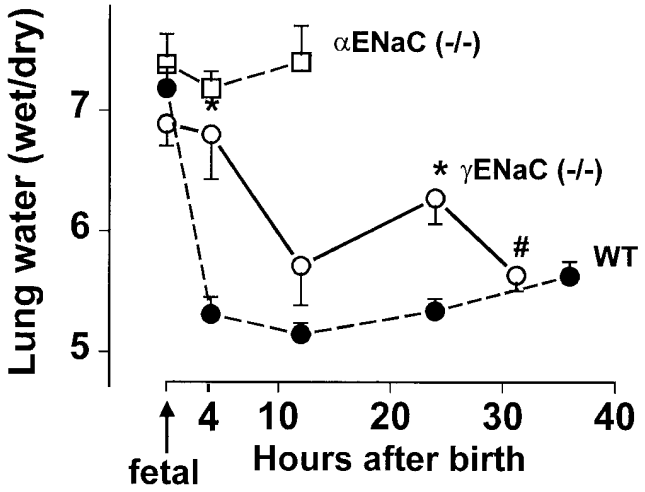

Figure 4. Water content of lungs from γENaC (−/−), αENaC (−/−), and wild-type (*WT*) (γENaC +/− and γENaC +/+) mice expressed as wet/dry. Asterisks denote wet/dry in γENaC (−/−) were significantly different from WT at the time samples were obtained. # denotes wet/dry value obtained from αENaC (−/−) pups that died spontaneously between 24 and 36 h. The data for αENaC (−/−) mice are given for reference and were reported previously by Hummler et al. (14).

The high serum [K^+] of γENaC (−/−) mice would be expected to interfere with cardiac function, decrease cardiac output (possibly responsible for the small volumes of serum collected from these mice), and, ultimately, induce arrythmia or cardiac arrest. The cause of hyperkalemia in γENaC (−/−) mice was probably renal K^+ retention. The [K^+] of urine from γENaC (−/−) mice was lower than that of littermates with other genotypes. However, because urinary [Na^+] of γENaC (−/−) mice tended to be raised and γENaC (−/−) newborns lost rather than gained weight, we cannot dismiss the possibility that depletion of extracellular volume, secondary to Na^+ wasting, contributed to the high serum [K^+].

Another important finding of this study was the ability of γENaC (−/−) lung to clear lung liquid during the first 24 h of life, albeit slowly. This residual absorptive function contrasts with the complete absence of liquid absorption from the αENaC (−/−) mouse lung (14). Although little liquid was absorbed from either αENaC (−/−) or γENaC (−/−) lungs during the first 4 postnatal hours, there was detectable clearance of liquid from γENaC (−/−) lungs thereafter. By the time of death (24–36 h), the water content (water/dry weight) of ENaC (−/−) lungs was not different from that in lungs of wild-type or heterozygote mice of the same age. In contrast, the water content of αENaC (−/−) lungs after 4 h tended to rise rather than fall and was not different from that of fetal lungs. We attribute the difference in timing of death between αENaC (−/−) and γENaC (−/−) mice to differences in the capacity for liquid clearance in the two genotypes. None of the γENaC mice died in the first 24 h, whereas 50% of αENaC mice died within this time.

A number of putative mechanisms may explain the difference in the lung liquid clearance between α- and γENaC-deficient mice. One possibility is that the alteration introduced

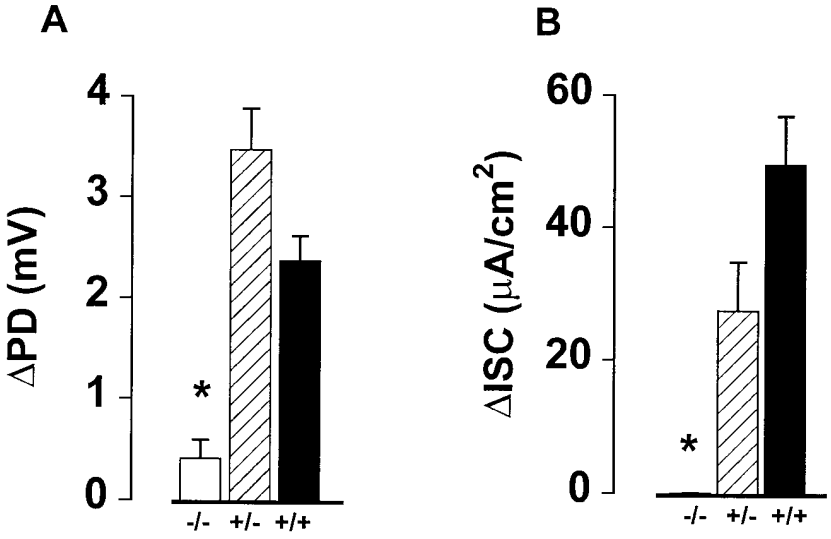

Figure 5. (*A*) Change of basal PD (*ΔPD*) after intralumenal injection of amiloride (10^{-4} M) into tracheal explants from knockout (−/−), heterozygote (+/−), and wild-type (+/+) fetal and newborn mice. (*B*) Change of short-circuit currents (ΔISC) after addition of amiloride (10^{-4} M) to apical membrane of freshly excised colon from knockout (−/−), heterozygote (+/−), and wild-type (+/+) newborn mice. Asterisks denote (−/−) values that are different from (+/−) and (+/+) values.

into the gamma gene did not create a null allele. While it is clear that no normal γENaC transcript was present in γENaC (−/−) mice, transcripts of a smaller size hybridized to a full-length cDNA were observed in Northerns of γENaC (−/−) kidney (Fig. 1 *C*). While we cannot formally rule out the possibility that this mRNA encodes a protein with some residual ENaC function, this seems unlikely given that the targeted deletion removes the entire first exon. Furthermore, a similar region of αENaC was targeted with quite different phenotypic expression.

