

## Dysfunction of epithelial sodium transport: From human to mouse

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**Dysfunction of epithelial sodium transport: From human to mouse.** The highly amiloride-sensitive epithelial sodium channel (ENaC) is an apical membrane constituent of cells of many salt-absorbing epithelia. In the kidney, the functional relevance of ENaC expression has been well established. ENaC mediates the aldosterone-dependent sodium reabsorption in the distal nephron and is involved in the regulation of blood pressure. Mutations in genes encoding ENaC subunits are causative for two human inherited diseases: Liddle's syndrome, a severe form of hypertension associated with ENaC hyperfunction, and pseudohypoaldosteronism (PHA-1), a salt-wasting syndrome caused by decreased ENaC function. Transgenic mouse technologies provide a useful tool to study the role of ENaC in vivo. Different mouse lines have been established in which each of the ENaC subunits was affected. The phenotypes observed in these mice demonstrated that each subunit is essential for survival and for regulation of sodium transport in kidney and colon. Moreover, the  $\alpha$  subunit plays a specific role in the control of fluid absorption in the airways at birth. Such mice can now be used to study the role of ENaC in various organs and can serve as models to understand the pathophysiology of these human diseases.

The epithelial sodium channel (ENaC) belongs to the DEGenerin/ENaC gene superfamily, which shares several regions of homology, such as two transmembrane and cysteine-rich domains. The members of this family are implicated in several cellular functions, including mechanosensation, proprioception, pain sensation, gametogenesis, and sodium transport [1]. In tight epithelia found in kidney, colon, lung, and ducts of sweat and salivary glands, electrogenic entry of sodium ions from the lumen into the cell is mediated by the ENaC located in the apical membrane of the cell. On the basolateral side, the Na,K-ATPase actively transports sodium out of the cell into the extracellular compartment and provides the driving force for sodium reabsorption [2]. ENaC consists of three homologous subunits,  $\alpha$ ,  $\beta$ , and  $\gamma$ , which have been cloned from different species, including

humans, mouse, rat, bovine, and chicken [reviewed in 2, 3]. This has permitted the study of the molecular properties of this channel in various expression systems. In *Xenopus* oocytes, all three ENaC subunits are required to reproduce channel characteristics similar to those described in native tissues [2], although small amiloride-sensitive sodium currents can be measured in oocytes injected with  $\alpha$ ,  $\alpha\beta$ , or  $\alpha\gamma$ ENaC cRNAs [4–6]. A heterotetrameric structure ( $\alpha\beta\alpha\gamma$ ) has been recently proposed for ENaC [7, 8]. It is therefore likely that all cation channels belonging to this gene family are tetrameric, even though a nonameric architecture has been proposed by others [9]. In humans, an additional subunit ( $\delta$ ENaC) has been reported, but little is known about its function to date [10].

