Genetic Disorders of Membrane Transport
V. The epithelial sodium channel and its implication in human diseases*

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The epithelial sodium channel (ENaC) is a membrane protein made of three different but homologous subunits (α, β, and γ) present in the apical membrane of epithelial cells of the distal nephron (cortical and medullary collecting tubule) and distal colon and in the airways and in the excretory ducts of several glands. It provides a controlled entry pathway for Na\(^+\) from the lumen of these organs into the epithelial cells, and, together with the Na\(^+\)-K\(^+\)-ATPase located in the basolateral membrane of the same cells, it is responsible for the active, vectorial transport of Na\(^+\) from the external medium through the epithelial cells into the extracellular fluid and toward the blood (7, 12).

The ENaC has different functional roles in various organs in which it is expressed. In the kidney (collecting tubule), the modulated reabsorption of Na\(^+\) through ENaC provides the primary mechanism of the regulation of urinary Na\(^+\) excretion and thus allows the fine control of the whole organism Na\(^+\) balance under the hormonal control of aldosterone. By its depolarizing effect on the apical membrane potential, the Na\(^+\) channel also provides the driving force for tubular K\(^+\) secretion. Specific inhibitors of ENaC promote urinary Na\(^+\) excretion and inhibit K\(^+\) secretion; these drugs (amiloride, triamterene) are therefore used as K\(^+\)-sparking diuretics. ENaC has a similar functional role in the distal colon, preventing excessive Na\(^+\) loss in the stools. In airways, a most important role is the reabsorption of the fluid that fills the airways at birth, promoting the shift from fluid secretion (before birth) to fluid reabsorption (postnatal). With the cystic fibrosis transmembrane conductance regulator, it also participates in the delicate regulation of the fluid balance in the airways that maintains a thin mucosal fluid film necessary for mucus clearance (31). In the excretory ducts of salivary and sweat glands, the activity of ENaC tends to decrease the luminal Na\(^+\) concentration, allowing the excretion of a less salty saliva and preventing major loss of Na\(^+\) in the sweat fluid.

**MUTATIONS IN HUMAN ENaC SUBUNIT GENES**

The pathophysiological consequences of the mutations known in humans are essentially related to the role of ENaC in the kidney and less to the role in other organs, probably because of the predominant function of ENaC in renal Na\(^+\) and K\(^+\) excretion and possibly because of partial redundancy with other Na\(^+\) transport systems in other organs (although no data are available to support this last point). Mutations of the ENaC subunit genes are responsible for two syndromes that have different effects on channel function. Mutations resulting in a decrease or loss of function result in urinary salt loss and decreased capacity to secrete K\(^+\) in urine, whereas mutations resulting in gain of function produce Na\(^+\) retention, hypertension, and excessive K\(^+\) urinary loss resulting in hypokalemia.

Mutations causing a reduced ENaC activity. Pseudohypoaldosteronism type 1 (PHA-1) is a heterogenous clinical syndrome characterized by mineralocorticoid end organ resistance, i.e., urinary loss of Na\(^+\) and reduced K\(^+\) excretion despite an elevated level of aldosterone (18). A severe form of this syndrome is inherited as an autosomal recessive trait, results in sometimes lethal episodes of hyponatremia, hypoten-

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sion, and hyperkalemia, and shows alteration of Na⁺ transport in several organs, kidney, salivary glands, sweat glands, and colon (11). In several families showing this form of PHA-1, links to mutations in any one of the three ENaC subunits (5, 30) were found, as shown in Fig. 1. A causal link between these mutations and the PHA-1 syndrome was further supported by the demonstration that these mutations resulted in decreased ENaC activity in an artificial expression system (8). A less severe form of PHA-1 with an autosomal dominant mode of inheritance is symptomatic mostly during infancy and improves with age (17).