An alternative explanation for difference in rates of lung liquid clearance by lungs of αENaC (−/−) and γENaC (−/−) neonates is suggested by studies of exogenous ENaC subunit mRNA expression in xenopus oocytes. Oocytes injected with αENaC mRNA in combination with βENaC mRNA exhibited ∼ 5% of amiloride-sensitive absorption compared with oocytes expressing all three subunits, whereas coinjection of β- and γENaC subunits resulted in no detectable current (1). If these data can be extrapolated to mouse lung epithelium, aggregates of α- and βENaC subunits at the lumenal surface may provide low levels of Na^+ absorption that could account for the slow liquid clearance that was seen in γENaC (−/−) mice. We think that it is unlikely that residual Na^+ absorption was related to a compensatory increase in α- and βENaC subunits in γENaC knockout mice. We tested for this possibility in αENaC (−/−) mice (14) and found no differences in γ- and βENaC subunit expression levels between wild-type and knockout mouse kidney.

Pathways that affect net liquid balance in the lung but do not depend on ENaC subunits (e.g., aquaporins and other channels/transporters) also should be considered as mechanisms of liquid clearance in the newborn lung, particulary since human infants with PHA do not manifest signs of respiratory distress at birth. In addition, the probable loss of fetal epithelial Cl^- secretory function soon after birth may lessen the need for active reabsorption in the postnatal lung. The lack of liquid clearance by αENaC (−/−) lungs between 4 and 12 h (the period when liquid was cleared from γENaC [−/−] lungs) suggests that, in the mouse model, any alternative mechanisms may depend on the presence of the αENaC subunit. This suggestion is supported by the observation that the presence of αENaC is required for expression of other ENaC subunits at the cell surface (28).

The significant respiratory phenotype observed in the αENaC model and apparent absence respiratory problems in human neonates with PHA raises questions about the applicability of these mouse models to human PHA. However, the apparent species differences in phenotype may be accounted for by residual Na^+ transport function by human mutant ENaC. Our αENaC knockout model may result in more complete loss of subunit function than human ENaC mutations. Although patients have been reported with very early (codon 68) mutations in the αENaC subunit (7), we speculate that short fragments of αENaC subunit might be sufficient to chaperone βENaC and γENaC subunits to the plasma membrane and form a functional channel that has sufficient Na^+ transport for the needs of the perinatal lung.

It is possible that, in humans with PHA, mutant ENaC subunits permit sufficient residual Na^+ absorption to clear fetal lung at birth but not to maintain electrolyte homeostasis thereafter. This speculation is supported by our recent study of αENaC (−/−) mice complemented with a rat αENaC transgene (αENaC −/− Tr; 29). Lung liquid was cleared within 12 h of birth in γENaC −/− Tr, but Na^+ absorption rate by tracheal explants was only ∼ 20% that of littermate controls, and this degree of complementation was not sufficient to prevent PHA and metabolic disturbances.

Major differences in anatomical maturity between the human and rodent newborn lung (30) may also be relevant to interpretation of the presence or absence of lung disease. The absence of alveolarization in the newborn mouse lung may be expected to leave this species more vulnerable to delayed liquid clearance than the more developed human newborn lung, which has a much larger gas exchange surface area. The respiratory problems encountered by the full-term αENaC −/− mouse lung may be more reflective of events in the very preterm human lung. A previous study of upper airway Na^+ transport in preterm human infants (31) suggested that immaturity of ENaC expression with consequent delayed neonatal lung liquid clearance may contribute to the pathogenesis of respiratory distress syndrome of the newborn. Based on our experience with the α- and γENaC knockout mice, and the

αENaC (−/−) Tr mouse, we conclude that the requirements for α and γENaC–dependent Na^+ transport for neonatal survival are considerably less in the lung than in the kidney. αENaC is more critical for efficient Na^+ transport in the lung than is γENaC, but in the kidney, loss of either αENaC or γENaC results in major metabolic disturbances. Both the γENaC (−/−) and αENaC (−/−) Tr mice provide models of human PHA1 and suggest that the absence of a neonatal lung phenotype in PHA1 may be due to small, undetected residual Na^+ transport in these patients and differences in mouse and human lung architecture.

The phenotype of our model of targeted γENaC deletion is closer than that of the αENaC knockout mouse to the early human PHA1 phenotype. Although mutations of the γENaC subunit have been reported to cause PHA1 in three families (8), the clinical phenotype in these cases has not been described in detail. It is known, however, that there is wide range of expression of PHA1 from severely affected infants to apparently asymptomatic individuals. As more genetic and clinical information becomes available on PHA1 patients, it may be possible to make better links between specific mutations and clinical phenotype and to ascribe specific function to individual ENaC subunits.

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