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Disturbances of Na/K Balance: Pseudohypoaldosteronism Revisited

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Introduction

Pseudohypoaldosteronism (PHA) is a heterogenous syndrome resulting from the inability for aldosterone to produce one of its physiologic effects, namely the promotion of potassium and hydrogen secretion. It is thus characterized by three essential features: (*1*) hyperkalemia, (*2*) metabolic acidosis, and (*3*) abnormally elevated plasma aldosterone concentrations. It has been classified by Kuhnle (1) into three distinct diseases or types (Table 1).

Type I PHA

Type I PHA is characterized by early and severe manifestations of salt-wasting, life-threatening hyperkalemia, metabolic acidosis, dehydration, and activation of the renin-angiotensin-aldosterone system (RAAS) associated with normal adrenal function. Plasma aldosterone concentrations are elevated, as is plasma renin activity, thus demonstrating the peripheral resistance of target tissues to these hormones. Treatment by the potent mineralocorticoid fludrocortisone is without effect. Salt supplementation alone is sufficient to compensate for sodium wasting. Type I PHA is heterogeneous and was divided into at least two entities on the basis of inheritance and phenotype of the disease (2). The identification of two distinct genetic defects strengthened this subdivision.

Type II PHA

PHA II is also known as familial hyperkalemia and hypertension or Gordon syndrome (3,4). The classification of this heterogeneous syndrome as PHA is, however, controversial because plasma aldosterone concentrations are highly variable, usually almost normal, and patients respond adequately to mineralocorticoid hormone (5).

The hallmarks of PHA II are hypertension, hyperkalemia, and correction of these abnormalities by low doses of thiazide diuretics (6). Mild hyperchloremia, metabolic acidosis, and suppressed plasma renin activity are also associated with the syndrome. The GFR and adrenal function are normal. An autosomal dominant inheritance has been reported in these families. Three loci were linked to the disease, demonstrating the genetic heterogeneity of the syndrome, which underscores the heterogeneity of the clinical phenotype. Moreover, additional pedigrees with PHA II could not be linked to these loci, implying a wider genetic heterogeneity of the syndrome (7). One locus is on chromosome 1 (8); a second locus is on chromosome 12 (6); the third is on chromosome 17 (6). Interestingly, this latter locus overlaps with a syntenic segment of rat chromosome 10 that contains a BP quantitative trait locus (8). Online Mendelian Inheritance in Man (OMIM) proposes to symbolize the chromosome 1q31-q42 locus involved in type II PHA as PHA2A, the chromosome 17p11-q21 locus as PHA2B, and the locus on chromosome 12p13 by PHA2C.

Thanks to the study of a large PHA II kindred, the molecular basis of the syndrome was recently elucidated (9). Mutations or deletions in two members of the WNK serine-threonine kinase family (WNK1 and WNK4) were identified. The precise pathogenic mechanisms by which these mutations or deletions lead to the PHA II phenotype are not yet understood. The deletions (22 and 41 kb) of the intron 1 of the *WNK1* gene lead to fivefold overexpression of the *WNK1* transcript in leukocytes of affected patients. The WNK1 protein was localized in the cytoplasm of the cells bordering the distal convoluted tubule (DCT), the cortical collecting duct (CC,D) and the medullary collecting duct. Four missense mutations of the WNK4 gene involve a highly conserved coil-coiled region of the protein. Interestingly, the WNK4 protein is expressed both in cytoplasm and at intercellular junctions in the CCD and exclusively in the tight junction complex in the DCT. Further studies are needed for a better understanding of the pathogenesis of this syndrome.

Type III PHA

Type III PHA is *transient* and *secondary* to different pathologies related to kidneys or other organs (Table 1). Rare cases of major intestinal resection (10) or sweat gland dysfunction associated with excessive loss of sodium (11) have been described as leading to PHA III. However, renal causes are encountered more frequently. Nephropathies such as obstructive uropathy (12) or urinary tract infections (13) were reported as causes of transient aldosterone resistance (1). The main characteristic of this type of PHA is a decreased GFR. The mechanism of the renal aldosterone resistance is not well understood, but a relationship between the increase in amounts of transforming growth factor–β (TGF-β) produced in inflammatory states and the known inhibitory effect of TGF-β on the

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Table 1. The three types of pseudohypoaldosteronism[a]

Type	Inheritance	Clinical and Biochemical Characteristics	Molecular Pathology	OMIM
I	AR	Renal: salt wasting, hyponatremia, hyperkalemia, metabolic acidosis elevated PAC and PRA, treatment by salt supplementation for life Lung: chest congestion, cough and tachypnea Na^+ and Cl^- elevated in sweat, saliva, and stool	Mutations on the three genes coding for the epithelial sodium channel	264350
	AD	Renal: salt wasting, hyponatremia, hyperkalemia, metabolic acidosis, elevated PAC and PRA, treatment by salt supplementation Spontaneous remission over time.	Mutations on the gene coding for the mineralocorticoid receptor	177735
II[b]	AD	Hyperkalemia, hypertension, hyperchloremic acidosis, normal PAC, low PRA, treatment by thiazide diuretics	At least three subtypes: subtype a: associated with locus on chromosome 1q31–q42 (gene unknown) subtype b: mutations on the gene coding for WNK4 subtype c: mutations on the gene coding for WNK1	 145260 601844 605232
III[c]	No	Hyperkalemia, acidosis, elevated PAC and PRA, low glomerular filtration rate.	Secondary to: excessive salt loss: intestine, sweat nephropathies: obstructive uropathy, sickle cell and lead nephropathy, amyloidosis, urinary tract infections.	

[a] AR, autosomal recessive; AD, autosomal dominant; PAC, plasma aldosterone concentration; PRA, plasma renin activity.
[b] Gordon syndrome or familial hyperkaliemic hypertension.
[c] Transient PHA.

sensitivity of the collecting duct to aldosterone has been suggested (5,14).

PHA-1 and PHA III share common features and are primarily salt-losing syndromes. PHA II is a salt-retaining syndrome with a hypertensive phenotype. As discussed below, we propose to abandon this classification and restrict the discussion to PHA as a salt-losing syndrome with strong activation of RAAS. In this review, we will focus on PHA-1, and we will discuss the molecular basis of the disease in describing the relevant molecular mechanisms that allow mineralocorticoid-dependent Na/K balance that takes place in CCD. We will then discuss the genetic basis of the two forms of PHA-1 in an attempt to understand the pathogenic mechanisms of the disease.