Gain of function mutations. Liddle’s syndrome, an autosomal dominant hereditary form of hypertension, is characterized by an early and severe hypertension, often accompanied by metabolic alkalosis and hypokalemia (19), all signs that are characteristic of an excess of aldosterone (Conn’s syndrome). The plasma level of aldosterone is however low. For this reason, Liddle’s syndrome is also called pseudoaldosteronism. This severe form of hypertension is remarkably responsive to treatment with a low-salt diet and Na⁺ channel inhibitors (K⁺-sparing diuretics), suggesting a primary defective regulation of the ENaC. Indeed, linkage studies showed that this trait was due to mutations in the genes of ENaC subunits (28). As illustrated in Fig. 1, these mutations are all localized in the COOH-terminal region of the β-subunit (10, 15, 16, 28, 32) and γ-subunit (9, 30). Furthermore, these mutations all modify the so-called “PY” motif (the consensus sequence PPXY, see Fig. 1) either by missense mutations, by introduction of an upstream frameshift, or by a stop codon that results in elimination of the PY motif. In expression systems, these mutations have been shown to result in the overexpression of Na⁺ channels that are hyperactive compared with the wild-type ENaC (25, 26). More precisely, Kellenberger et al. (17) have shown that these mutations prevent the downregulation of the channel that normally occurs with a rise in intracellular Na⁺: ENaC channels bearing the Liddle’s mutation remain in a highly active state despite a high intracellular Na⁺ concentration. The mechanism of this downregulation is not yet completely elucidated but may involve the binding of the ubiquitin ligase Nedd-4 through a direct interaction of the PY motif in the COOH terminus of ENaC subunits with the WW domain of Nedd-4 (29) and/or clathrin-mediated endocytosis (27).

TRANSGENIC MOUSE MODELS WITH ALTERED ENaC FUNCTION

Both the loss of function and the gain of function mutations in ENaC genes are extremely rare in humans, and no homologous spontaneous mutations are known in animals. It is therefore very difficult to study the pathophysiology of these diseases. However, mutant animals provide a means to explore in detail the consequences of these mutations in a living organism. The deletion or modification of ENaC subunits in vivo would show possible functional redundancy and requirements of different ENaC subunits for its functional role in different organs. Thus the relative importance of each ENaC subunit is now experimentally testable in animal models, and mouse models bearing mutations in all three subunits (α-, β-, and γ-ENaC) have been generated that result in reduced or complete abolishment of ENaC activity (2, 13, 21). This allows us to address the questions of how much ENaC activity is sufficient to maintain Na⁺ balance or pulmonary fluid balance in vivo. As described below and summarized in Table 1, reduced ENaC activity in these (ENaC-mutant) mice leads to clinical symptoms similar to the PHA-1 phenotype, ranging from mild to severe forms of this disease.

Inactivation of the α-ENaC subunit gene locus. Inactivation of the mouse α-ENaC gene locus demonstrated that α-ENaC is indispensable for lung liquid clearance at birth. Absence of ENaC function, in α-ENaC(-/-) mice, led to a respiratory distress syndrome and neonatal death (13). The amiloride-sensitive electrogenic Na⁺ transport in airway epithelia was completely abolished.
indicating that without α-ENaC no functional Na\(^+\) channels are expressed. Furthermore, the newborns showed metabolic acidosis with significantly lower blood pH and HCO\(_3^-\) concentrations. Unfortunately, these mice die so early that it is impossible to evaluate either the extent of electrolyte disturbances or the epithelial function in kidney and colon. Using a transgenic approach, we therefore attempted to rescue the α-ENaC deficiency by reintroducing the rat α-ENaC cDNA under the control of an heterologous (cytomegalovirus) promoter. The function induced by the transgene in the airways was sufficient to alleviate the respiratory distress syndrome, and about one-half of the transgenic (Tg) animals survived to adult age. Surviving α-ENaC(-/-)/Tg mice developed a PHA-1 with growth retardation, prominent salt-wasting in the first weeks of life, and metabolic acidosis (14). The 50% early mortality in α-ENaC(-/-)/Tg mice also demonstrated that the neonatal period is quite sensitive to electrolyte disturbances, as observed in mice deficient for the mineralocorticoid receptor (MR) (3). Adult α-ENaC(-/-)/Tg mice escaped from this severe PHA-1, and their metabolic profile changed with age. In these animals, blood gases and serum and urinary electrolyte concentrations were in the normal range despite plasma aldosterone levels that were elevated sixfold (14), suggesting that these animals were constantly hypovolemic. This course of the disease is similar to that of patients with PHA-1, in which supplementary Na\(^+\) requirements diminish over time. The mechanism of this adaptation to distal nephron Na\(^+\) loss is not yet well understood (23). Redundant mechanisms of salt transport may be relatively more efficient in the kidney, colon, and lung of adults.