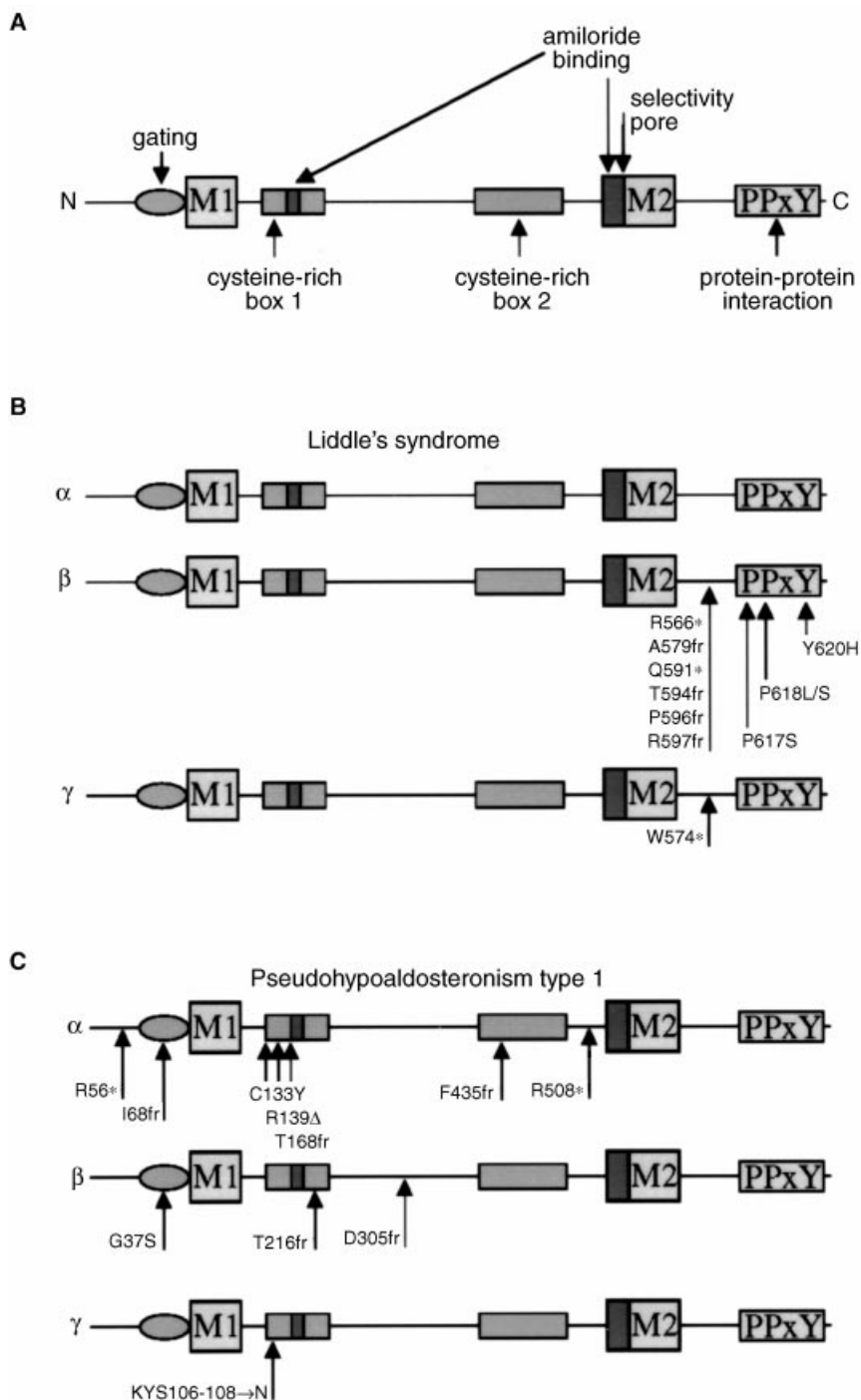
Recent advances in the understanding of the structure–function relationship of ENaC were obtained by analyzing mutations found in genes coding for human ENaC subunits. A putative gating domain was identified within the highly conserved N-terminus (Fig. 1A) [11]. The role of the large extracellular loop is not well established [12]. A short segment preceding the second membrane-spanning domain is involved in amiloride sensitivity and in selectivity of the channel to different ions, suggesting an implication of this domain in the pore structure of ENaC [13–16]. In the C-terminus, which is located intracellularly, mutations leading to deletion of a well-conserved PPxY motif lead to hyperactivity of the channel probably because of altered protein–protein interaction, for example, of Nedd4 protein [17, 18]. Interference of such mutant proteins with the clathrin-mediated endocytosis pathway has been discussed as well [19].

### IMPLICATION OF THE EPITHELIAL SODIUM CHANNEL IN HUMAN DISEASES

Molecular analysis of two human genetic diseases, Liddle's syndrome and pseudohypoaldosteronism type 1 (PHA-1), has revealed the importance of ENaC in the salt homeostasis and thus in the control of the extracellular volume and blood pressure.