Sodium and Potassium Balance: Aldosterone Control

The final concentration of sodium and potassium in urine is determined during its passage through the collecting duct; this process is mainly controlled by aldosterone (15). In the collecting duct, sodium is reabsorbed from the urinary lumen into the extracellular compartment through a tight epithelium formed by principal and intercalated cells. Aldosterone-dependent, amiloride-sensitive electrogenic sodium transport takes place in the principal cell and is generated by the selective entry of sodium trough an epithelial sodium channel (ENaC) located at the apical membrane. Sodium is then actively extruded out of the cell by the sodium pump or Na,K-ATPase restricted to the basolateral membrane. This electrogenic sodium transport is the driving force for the coupled secretion of potassium through a selective potassium channel (ROMK1) co-expressed at the apical membrane with ENaC. The classic model of the mechanism of aldosterone action in tight epithelia proposes the following steps: aldosterone crosses the plasma membrane and binds to its cytosolic receptor, either the mineralocorticoid (MR) or glucocorticoid (GR) receptor. MR and GR are protected from illicit occupation by high levels of plasma glucocorticoids (cortisol or corticosterone) thanks to the metabolizing action of 11-β-HSD2, which transforms the active cortisol into cortisone, an inactive metabolite that is unable to bind to either MR or GR (16,17). The receptor-hormone complex is translocated to the nucleus, where it interacts with the promoter region of target genes activating or repressing their transcriptional activity. Aldosterone-induced or -repressed proteins (AIPs or ARPs) mediate an increase in transepithelial sodium transport. Early effects are produced by the activation of preexisting transport proteins (ENaC, Na,K-ATPase) via yet-uncharacterized mediators (15). We will discuss MR and ENaC as the two key limiting factors in aldosterone action that are mutated in PHA-1 and emphasize the main

features relevant to understand the pathophysiology of the disease.

Mineralocorticoid Receptor

General Features. A number of excellent reviews have recently been published in this field (15,18–21). We will describe some essential features of the structure and the function of the mineralocorticoid receptors that are necessary to understand the pathophysiology of PHA-1.

There are two types of classical corticosteroid receptors: the high-affinity type 1 or mineralocorticoid receptor (MR [22]) and the lower affinity type 2 or glucocorticoid receptor (GR [23]), which are structurally highly homologous. The glucocorticosteroid hormone cortisol (corticosterone in rodents) binds to MR with a high affinity that is similar to that of the mineralocorticoid hormone aldosterone (24); conversely, aldosterone binds to the human GR with a lower affinity that is similar to that of cortisol. Molecular cloning of the glucocorticoid and mineralocorticoid receptors allowed the determination of their primary amino acid structures and prediction of common functional domains. GR and MR display a high degree of identity in their amino acid sequences, with the exception of the variable N-terminal region. The human MR gene encodes a protein of 984 amino acid residues with a predicted molecular size of 107 kD. Structurally and functionally defined domains are observed within the MR and GR receptors (25). The amino-terminal part contains the domain A/B, which is involved in transcriptional activation. The central part includes the DNA-binding domain (DBD or C), which is responsible for DNA binding and recognition of the specific HRE sequences. It consists of two zinc fingers and represents the most highly conserved part of the molecule. Domain E, which represents the ligand-binding domain (LBD or E) also contains sequences that are involved in nuclear translocation, receptor dimerization, hormone-regulated transactivation, and interaction with heat shock proteins.

Ligand Binding Domain. The crystal structure of different nuclear receptors is known (26). The human MR LBD has not yet been crystallized but has been modeled on the basis of the retinoic acid receptor gamma and the progesterone receptor crystal structure, allowing for good predictions on the structure-function relationship of the ligand binding pocket and on the specific requirements for binding agonists or antagonists (18,21). Upon binding to an agonist, the receptor undergoes a major conformational change (transconformation). A number of contact sites are required for proper transconformation and have been identified by site-directed mutagenesis (27) and by the discovery of a gain-of-function mutation leading to a constitutively active receptor causing a severe hypertensive phenotype in pregnant women (28).

DNA Binding. Dimerization of steroid receptor is a prerequisite for binding to specific hormone-responsive elements (HRE) lying in the promoter region of the target gene. In aldosterone target cells, MR is always coexpressed with GR (no tissue expresses only MR), and it has been proposed that MR can heterodimerize with GR, allowing more diversity in the physiologic response to mineralo- or glucocorticoid hormones, but the *in vivo* relevance of the phenomenon remains to be established (29,30).

Epithelial Sodium Channel (ENaC)

ENaC is characterized by a high selectivity for sodium over potassium, a low unitary conductance, long open and closed time, and a high affinity for the potassium-sparing diuretics amiloride and triamterene (31). The genes coding for ENaC were identified by functional expression cloning (32–34). Genes coding for ENaC are members of the DEG/ENaC family that include genes involved in mechanosensation, neurodegeneration, epithelial sodium transport, or pH sensation (see reference 35 for review).

Subunit Topology and Stoechiometry. ENaC is a heteromultimeric protein made of three subunits, termed α, β, and γ ENaC (34). All three subunits share about 35% homology at the amino acid level and adopt the same topology, with two transmembrane domains, short intracellular amino- and carboxy- ends and a large extracellular loop corresponding to about 70% of the protein mass. When all three subunits are expressed in the same cell, they assemble according to a preferential heterotetrameric structure (36). The number of subunits lining the pore is still under debate. Four ($\alpha\beta\alpha\gamma$) (36–38), eight (39), or nine ($\alpha3\beta3\gamma3$) (40) subunits stoechiometries were proposed for ENaC. A homotetrameric stoechiometry for a related member of the same gene family, the FMRF-amide-gated sodium channel (FaNaC) cloned in *Helix Aspersa*, has been proposed (41).

Specific Role of αENaC Subunit in the Channel Complex. If all three subunits are homologous and participate in the pore structure, they do not have the same relative function. When injected alone in the *Xenopus* oocyte, the α subunit produces small but significant amiloride-sensitive sodium currents (32). By contrast, neither β nor γ alone are able to induce similar currents. When $\alpha\beta$ or $\alpha\gamma$ are co-injected in the oocyte, the measured sodium current reached 30% and 10% of wild-type currents respectively, but with a 2-d delay in the appearance of the maximal current (Figure 1) (42). In oocytes injected with β and γ subunits together, small but significant amiloride-sensitive sodium currents are recorded, reaching about 3 to 5% of wild-type currents. They appear with a long delay (5 to 6 d) compared with wild type. Binding experiments demonstrated a close correlation between the number of channel molecules present at the oocyte surface and the current expressed in individual oocyte (43). Complexes comprising α subunits reach the cell surface, though less efficiently for $\alpha\beta$, $\alpha\gamma$, and α alone compared with $\alpha\beta\gamma$-injected oocytes, while no signal could be detected on oocytes injected with either β or γ subunits alone. Other studies have revealed that the α subunit plays a role in the trafficking of the channel to the cell surface (42) or in its anchoring to the cytoskeleton (44). A fourth subunit sharing 37% homology with α and 25 to 30% with β and γ subunits has been cloned in human (45). When expressed in the oocyte, it assembles with β and γ subunits like an α subunit and gives rise to sodium currents with slight biophysical differences when compared with the $\alpha\beta\alpha\gamma$ channel. The amiloride sensitivity is lower, and selectivity of lithium over