Disruption of the β-ENaC gene in mice. A mutation was introduced into the mouse β-ENaC gene locus, which led to low β-ENaC RNA levels (<4%) and absence of detectable β-ENaC protein (21). With normal salt intake, these mice, designated as β-ENaC(m/m), showed normal growth rates, no respiratory phenotype, but exhibited a lower Na\(^+\) transport in colon. Urinary Na\(^+\) and K\(^+\) concentrations were elevated, and the mice showed a mild PHA-1 with compensated metabolic acidosis and slightly elevated plasma aldosterone levels. With low-salt diet (0.1 g Na\(^+\)/kg dry food), these mice developed clinical symptoms of an acute PHA-1 with continuous weight loss, hyperkalemia, and decreased blood pressure (21). Thus, under certain conditions, like low-salt intake, there was a failure of ENaC function because of the absence or low amount of β-ENaC, even though in colon Na\(^+\) transport measured as the amiloride-sensitive potential difference (PD) was increased. This indicates that the β-ENaC subunit is required for a full Na\(^+\) conservation capacity during salt deprivation.

Inactivation of the γ-ENaC gene locus. In mice, inactivation of the γ-ENaC subunit resulted in early death of homozygous γ-ENaC knockout mice at ~36 h after birth. The death was mainly caused by severe disturbances of Na\(^+\) and K\(^+\) balance (2). Shortly after birth, these mice exhibited a severe neonatal PHA-1 syndrome with urinary Na\(^+\) wasting, low urinary K\(^+\) excretion, and a large increase in plasma K\(^+\) concentration. Surprisingly, these γ-ENaC knockout mice did not present any respiratory distress syndrome.

Comparison of the results obtained with the α-, β-, and γ-subunit knockout animals suggests that the α-subunit is the most essential subunit, which is required for channel function in lung as well as for kidney and colon. A similar observation was made in the Xenopus laevis oocyte expression system in which amiloride-sensitive currents are observed when a combination of subunits including α is expressed but not when β alone, α alone or βγ are expressed without α (4).

The less severe airway phenotype in β and γ knockout mice indicates that either assembly of the remaining subunits (αγ or αβ, respectively) may yield enough transport function in the lung or that the β- and γ-subunits can be substituted for by another subunit; this is not possible with the α-subunit.

So far, only gene disruption resulting in complete inactivation of ENaC genes has been explored. Less drastic modifications leading to less severe phenotypes might result in pathophysiological changes more closely related to the milder forms of human PHA-1, which can be clinically silent or apparent only when subjected to specific environmental challenges such as low-salt diet (20, 21). ENaC genes and physiological pathways implicated in severe forms of hypertension may also be involved in the milder, but more common, forms of hypertension.

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### Table 1. Human PHA-1 and transgenic mouse models with dysfunction in ENaC activity

<table>
<thead>
<tr>
<th>Human PHA-1 (see text)</th>
<th>α-ENaC(-/-) (Ref. 13)</th>
<th>α-ENaC(-/-)/Tg (Ref. 14)</th>
<th>β-ENaC(m/m) (Ref. 21)</th>
<th>γ-ENaC(-/-) (Ref. 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary salt wasting</td>
<td>+ to +++</td>
<td>ND†</td>
<td>+‡</td>
<td>+++</td>
</tr>
<tr>
<td>Hyperkalemia</td>
<td>+</td>
<td>ND†</td>
<td>–</td>
<td>+†</td>
</tr>
<tr>
<td>Metabolic acidosis</td>
<td>+</td>
<td>ND†</td>
<td>+*</td>
<td>Compensated</td>
</tr>
<tr>
<td>Hypokalemia</td>
<td>+</td>
<td>ND†</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Reduced blood pressure</td>
<td>+</td>
<td>ND†</td>
<td>+‡</td>
<td>ND</td>
</tr>
<tr>
<td>Aldosterone level</td>
<td>++</td>
<td>ND†</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Failure to thrive</td>
<td>+</td>
<td>†</td>
<td>+‡</td>
<td>†</td>
</tr>
<tr>
<td>ENaC activity</td>
<td>Reduced or abolished§</td>
<td>Abolished</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
</tbody>
</table>