**Key words:** epithelial sodium channel, pseudohypoaldosteronism, Liddle's syndrome, gene targeting, aldosterone.

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**Fig. 1.** Linear model of the epithelial sodium channel (ENaC) subunit consisting of short intracellular N- and C-termini, two trans-membrane domains (M1, M2) and a large extracellular loop containing two cysteine-rich boxes. Known functional domains are indicated in (A). Identified mutations in human genes encoding ENaC subunits causing Liddle's syndrome (B) and pseudohypoaldosteronism type 1 (C) are summarized. Symbols are: \*stop codon; fr, frameshift;  $\Delta$ , deletion.

Liddle's syndrome (pseudaldosteronism) is characterized by constitutive hyperactivity of ENaC and is inherited in an autosomal dominant fashion [20]. It is characterized by an early and severe hypertension in conjunction with hypokalemia and metabolic alkalosis suggesting hyperaldosteronism, but low plasma aldosterone concentrations are measured. These patients respond to a low-salt diet and to potassium-sparing diuretics that act as ENaC

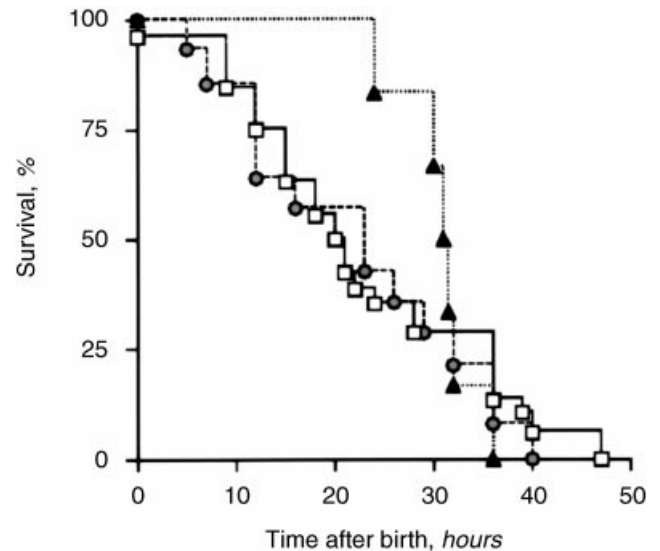
blockers (triamterene or amiloride). A direct link between genes coding for  $\beta$  and  $\gamma$  subunits and Liddle's syndrome has been established [21]. Subsequent studies have identified the PPxY domain of these subunits as the ENaC regulatory region affected by stop, point, and frameshift mutations (Fig. 1B) [reviewed in 22–24]. The phenotype of patients with these mutations is restricted to the kidney, although constitutive activation of ENaC

could be expected in all  $\beta$ - and  $\gamma$ -subunit-expressing tissues. No clinical symptoms related to ENaC hyperfunction in lung or colon have been described yet. Abnormalities of nasal potential difference [25] and sodium channel hyperactivity in human B lymphocytes isolated from Liddle patients [26] have been reported. However, the clinical relevance of these findings is limited to diagnostic procedures, but should encourage clinicians to search carefully for putative extrarenal phenotypes in those patients or to conclude a tissue-specific ENaC hyperactivity in these patients.