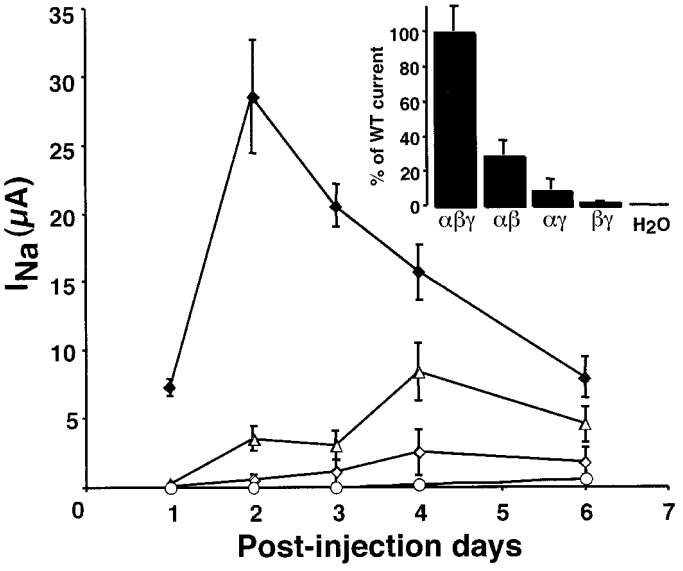

Figure 1. Time-course of epithelial sodium channel (EnaC) expression in the *Xenopus* oocyte system. Oocytes were injected with rENaC $\alpha\beta\gamma$ (◆), $\alpha\beta$ (△), $\alpha\gamma$ (◇), $\beta\gamma$ (○), or water and were measured by a two-electrode voltage clamp every day. In the upper panel, the maximal relative currents to the wild-type (WT) are expressed. At day 6, $P < 0.05$ for all the conditions over water-injected oocytes.

sodium is decreased. The delta subunit is mainly expressed in the testis, pancreas, ovary, brain, and heart. By contrast to $\alpha\beta\gamma$ subunits found in several species (human, rat, mouse, bovine, chicken, rabbit, chimpanzee, guinea pig, pig, dog, frog), the δ subunit was found only in human and chimpanzee.

Structure/Function. In the absence of a three-dimensional structure for DEG/ENaC proteins, site directed mutagenesis provides the best tool to explore structure/function relationships of the channel (see reference 46 for review). In the cytoplasmic N-terminal domain, a highly conserved region preceding the first transmembrane domain is involved in the gating of the channel (47,48). The relative importance of this domain on gating is not similar between subunits ($\alpha >> \beta > \gamma$). Another important feature of the amino-terminal region is the presence of numerous lysine residues. Staub *et al.* (49) demonstrated that these residues (especially on the γ and α subunits) can be ubiquitinated and are key elements determining the half-life of the channel. Chalfant *et al.* (50) reported that a motif present on the rat α subunit (KGDK) could play a role in regulating the endocytosis of the channel. Finally, the N-terminal domain was reported to be involved in channel assembly as demonstrated for the γ subunit (51). The extracellular loop is the largest domain of the protein, encoded by ten different exons, in comparison with the N- or the C-terminus which are each encoded by one exon. It contains several glycosylation sites (52,53). Two cysteine-rich boxes (CRB1 and CRB2) are also present in the loop. CRB1 contains six cysteines and CRB2 ten cysteines. Firsov *et al.* (54) demonstrated that interaction between cysteines 1 and 6 in the first CRB and cysteines 11 and 12 in the second CRB are critical for channel trafficking to the cell membrane. Using antiamiloride antibodies, Ismailov *et al.* (55) identified an amiloride binding site in the extracellular loop, but its functional relevance is challenged by others (56). Waldmann *et al.* (57) and Schild *et al.* (58) highlighted the importance of the pre-M2 domain in the amiloride block and the selectivity filter. Schild *et al.* (58) found point mutations that substantially affect the amiloride sensitivity on the three subunits. Kellenberger *et al.* (56,59) recently solved the molecular aspects of the selectivity filter and proposed a model of the external pore of the channel. The intracellular carboxy-terminus contains several functional domains involved in the regulation of the number of channels present at the cell surface. A PPPxY motif is present on all three ENaC subunits, but curiously not on the δ subunit. Deletion or missense mutation of this motif on the β and γ subunits are found in patients affected by the Liddle syndrome, underscoring its importance in channel regulation (60–64). In Liddle syndrome, the channel is hyperactive because of two factors: an increased number of channels present at the cell surface and an increased intrinsic activity of ENaC. Staub *et al.* (65) demonstrated that the so-called PY motif is the target of Nedd4, a ubiquitin-protein ligase, which binds to the PY motif through its WW domains. The binding allows the ubiquitination of ENaC and its degradation. In Liddle syndrome, this interaction between Nedd4 and the PY motif of the β and γ subunits is no longer possible, and this leads to a higher number of hyperactive channels at the cell surface (see reference 66 for review). An alternative explanation for the regulation of the number of channels present at the cell surface was pointed out by Shimkets *et al.* (67). They propose that the PY motif plays a role as endocytic signal. Another possibility of ENaC regulation is phosphorylation. Serines and threonines, but not tyrosines, were found to be phosporylated on the C-termini of β and γ subunits in the basal state, when channel subunits were stably expressed in MDCK cells (68). In these cells, aldosterone and insulin increased basal phosphorylation of the β and γ subunits, but not the α subunit. However, the phosphorylated residues are not yet identified and the functional relevance not established. Finally, a proline rich domain in the C-terminus of the α subunit that resembles a SH3 protein-protein interaction domain was involved in the interaction with an element of the cytoskeleton, α-spectrin (44). This interaction could play a role in the localization and/or anchoring of the channel at the cell surface.

Tissue- and Cell-Specific Expression of ENaC. All three subunits are expressed in the main aldosterone-sensitive target cells or tissues, namely the last part of the nephron in the kidney (69–72), in the distal colon, in the ducts of salivary and sweat glands (69), and in the lung, where they could be expressed differentially along the pulmonary tree (73–77). However, in some tissues, only one or two subunits are expressed, leading to speculations concerning the stoechiometry of the channel and their physiologic function. In the thyroid, the α subunit alone is apparently expressed, as well as in the liver and the pancreas. In the eye, the α subunit was found in different cells, but its function remains unknown (78–80). In the colon, the α subunit is present alone as long as aldosterone concentrations are low and β and γ subunits are induced under

low-salt diet (81,82). The β subunit is expressed in the placenta (33). β and γ subunits are present in baroreceptor nerve endings (83), the rat foot pad (84), in skin sensory corpuscules (84), and in the urinary bladder of the rat (85,86), where they could play a role in mechanosensation. In the skin, all three subunits are expressed in keratinocytes and hair follicles (87). A recent report showed no sodium transport through cultured human keratinocytes (88,89). These authors and others (90) suggest that ENaC might be involved in cell differentiation in the skin.