PHA-1, pseudohypaldosteronism type 1; ENaC, epithelial sodium channel; ND, not determined; +, ++, and +++ indicate severity of phenotype. *Only in the neonatal period. †Neonatal death. ‡Only on low-salt diet. §Measured by expression of the corresponding ENaC mutant in Xenopus oocytes.
be PHA-1-type protected from environmental or genetic hypertensive factor.

Mouse models with reduced ENaC activity. Mice heterozygous for an inactivating mutation in the α-, β-, or γ-ENaC subunit are expected to produce 50% of RNA transcripts from the corresponding gene but do not show obvious clinical symptoms of PHA-1. They exhibit normal blood Na\(^+\), K\(^+\) and HCO\(_3\)\(^-\) concentrations and pH values, and urinary electrolytes are in the normal range. Although measurements of amiloride-sensitive short-circuit current in airway epithelia showed nearly normal ENaC-mediated Na\(^+\) transport, in vivo measurements of amiloride-sensitive transepithelial PD in colon (ΔPD\(_\text{amil}\)) showed up to 50% reduction in ENaC activity in β- and γ-ENaC heterozygous mutant mice (2, 21). In α-ENaC heterozygous mutant mice, mean values of PD\(_\text{amil}\) were not different from wild-type mice, but the normally observed diurnal cyclicity of ΔPD\(_\text{amil}\) was abolished (33). However, treatment with an ANG II receptor blocker (irbesartan) reduced mean blood pressure in α-ENaC(+/-) mice, indicating that Na\(^+\) balance and blood pressure were maintained possibly as a result of an increased responsiveness to ANG II (34). In summary, the ENaC has been shown to be essential in the vital function of Na\(^+\) balance regulation by the kidney and colon and in the control of fluid balance in the airways. Its role and physiology in colon and exocrine glands are only starting to be understood.

Perinatal death in mice heterozygous for an inactivating mutation in the MR receptor was inactivated developed ascribed to alteration of the MR gene (1), and mice in which the MR receptor was inactivated developed severe symptoms of PHA with failure to thrive, weight loss, severe Na\(^+\) and water loss, and a highly stimulated renin-ANG system (3). These MR(−/−) mice die around 10 days after birth because they are not able to compensate for Na\(^+\) loss. Interestingly, amiloride-sensitive Na\(^+\) absorption is reduced, but the abundance of the mRNAs encoding for ENaC and Na\(^+\)-K\(^+\)-ATPase subunits is unchanged, indicating that regulation of Na\(^+\) absorption via MR is not achieved by transcriptional control of ENaC and Na\(^+\)-K\(^+\)-ATPase. Daily injections of the glucocorticoid betamethasone from day 5 after birth onward prolonged survival of MR(−/−) mice but could not completely replace MR function. In lung, ENaC transcription is controlled by glucocorticoids (22), and inactivation of the glucocorticoid receptor in vivo resulted in perinatal death due to respiratory failure (6); lung maturation was severely retarded, and the abundance of mRNA encoding the amiloride-sensitive ENaC was diminished.

Perspective

Gene-targeting experiments have already revealed some of the mechanisms underlying the control of Na\(^+\) balance and blood pressure regulation. Animals deficient in genes can be readily intercrossed to explore their functional relationship. Transgenic expression of the enzyme Cre recombinase can induce tissue-specific gene targeting, resulting in selective reactivation or inactivation of the gene (22, 24). These new strategies can be used to overcome lethal phenotypes (e.g., in α- and γ-ENaC knockout mice) and to study tissue-specific functions of ENaC at any given time during development or adulthood. The engineering of new animal models based on mutations identified in humans will provide novel opportunities to examine the effect of particular mutations in different defined environments and to explore the physiological consequences of different combinations of mutant genes.

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References