Type 1 pseudohypoaldosteronism is an inherited disease presenting soon after birth by failure to thrive, lethargy, anorexia, vomiting, and dehydration. Hyperkalemia, hyponatremia, and metabolic acidosis suggest hypoaldosteronism, but plasma aldosterone concentrations are highly elevated [27]. Two kinds of inheritance have been described with distinct clinical and pathophysiological features [28]: (1) the autosomal dominant renal form is caused by mutations in the gene coding for the mineralocorticoid receptor [29], and (2) the autosomal recessive systemic form is caused by mutations in all three genes encoding ENaC subunits (Fig. 1C) [12, 30–32]. In all tissues expressing ENaC, a severe defect in sodium transport is characteristic for this PHA-1 form. In sweat, concentrations of sodium are elevated contributing to the exacerbation of the salt wasting, and permitting the differentiation of this form from the renal form [33]. A lung phenotype has been recently described [31, 33] and is manifested by recurrent viral-like infections and wheezing, which is attributed to the increased amount of fluid present in the lung. To date, no colonic phenotype has been described in these patients, despite the presence of ENaC activity in this tissue. Likewise, although ENaC expression has been demonstrated in the skin [34], no cutaneous phenotype has been reported, except irritation of the nasal filtrum area chronically exposed to nasal discharge of clear liquid [31]. Other ENaC expressing tissues seem free of any clinical manifestation (pancreas, liver, thymus, eye, urinary tract, placenta). Interestingly, cases of cholelithiasis were reported in three young PHA-1 patients [35, 36]. These phenotype differences between ENaC-expressing tissues in humans carrying these mutations in ENaC could be related to differential expression and/or regulation of ENaC subunits in these tissues or to subclinical symptomatology not yet reported.

#### MOUSE MODELS WITH ALTERED EPITHELIAL SODIUM CHANNEL FUNCTION

The use of transgenic mouse technologies allows the experimental analysis of ENaC gene defects in vivo and provides the possibility of comparing mouse phenotypes with those observed in patients with alterations of ENaC



**Fig. 2.** Survival curve of  $\alpha(-/-)$  (●),  $\beta(-/-)$  (□), and  $\gamma(-/-)$  (▲) mice according to previously reported data [37–39].  $\alpha(-/-)$  mice die mainly from respiratory insufficiency, and  $\beta(-/-)$  and  $\gamma(-/-)$  mice die from kidney dysfunction resulting in hyperkalemia, metabolic acidosis, and severe dehydration. After birth, the  $\alpha(-/-)$  and  $\beta(-/-)$  mice show a similar steady decline in survival, with a maximum survival time of about 48 hours.

function. Inactivation of the genes coding for  $\alpha$ ,  $\beta$ , and  $\gamma$ ENaC have clearly demonstrated that all three ENaC subunits are essential for survival (Fig. 2) [37–39]. These modifications led to reduced or even abolished ENaC activity and permit addressing the question of how much ENaC activity is necessary to maintain sodium transport in the various tissues (Table 1).

#### The lung

Mice deficient for the  $\alpha$ ENaC gene locus [ $\alpha(-/-)$  mice] present poor feeding, costal retractions, and cyanosis a few hours after birth. They die within 40 hours with their lungs filled with fluid, demonstrating the importance of the  $\alpha$  subunit in the perinatal lung liquid clearance [37]. Measurements of the amiloride-sensitive transepithelial potential differences in  $\alpha(-/-)$  mice revealed that ENaC activity is completely abolished. This suggests that channels made of  $\beta\gamma$  subunits do not confer enough activity to be the surrogates of the ENaC function in the lung. Rescue of these  $\alpha(-/-)$  mice by reintroducing a transgenic rat  $\alpha$ ENaC cDNA [ $\alpha(-/-)$ Tg mice] revealed that a slight decrease in ENaC activity does not lead to neonatal respiratory problems [40]. Mice deficient for either  $\beta$ ENaC [ $\beta(-/-)$ ] [38] or  $\gamma$ ENaC [ $\gamma(-/-)$ ] [39] showed increased lung water contents measured as lung wet/dry ratio after birth, but otherwise did not exhibit the symptoms of respiratory distress like  $\alpha(-/-)$  mice. This is particularly remarkable, since in the  $\gamma(-/-)$  pups, the residual ENaC activity measured