ENaC Regulation. ENaC is regulated by intracellular as well as by extracellular signaling pathways. Hormones like aldosterone, vasopressin, insulin, or glucocorticoids regulate ENaC expression and/or activity by intracellular signaling cascades (see references 15 and 31 for review). A high intracellular concentration of sodium inhibits ENaC by a feedback mechanism (91). This regulation is defective in Liddle mutants expressed in the *Xenopus* oocyte (92,93), but this is still poorly understood. A low intracellular pH has a direct inhibitory effect, as demonstrated by the cut-open method on oocytes (91). Several kinases have been implicated in the regulation of ENaC (31). In particular, the aldosterone-induced sgk-kinase was shown to increase ENaC activity and the number of channels present at the cell surface (94–97); this effect is mediated by the phosphorylation of Nedd4–2 (98). Cytoskeleton elements could also play a role in regulating ENaC function. A-spectrin (44), actin (99), and syntaxins (100,101) were reported to influence ENaC function.

Luminal high sodium concentrations have been described to downregulate ENaC by self-inhibition (102). Extracellular serine proteases (trypsin and the channel-activating protease [CAP-1]) activate ENaC by an extracellular signaling pathway (103–105), but the molecular mechanisms of their effect have not yet been elucidated.

ENaC and Sodium Balance in Human Monogenic Diseases. The cloning of ENaC led to the discovery that a hereditary monogenic form of hypertension, namely the Liddle syndrome, was caused by mutations deleting the PY motif present in the C-terminus of the β- or the γ-ENaC subunits (60–62). Soon after, a salt-wasting syndrome in infancy, PHA-1, was also demonstrated to be caused by genetic defects in the three genes coding for the ENaC subunit (106). This emphasized the importance of ENaC in salt homeostasis and demonstrated that either gain-of-function or loss-of-function mutations could arise on the same genes and lead to mirror phenotypes. Implication of ENaC in tight regulation of salt homeostasis, control of extracellular volume, and BP has opened a new field of investigation and pointed out ENaC and all its regulating factors as candidate proteins potentially involved in salt sensitivity and salt resistance (107).

Pseudohypoaldosteronism Type 1

Renal Type 1 PHA

Autosomal dominant pattern of inheritance is associated with a renal phenotype characterized by salt-wasting, hyperkalemia, hyponatremia, and metabolic acidosis. It has a mild course, and spontaneous remissions are observed over time (108). Geller *et al.* (109) studied five of such kindreds and found four mutations in the gene coding for the mineralocorticoid receptor (hMR), introducing frameshift, premature stop codon, or splice donor site deletion, all probably leading to a dysfunctioning mineralocorticoid receptor. Another group found a missense mutation in a family with autosomal dominant PHA-1 (110). This heterozygous L924P mutation leads to a complete abolition of the MR function. Viemann *et al.* (111) found an insertion (Ins2871C, codon 958) in a sporadic form of renal PHA-1, leading to a frameshift from codon 958 to stop codon 1012 (on 984 amino acid residues for the wild-type protein). Interestingly, one mutation described by Geller *et al.* and the mutation of Viemann are *de novo* mutations, suggesting that sporadic cases could be present in the trait. More striking is the fact that only one mutated allele of the mineralocorticoid receptor is sufficient to lead to the renal phenotype. By contrast, heterozygous knockout mice for the mineralocorticoid receptor (MR+/−) grew and bred normally (112). Only homozygous knockout mice (MR−/−) display a renal phenotype with a severe salt wasting, dehydration, and a rapid death about 10 d after birth. Such severe phenotype is not observed in humans, often asymptomatic at birth. These MR−/− mice could be rescued by subcutaneous salt injection until the animals had reached a body mass of 8.5 g (113). Most of these animals survive with this regimen and cope with their defect. This indicates that, as in humans, survival is possible after the first neonatal critical times, where the kidney is too immature to handle sodium and where NaCl supply in milk feeding is insufficient to compensate the renal losses. In that respect, differences in maturation of the kidney in the neonatal period between human and mice could explain the observed species differences.

Systemic Type 1 PHA

The form of type I PHA inherited as an autosomal recessive mode is characterized by a renal phenotype as in the renal autosomal dominant form. However, no spontaneous remission is observed over time. In addition, other organs than kidneys present defects in salt reabsorption, and this is the reason why this form is called systemic. Lung abnormalities were described as recurrent respiratory infections within weeks or months after birth, which are characterized by cough, tachypnea, fever, and wheezing (114–119). Cultures are sometimes negative, but CMV, RSV, and *S. aureus*, *H. influenzae*, *S. pneumonia*, *M. catarrhalis*, and even *P. aeroginosa* were isolated in others. Kerem *et al.* (118) measured the volume of the airway surface liquid in PHA-1 patients and found an increased volume of more than twice the normal. They hypothesized that the liquid could narrow airways lumen and/or dilute surface-active materials that stabilize small airways, thus explaining the phenotype. The excess of liquid in the airways of these patients results from an absence of electrogenic sodium transport as demonstrated by assessment of the nasal transepithelial voltage difference. Interestingly, no neonatal respiratory abnormality was associated with this form of PHA-I. All PHA-1 patients are born with normal Apgar score. A case of respiratory distress syndrome has been associated to pseudohypoal-

dosteronism (120). However, some features of this case are incompatible with an EnaC-related PHA-1. First, the patient was a premature, and it is well established that premature infants are more susceptible to develop respiratory distress syndrome without a genetic defect (121). Second, he responded well and quickly to a mineralocorticoid treatment, which is an exclusion criteria for PHA. Finally, systemic phenotype and putative consanguinity of the parents were unfortunately not reported. The genotype was not tested. Prince *et al.* (122) measured the nasal transepithelial voltage difference in a child affected by PHA-1. They found a basal value of zero and the absence of an amiloride-sensitive voltage. No mutations were found on genes coding for ENaC, but the patient is clearly affected by the systemic form of PHA-1.

Other phenotypes in systemic PHA could result from ENaC expression in other tissues. These include gallbladder, where cholelithiasis was described (123,124), Meibomian glands (1), skin (125,126), and placenta with association with polyhydramnios (127–129). Only cases reported with skin or Meibomian glands phenotypes were clearly associated with the systemic form of PHA-1. The relevance of other findings and their link with the syndrome are not yet clear. On the other hand, expression of ENaC in tissues like inner ear or thymus suggest that other clinical phenotypes may have to be unraveled (possibly deafness or immunologic disorder). One might also expect some phenotypic expression of long-term exposure to high plasma aldosterone in the absence of hypertension. High plasma aldosterone has been associated with cardiac fibrosis, but it is unclear whether this relates to the toxic effect of aldosterone or to high BP *per se*, which commonly accompanies aldosteronism.

ENaC Mutations and the Systemic Type I PHA

Systemic PHA-1 was linked to chromosome 12p and 16p in human (130). Chang *et al.* (106) identified three homozygous mutations in five kindreds from Near-East origin (Table 2). All subjects were issued from consanguineous unions and have all the characteristics of the systemic PHA-1. Deletion of 2 bp on the α subunit led to a frameshift at position I68 (αI68fs). A premature stop codon in the extracellular loop of the α subunit predicts a truncated α lacking the second transmembrane domain and the intracellular C-terminus (αR508x). A missense mutation in a highly conserved domain in the intracellular N-terminus of the β subunit (βG37S) reveals genetic heterogeneity of systemic PHA-I. Heterogeneity was reinforced by the report of a splice-site mutation on the γ subunit, leading to abnormal splicing that resulted either in a truncated γ protein or into the replacement of 3 conserved amino acid residues by a novel one (131). Other mutations were since described by different groups (see Table 2). Most of them are on the α subunit, thus illustrating the importance of this subunit in the function of ENaC. By contrast, activating mutations of the Liddle syndrome were found only in the C-terminal part of the β and γ subunits.

Compound heterozygous mutations on the genes coding for the ENaC subunits were reported in PHA-1 patients, adding more complexity in the analysis of the trait (118,119). In particular, these PHA-1 patients can present not only autosomal recessive inheritance, but can also appear as sporadic cases. This is the reason why we prefer to name the form associated to ENaC as the systemic form and not the autosomal recessive one.

Other groups also reported *polymorphisms* on the genes

Table 2. Reported human ENaC mutations causing the autosomal recessive form of PHA-1

Subunit	Mutation	Type	Genotype Reference	Clinical Description Reference
α	I68 frameshift	Homozygous	(106)	(156)
	C133Y	Homozygous	(54, 139)	Lifton RP, personal communication
	S483 frameshift	Homozygous	(119)	(119)
	R492 stop	Homozygous	Bonny O, manuscript in preparation	Bonny O, manuscript in preparation
	R508 stop	Homozygous	(106, 118)	(2,115,157)
	R56 stop and R139 deletion	Compound heterozygous	(118)	NR[a]
	T168 frameshift and F435 frameshift	Compound heterozygous	(118)	NR
	S243 frameshift and S483 frameshift	Compound heterozygous	(119)	(116)
	S562L and S483 frameshift	Compound heterozygous	(119)	(119)
β	G37S	Homozygous	(106)	(106)
	T216 frameshift and D305 frameshift	Compound heterozygous	(118)	NR
γ	KYS106–108→N and 134stop	Homozygous	(131)	(130)
	V543 frameshift and acceptor splice site mutation exon 13	Compound heterozygous	(153)	(153)

[a] NR, not reported.

coding for ENaC and suggested that they could be related to type I PHA (132,133), especially if associated with polymorphisms present in the gene coding for the mineralocorticoid receptor. Polymorphisms on ENaC that do not lead to a loss-of-function *per se*, but only when associated with polymorphisms on other genes, could be of high relevance. They may confer a protection against excessive salt consumption and salt-sensitive hypertension.

Recently, Iwai *et al.* (134) found a polymorphism on the promoter region of the γ subunit (G(-173)A), which was associated to hypotension in a large Japanese population. The AA genotype was associated with an 11-mmHg decrease in systolic BP and with a higher prevalence of hypotension. In a luciferase assay in MDCK and HRE cells, they showed that the allele A was associated with a drop of the promoter activity of about 50% compared with the G allele activity. This highlights the importance of potential polymorphisms in the promoter region of ENaC genes to influence BP. However, the clinical characteristics of the patients bearing the polymorphism were not detailed, and we are not aware whether they have a salt losing nephropathy, a higher aldosterone concentration or other features of pseudohypoaldosteronism.

Even if many PHA-1 patients reported in the literature have not been genotyped, it seems that not all cases of systemic PHA-1 are due to mutations of ENaC genes. In the series of Chang *et al.* (106), no ENaC mutations were found on 2 of 7 kindreds. In the series of Kerem *et al.* (118), no ENaC mutation was found in 3 of 9 of the kindreds. This 30% of unknown cases of PHA-1 could be the result of a low sensitivity of the method used for the screening or could arise from mutations of the promoter or of regulatory proteins that were missed by the screening. Indeed, we could imagine loss-of-function mutations on genes upregulating ENaC (CAP-1) or transducing the mineralocorticoid response from the mineralocorticoid receptor to ENaC (the aldosterone-induced protein, sgk kinase, for instance). On the contrary, genes involved in the repression of ENaC could be hyperactivated and become candidate genes for PHA-1 (Nedd-4 or other). Further studies will help in resolving this issue.

Intermediate Phenotype in Heterologous Expression Systems

Some of the human PHA-1 mutations were functionally tested in heterologous expression systems (Table 3). *Xenopus* oocyte is the most convenient system, but cell lines or even mice have been used. This review will focus on results obtained by expression of ENaC mutants in different heterologous systems.

Mutations on the α Subunit

Whereas all the mutations responsible for Liddle syndrome are on the β and γ subunits, most of the mutations identified in human patients affected by systemic PHA-1 are on the gene coding for the αENaC subunit (Table 2). This underlines the importance of the α subunit in the channel complex. Mutations of the α subunit are mainly frameshift or premature stop codon insertion. In addition, two missense mutations were reported: one is homozygous (αC133Y) ,and the other is a compound heterozygous mutation (αS562L) associated with a frameshift on the other allele (αS483fs). Frameshift mutations disrupt the reading frame and lead to aberrant and prematurely interrupted proteins. The effect of nonsense mutations on mRNA levels is variable. The levels of some mRNAs are not affected, and truncated proteins are produced (135), whereas the levels of others are severely decreased and null phenotypes are observed (136,137). In PHA-1 patients, no studies on αENaC mRNA levels were reported until now, leaving speculations opened concerning the stability of the mRNA and the relevance of a putative residual activity of the prematurely interrupted protein.

Whereas no clinical parameter has a sufficient power to predict the outcomes of a mutation *in vivo*, the mutant ENaC function can be assessed in the *Xenopus* oocyte expression system. Some of the mutants identified in the α subunit were tested in that heterologous expression system (Table 3). We previously tested the shorter homozygous frameshift described by Chang *et al.* (αI68fs). When co-injected with β and γ rat subunits and measured 48 h later, the human αI68fs gave rise to small amiloride-sensitive currents, representing about 0.1%

Table 3. Mutations tested in the *X. laevis* oocyte system

Subunit	Human Mutation	Tested in the *X. laevis* Oocyte as Expression System	% of the WT[a]	Reference
α	I68 frameshift	Human αI68fr ratβ ratγ	0.1%	(138)
	C133Y	Rat αC158Y$\beta\gamma$	71% at 19°C 13% at 30°C	(139)
	R492 stop	Human αR492*$\beta\gamma$	2%	Bonny O, manuscript in preparation
	R508 stop	Human αR508*$\beta\gamma$	8%	(42)
		Rat αL535*$\beta\gamma$	13%	
	S562L	Rat S589L$\beta\gamma$	<0.1%	Gautschi I, Schild L, Kellenberger S, personal communication
β	G37S	Rat $\alpha\beta$G37Sγ	50%	(47, 106)

[a] WT, wild-type ENaC.

of the wild-type activity. These currents were, however, significantly higher than currents recorded from oocytes injected with β and γ subunits only and measured 48 h after injection (138).