**Table 1.** Tissue specific anomalies in human and mouse with ENaC mutations

	Airways	Kidney	Other organs
Human PHA-1 (autosomal recessive)	Viral-like infections Wheezing Nasal discharge	Salt wasting Hyperkalemia Metabolic acidosis	Increased sweat and saliva electrolytes concentrations (no reported symptom) Cholelithiasis
Mouse $\alpha(-/-)$	Lethal respiratory distress syndrome (RDS)	Metabolic acidosis	Not yet determined
Mouse $\beta(-/-)$	Delayed lung liquid clearance at birth, but no RDS	Salt wasting Lethal hyperkalemia Metabolic acidosis	Not yet determined
Mouse $\gamma(-/-)$	Delayed liquid clearance at birth, but no RDS	Salt wasting Lethal hyperkalemia Metabolic acidosis	ENaC activity ( $\Delta I_{sc_{ami}}$ ) abolished in colon, without reported symptom
Mouse $\alpha(-/-)$ Tg (rescued $\alpha(-/-)$ mouse)	Low $\Delta PD_{ami}$ in tracheal explants Slight decrease in ENaC function ( $\Delta PD_{ami}$ and $\Delta I_{sc_{ami}}$ ) without lung phenotype reported	Salt wasting Hyperkalemia Metabolic acidosis	ENaC hypofunction recorded in colon ( $\Delta PD_{ami}$ and $\Delta I_{sc_{ami}}$ ), without reported symptom
Mouse $\beta(m/m)$ (low level of $\beta$ mRNA)	Slight delay in neonatal lung liquid clearance Decreased ENaC function ( $\Delta I_{sc_{ami}}$ )	Salt-restriction induced PHA-1 (renal phenotype)	ENaC hypofunction recorded in colon ( $\Delta PD_{ami}$ ), without reported symptom
Human Liddle's syndrome	Abnormalities of nasal potential difference	Sodium retention Hypertension Hypokalemia Metabolic alkalosis	In vitro hyperactivity in B lymphocytes

as amiloride-sensitive potential difference in tracheal explants was only about one sixth of wild-type animals [39]. These observations are confirmed by another mouse model expressing low levels of the  $\beta$ ENaC subunit mRNA [ $\beta(m/m)$ ] [41], suggesting that a low residual ENaC activity in the lung is sufficient to circumvent the neonatal lung phenotype. Thus far, the role of ENaC in the adult mouse lung is not yet well established.

It is of great interest to compare the lung phenotype in ENaC-deficient mice and humans. Barker et al suggested that ENaC deficiency in the lung could be a cause of respiratory distress syndrome of very premature infants [42]. However, no case of neonatal respiratory distress syndrome has been reported in PHA-1 patients. In these patients, the lung phenotype manifests later (a few months after birth) and is not as dramatic as in the mouse [31]. At least two explanations could account for this difference between mouse and human lung phenotype: (1) species-specific differences such as anatomical immaturity of newborn mouse lung [43], or the presence of a  $\delta$  subunit [8], or splice variants of the  $\alpha$  subunit [44], which are not yet identified in rodents (our unpublished data) [45]; and (2) the mutations involved in human PHA-1, which could allow for a sufficient residual ENaC activity to rescue or attenuate the human lung phenotype [6]. Further studies are necessary to solve this apparent contradiction between human and mouse lung phenotype.

### The kidney

In contrast to  $\alpha(-/-)$  mice, which die mainly from pulmonary problems,  $\beta(-/-)$  and  $\gamma(-/-)$  mice exhibit a significant defect in renal function appearing a few hours after birth and leading to death within 48 hours. Lethargy and failure to thrive are associated with urinary