Two nonsense mutations were recently studied in the oocyte system (αR492stop [Bonny *et al.*, unpublished observation] and αR508stop) (42). Both led to significant residual current, reaching about 2 and 8% of the wild-type activity, respectively. We were puzzled by such a residual activity, when the second transmembrane domain of the α subunit forming the channel pore is deleted. We demonstrated that the truncated α is co-assembled with wildtype β and γ and that all three subunits are present at the cell surface, although to a lesser extent than wildty-pe channels. The diminution of the macroscopic current of these mutants expressed in the *Xenopus* oocyte was attributed to the parallel decreased number of channels present at the cell surface. This was demonstrated by the single channel conductance, which was only slightly diminished. More striking was the discovery that $\beta\gamma$ channel activity was expressed after a long delay (see Figure 1) and has about the same characteristics as the mutant channel, suggesting that αR508stop$\beta\gamma$ channels could have a pore made by $\beta\gamma$ subunits only. Taken together, these data suggest that despite truncations of an important part of one of the numerically more important subunit (αENaC) of the ENaC complex, some residual ENaC activity could be recorded in the *Xenopus* oocyte. This residual activity could be of particular relevance in the lung at birth, helping the clearance of lung liquid and explaining the absence of respiratory distress syndrome at birth.

The missense mutation αC133Y was identified in a Pakistan kindred (139). The αC133 belongs to the first cysteine-rich box of the extracellular loop. The most notable feature of the extracellular loop is the presence of two cysteine-rich boxes covering about 50% of the sequence. All the extracellular cysteines are conserved among ENaC subunits cloned from different species, suggesting that they are involved in disulfide bond formation. Firsov *et al.* (54) studied these cysteines by mutational analysis and identified the αC133 as a member of a pair of cysteines essential for the channel routing to the plasma membrane. When these authors studied a serine substitute at the equivalent position on the rat cDNA (αC158S$\beta\gamma$) in the *Xenopus* system, they found that the mutation is thermosensitive (35% of the wild-type current at 19°C and only 5% of the wildtype current at 30°C). The equivalent mutation of αC133Y (rat αC158Y) was tested in the oocyte system. The mutation is also temperature-sensitive. When αC158Y$\beta\gamma$-injected oocytes were incubated at 19°C, the mutation led to a decrease of the amiloride-sensitive current by about 30%. By contrast, when incubated at 30°C, the decrease reached 87%. The thermosensitivity of this mutation confirms the possible implication of these cysteines of the extracellular loop in the folding of the protein and in the ENaC routing to the plasma membrane.

Recently, Schaedel *et al.* (119) identified three new αENaC mutations in four Swedish patients (Table 2). One of these mutations (α483fs) is carried by all subjects, indicating a putative common origin for all these Swedish PHA-1 patients. One missense mutation (αS562L) is of special interest for two reasons. First, the S562L is a missense mutation in the second transmembrane domain. Position 562 corresponds to position 589 in rat, and this latter mutant (rαS589) was extensively studied by Kellenberger *et al.* (59). They found that this residue is the key determinant of the selectivity filter of the channel. When mutated in aspartate (rαS589D), the channel pore is enlarged and potassium currents were recorded. When mutated in leucine (rαS589L), a complete inactivation of the channel was observed in the oocyte system. This was due to the inability of the mutant channel to traffic to the oocyte surface, as demonstrated by a binding assay (I. Gautschi, S. Kellenberger, and L. Schild, personal communication, 2002). Second, this mutation is of special interest because it was found to be associated with the αENaC W493R polymorphism. This polymorphism was described by Persu *et al.* (140,141) and was found in 3.1% of a normal population. No association with essential hypertension was found, and no significant increase in amiloride-sensitive current was observed after injection of the human αW493R$\beta\gamma$ENaC into *Xenopus* oocytes. However, some preliminary results indicating an increase in the activity of the rat equivalent (αW520R$\beta\gamma$) should motivate further investigations (L. Schild, personal communication, 2002).

We do not know the relevance of the residual ENaC activities from mutant channel *in vivo*. Extrapolation of the results obtained in the *Xenopus* oocyte system to human physiopathology may be hazardous. However, it is interesting to compare the phenotypes observed in PHA-1 patients with those obtained by a complete inactivation of the αENaC subunit in mice. Differences observed between human and mice will reflect either species differences or could be attributed to a residual ENaC activity due to $\beta\gamma$ channels or to the activity of the mutated α subunit co-assembled with β and γ (107).

Knockout mice for the α ENaC subunit ($\alpha(-/-)$) were obtained by homologous recombination that deletes the first translated exon of the α subunit (142). Only 23 amino acid residues of the α subunit remain in the recombined genomic DNA. A few hours after birth, $\alpha(-/-)$ pups were noted to have diminished mobility, poor appetite, and persistent chest wall retraction. Shortly before death, which intervenes between 5 to 40 h after birth, they were noted to be cyanotic. Autopsy revealed normal morphology, except for lungs, which appeared mottled and filled with water. The measurements of the whole lung wet/dry ratio was significantly higher in the $\alpha(-/-)$ compared with $\alpha(+/+)$ or $\alpha(+/-)$ animals, thus confirming the high water content of the knockout mice lungs. When measured as amiloride-sensitive potential difference of tracheal cysts, ENaC activity was abolished in $\alpha(-/-)$, meaning that αENaC subunit is limiting in the mouse lung for amiloride-sensitive electrogenic sodium transport. These $\alpha(-/-)$ mice also presented signs of acidosis associated with a decrease in HCO_3 concentration, probably reflecting altered ENaC function in the kidney (143). This means that death is not only due to the lung edema, but also to electrolyte disturbances resulting from kidney failure to handle sodium.

These mice seem to have no more ENaC activity in their lung. What about PHA-1 patients? Kerem *et al.* (118) measured nasal PD in a group of nine PHA-1 patients carrying

several different mutations on α (five patients), β (one patient), and three unidentified mutations. Individual values were not indicated, but the mean basal transepithelial PD was diminished to 35% of the normal value, and perfusion of amiloride did not change the PD significantly. Moreover, a patient with compound heterozygous mutations of the α subunit had a much lower bronchial potential difference than that of a normal subject, and this one was not affected by amiloride perfusion. This strongly suggests that individuals with truncations of the α subunit have almost no more ENaC activity in their lung, as measured by the nasal PD method, leading to an increased airway surface liquid and to the characteristic symptomatology. This indicates that $\beta\gamma$ channels or mutated α co-assembled with β and γ subunits play only a minor role in the lung. However, the residual activity of the truncated α subunit might be sufficient to prevent lung edema at birth. Studies of the lung phenotype of γENaC knockout mice (144) and of a mouse model in which the mRNA coding for the β subunit was not stable (145) are also informative to that respect. Both led to a greatly reduced ENaC activity in the lung, but without respiratory distress syndrome. Taken together, these results indicate that only a very low amiloride-sensitive sodium conductance is sufficient to clear lungs of amniotic liquid at birth and keeps them dry after birth.