$Na^+$  wasting,  $K^+$  retention, and increased plasma aldosterone concentrations, thus reflecting the renal phenotype found in PHA-1 patients. A comparison of survival curves of the  $\alpha(-/-)$  and  $\beta(-/-)$  mice (Fig. 2) suggests that  $\alpha(-/-)$  mice could also be affected by electrolyte disturbances. Indeed,  $\alpha(-/-)$  mice present low pH and low bicarbonate concentrations, suggesting a metabolic component added to the probable respiratory acidosis [40]. Transgenic rescue  $\alpha(-/-)$  mice [ $\alpha(-/-)$ Tg] present a renal phenotype evoking the human PHA-1, but with some differences compared with  $\beta(-/-)$  and  $\gamma(-/-)$  mice. The symptoms appear later (about 5 days after birth) and are less dramatic than in the  $\beta(-/-)$  and  $\gamma(-/-)$  mice (50% survival up to adulthood). In addition, survival differences between both sexes were noted because of the location of the transgene on the X chromosome [40]. The partial  $\beta$  knock-out mouse model [ $\beta(m/m)$ ] of Pradervand et al shows reduced ENaC activity and elevated plasma aldosterone levels [41]. Interestingly, these mice develop a renal phenotype with weight loss, hyperkalemia, and salt wasting under only salt restriction. The difference of severity between the renal phenotype observed in these  $\beta(m/m)$  mice compared with  $\beta(-/-)$  mice requires some explanations. The  $\beta(m/m)$  mice were obtained in the course of generating a mouse model of Liddle's syndrome. The resulting  $\beta$ ENaC mRNA expression is very low (about 1% in lung and kidney), probably because of a destabilization of mRNA. Moreover, the putative resulting  $\beta$ ENaC protein could be hyperactive, as it contains a Liddle mutation. This transcript level is sufficient to rescue both lung and kidney phenotypes under normal salt conditions. Therefore, a very low level of  $\beta$  subunit mRNA (even if the resulting protein could be hyperactive) seems to confer enough ENaC activity

to maintain salt and water homeostasis under normal salt conditions, but is limiting if salt restriction is imposed.

When present, the renal phenotype of these mouse models corresponds well to the human phenotype, with salt-wasting, hyperkalemia, and metabolic acidosis. However, slight variations in the time of appearance can be noticed between these models [ $\beta(-/-)$ ,  $\gamma(-/-)$ ,  $\alpha(-/-)$ Tg,  $\beta(m/m)$ ] and human PHA-1 patients. This could be related to the different level of residual ENaC activity and/or regulation depending on the model.

### The colon

Because of perinatal death, no experiments exploring colon physiology have been performed on  $\alpha(-/-)$  and  $\beta(-/-)$  mice. ENaC activity is reduced in colon of  $\gamma(-/-)$  pups [39], adult  $\alpha(-/-)$ Tg mice [40], and  $\beta(m/m)$  mice [41]. Nevertheless, no colonic phenotype was described, as in human PHA-1 patients, where no measurements are reported.

In summary, the severe lung phenotype in mice seems to be a consequence of the complete abolishment of ENaC activity, whereas the renal phenotype becomes evident in mice already with a slightly decreased ENaC activity. This might reflect a differential requirement in ENaC activity and/or regulation in various organs. Remarkably,  $\alpha(-/-)$  mice have focused the attention of clinicians and searchers on ENaC function in the lung. This proves that mouse models help us to acquire a better understanding of the pathophysiology of some human diseases.

### PERSPECTIVES

More refined mouse models will be developed expressing specific ENaC mutations. For example, a mouse model for Liddle's syndrome is expected to show hyperactivity of ENaC. This should establish the relationship between ENaC and salt-sensitive hypertension. The Cre/loxP-mediated recombination allows alteration of the ENaC genes in a time- and tissue-specific manner. This might be useful in circumventing the lethal phenotypes of  $\alpha$ ,  $\beta$ , and  $\gamma$ ENaC knockouts and in studying the role of ENaC hypoactivity and hyperactivity in other ENaC-expressing tissues.

### NOTE ADDED IN PROOF

Recently, a mouse model for Liddle's syndrome has been published [46], reproducing most of the characteristics of the human Liddle phenotype, like high blood pressure, metabolic alkalosis, and hypokalemia.

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