Relevance of the mutations occurring on the α subunit can also be reviewed through the study of the αENaC splice variants that result in truncations of the protein (Figure 2). A very premature truncation (h αENaCx) was described in a human pulmonary cell line and resulted in a loss of channel activity when expressed in the oocyte (146). Recently, Oh *et al.* (147) found two other splice variants in human resulting from the insertion of *Alu* cassettes in the junction of exons 8 and 9 (hαENaC+Alu and hαENaC+22). These splice variants led either to a premature termination of the α subunit at position 494 (hαENaC+Alu) or to insertion of 22 amino acid residues in the extracellular loop (hαENaC+22). When the truncated α was co-expressed in the oocytes with wild-type β and γ, no appreciable amiloride-sensitive current could be detected. In the chicken cochlea, Killick *et al.* (148) reported the presence of an α-like subunit and identified a splice variant of this subunit (cc αENaC) that predicts a truncated protein. In rat taste bud, two splice variants (r αENaCa and r αENaCb) were reported (149), both of them are predicted to delete the protein. Interestingly, the longer form (r αENaCa) was detected at the cell membrane, as determined by a [^{3}H] phenamil binding assay performed on transfected cells, but no amiloride-sensitive currents could be measured 48 h after injection in *Xenopus* oocytes. However, the physiologic function of these truncated splice variants is not yet established *in vivo*, but it is suggested that they could play a regulatory role in some specialized tissues. Using RT-PCR strategy, a novel N-terminal splice variant has been identified that deletes 49 amino acids in the N-terminal region of the mouse α ENaC subunit. In oocytes expressing the α ENaC splice variant, together with β and γ ENaC subunits, amiloride-sensitive currents were less than 20% of values obtained with the wild-type ENaC. The single-channel conductance and the ionic selectivity were similar, and there was only a minor decrease in the level of expression of the protein at the oocyte surface. These findings indicate that the deleted sequence in the N-terminal part of the mouse and rat α ENaC subunit might play a role in the regulation of the activity of expressed ENaC channels (150).

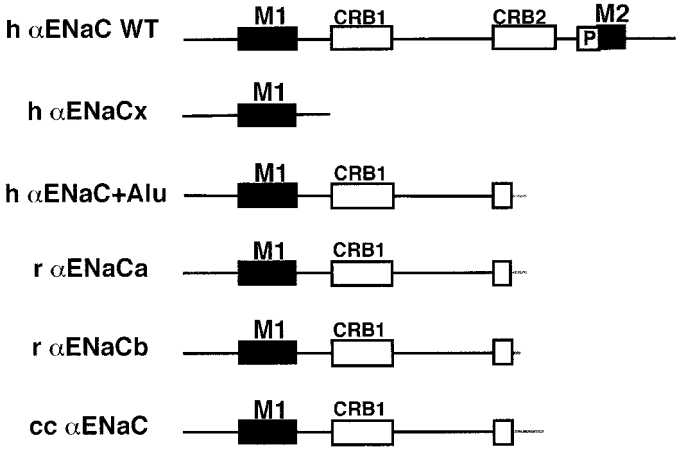

Figure 2. Splice variants of the α subunits leading to truncated proteins. A human splice-variant found in a pulmonary cell line (h αENaCx) results in an interrupted α subunit at the junction of exons III and IV. A splice-variant (h αENaC+Alu) amplified from pooled human cDNAs results in the insertion of an *Alu* cassette, leading to a frameshift and a putative truncated protein after exon VIII. Two splice-variants cloned from the rat taste bud (r αENaCa and b) lead to a truncation of the α subunit at the junction between exons VIII and IX, as a splice-variant of an α-like subunit identified in the chicken cochlea (cc αENaC).

Taken together, these results suggest that mutations of the α subunit found in PHA-1 patients lead to a drastic decrease of ENaC activity. However, a differential need for ENaC activity is encountered in the different tissues. Kidneys need a full ENaC activity, and even a slight decrease results in salt-wasting, hyponatremia, hyperkalemia, metabolic acidosis, and dehydration. The lung is more tolerant to a decreased ENaC activity, and only the complete knockout of the α subunit resulted in a phenotype in the mouse but curiously not in human, where only slight symptoms were noted in patients with severe truncations of the α subunit.

Mutations in the β ENaC Subunit

Two mutations were described in the β subunit. The βG37S is a homozygous missense mutation affecting a highly conserved glycine in a domain preceding the first membrane-spanning segment (106). Some clinical characteristics of the patient carrying that mutation are remarkable (106). The age at clinical presentation of PHA-1 was delayed compared with others, plasma aldosterone concentration was at the limit of the normal range, and the natremia was only slightly decreased. In total, this patient had only a mild PHA-1, but we are unfortunately not aware of the clinical evolution of this case. The mild phenotype was confirmed by expressing the mutant in the *Xenopus* oocyte system. The decrease in amiloride-sensitive current is surprisingly only about 50%. Thus, a discrepancy

here exists between the 50% residual ENaC activity associated with a PHA-1 phenotype and the heterozygote relatives who are free of symptoms. We have so far no explanation for that discrepancy. In searching for an explanation about the decreased ENaC function due to that mutation, Grunder *et al.* (47) demonstrated that the β mutated subunit is co-assembled with α and γ subunits and that the number of channels present at the oocyte surface was unchanged compared with wild-type channels. Moreover, the single-channel conductance was unaffected by the mutation. The authors suggested that a decrease in the open probability should explain the decrease in the macroscopic current, and thus they identified this domain as the gating domain of the channel. However, the importance of this domain for the gating of the channel seems to be variable, depending on the subunit. When the equivalent mutation is reproduced on the α subunit (αG95S), the residual function is only about 5 to 10% (47). The same mutation on the γ subunit led to a residual ENaC activity of more than 60%. Thus, the gating is mainly assumed by the α subunit, and a missense mutation present in that domain would predict to lead to a more drastic phenotype than the one observed on the patient carrying the βG37S mutation. Interestingly, an equivalent mutation to human βG37S was studied in *flr*-1, a *C. elegans* gene of the DEG/ENaC family involved in the control of the defecation rhythm (151). This mutation led to a hypofunction of that gene and a specific phenotype, thus confirming the conservation of the mechanism of gating of ENaC through the whole family.

Two compound heterozygous mutations βT216fs and βD305fs were identified in a patient described by Kerem *et al.* (118). This patient presented the kidney phenotype and the typical lung problems reported in systemic PHA-1. These frameshifts are predicted to interrupt the β subunit in the extracellular loop, deleting most of the loop and the intracellular carboxy terminus of the protein. This should result in a total disruption of the β subunit. If present, the remaining ENaC activity could be due to $\alpha\gamma$ channels, which were shown to have the same biophysical characteristics as the wild-type channel in the *Xenopus* oocyte system, even if their expression is only about 15% of the wild-type (Figure 1). β knockout mice are informative in that respect (152). They all died 48 h after birth, probably of hyperkalemia and metabolic acidosis, revealing that the β subunit is essential for the ENaC function *in vivo*. In contrast to αENaC knockout mice, they did not die of respiratory failure and did not show an abnormal breathing pattern. A small but significant increase in wet/dry lung weight was observed but was only half the increase reported for $\alpha(-/-)$ mice. What is interesting here is that the pulmonary phenotype is mild in the knockout mice. For the patient carrying these β mutations, the lung phenotype is the same as in other patients affected by the systemic PHA-1 with mutations on the α subunit, for instance. However, the phenotype is not detailed enough to draw general conclusions from this case.

Mutations in the γ Subunit

Strautnieks *et al.* (131) reported a mutation on the γ subunit in three Indian families. This homozygous mutation touches the 3' acceptor splice-site preceding the third exon of γ. This results with the production of two mRNAs predicted to create two mutant subunits with either three highly conserved amino acids replaced by a novel one (KYS106–108→N) or a premature truncation from 649 to 134 amino acids. Adachi *et al.* (153) recently reported a sporadic Japanese case of systemic pseudohypoaldosteronism type I. Compound heterozygous mutations of the γ subunit were found. The mother's allele contained a deletion in exon 13 (1627 del G) at the corresponding residue V543, leading to a frameshift from this position to position 597 (premature stop codon), if the mRNA is synthesized and translated. The father's allele carried a mutation of the acceptor splice site of exon 13. By RT-PCR on lymphocytes of the patient, the authors could amplify only the mother's allele, suggesting that the mutation in the acceptor splice site destabilized the mRNA.

No report relates the function of these mutations of the γ subunit in a heterologous expression system. If the mutations disrupt completely the γ subunit, a putative ENaC residual activity should be attributed to $\alpha\beta$ channels. When tested in oocyte, $\alpha\beta$ channels led to about 30% of the wildtype channel current (Figure 1). Their biophysical characteristics are different whether human or rat cDNA are used (42,154,155). Human $\alpha\beta$ channels have the same amiloride sensitivity and the same selectivity for potassium or lithium over sodium as wild-type channels. On the contrary, rat $\alpha\beta$ channels are less sensitive to amiloride and are no more selective between sodium and lithium ions compared with the rat wild-type. Their open probability is also different. We have no explanation for these species differences, but same differences were also observed with $\beta\gamma$ channels and the PHA-1 mutant channel αR508stop$\beta\gamma$ (42).

The γ knockout mice died within 36 h after birth of hyperkalemia, metabolic acidosis, and dehydration (144). This phenotype confirmed that all three ENaC subunits are needed for a full ENaC function, at least in the kidney. These mice, as $\beta(-/-)$ mice, have a predominant renal phenotype. They presented a slight but significant elevation of the wet/dry lung weight 4 h after birth, but no respiratory distress syndrome, by contrast to $\alpha(-/-)$ mice. Half of the $\gamma(-/-)$ knockout mice presented a tracheal transepithelial potential difference still inhibitable by amiloride by 8 to 15%. This residual activity could be attributed to $\alpha\beta$ channels or to a remaining function of the mutated γ subunit associated to α and β. In PHA-1 patients carrying a γENaC mutation, we are not aware of lung problems but their phenotype were not described.

Genotype-Phenotype Correlations

Does the diversity of mutations correspond to a heterogeneity of clinical phenotypes (Table 4)? Are all the PHA-1 phenotypes identical, or is there any phenotypic difference between mutations found on the α, β, or γ subunit? Is there any difference between patients carrying the missense mutation on the β subunit (βG37S) known to diminish by 50% the activity of the channel when compared with the mutation deleting almost the whole α subunit (αI68fs) and leading to a 0.1% residual activity? These are hard questions to answer because we have only few reported patients with well-

Table 4. Genotype-phenotype correlation when available[a]

Mutations	% of the WT Activity in the Oocyte	First Episode of PHA (d)	Na^+ (mmol/L)	K^+ (mmol/L)	PAC (μg/L)	Other Organs Involved than Kidney	Reference
αI68fr	0.1%	7	124	7.7	1.87	ND	(106)
αI68fr	0.1%	1	126	11.2	6.28	ND	(106)
αI68fr	0.1%	8	128	10.9	15.16	ND	(106)
αC133Y	13%	14	147	8.2	6.24	ND	Lifton RP, personal communication
αS483fr	ND	9	116	10.4	11.91	yes, sweat glands and lung	(119)
αR492stop	2%	7	124	9.7	6.50	yes, sweat glands	Bonny O, manuscript in preparation
αR508stop	8%	9	125	10	14.32	yes, lung, salivary and sweat glands	(106)
αS243fr/αS483fr	ND	5	124	10.4	11.15	Unknown	(119)
αS243fr/αS483fr	ND	4	129	8.4	4.8	yes, sweat glands and lung	(119)
αS562L/αS483fr	ND ($<$0.1% for S562L)	11	106	11.4	2.17	yes, sweat glands and lung	(119)
βG37S	50%	19	133	8.2	1.00	ND	(106)
γV543fr/splice site mutation	ND	7	116	8.6	10.73	yes, lungs	(153)

[a] PAC, plasma aldosterone concentration; to translate μg/L in nmol/L, multiply by 2.771; normal range for neonates, 0.20 to 1.00 μg/L. WT, means wild-type; ND, not determined.

described clinical characteristics and available genotype. The next few years should be informative on that respect. Clinicians should better characterize the phenotypes observed in PHA-1 patients and transmit blood for genotyping, and geneticists should be aware of the importance of a fine description of the clinical phenotype. In conclusion, no satisfactory genotype-phenotype correlation could be drawn from the data discussed here and no clinical parameter could predict the importance of the truncation of a subunit or its residual function when tested in a heterologous system such as the *Xenopus* system. Mice models are also not as close of the human systemic PHA-I as we could wish, as illustrated by the α knockout mice. The mouse model contributed significantly by delineating a new role of ENaC in the lung, which turned out to be also true in human.

Conclusions and Perspectives

The discovery of the molecular mechanisms of pseudohypoaldosteronism type 1 necessitated several steps. First, a fine clinical characterization permitted the subdivision of this heterogeneous syndrome into two main groups: the renal autosomal dominant and the systemic autosomal recessive. Second, the cloning of ENaC has allowed genetic studies that have resulted in the identification of ENaC as responsible for the Liddle syndrome, the mirror phenotype of systemic PHA-1. Third, several families affected by autosomal recessive PHA-1 were screened, and several mutations were identified on the three genes coding for ENaC. Finally, these mutations were tested in expression systems such as *Xenopus* oocytes or mice, confirming that they lead to a decrease in ENaC activity. The significant collaborative success between clinicians and researchers should not end here. PHA-1 patients still have no curative treatment for their innate disease. Salt treatment is sometimes not sufficient to prevent the salt loss due to the disease, and death can occur. Moreover, the quality of life of these patients is poor. Efforts to better understand the disease and treat it should be pursued.

In that perspective, an international PHA-I/Liddle syndrome consortium should be created to monitor the increasing number of ENaC mutations linked to these diseases. It should collect all information concerning genotype and phenotype and provide them to the scientific community. Moreover, it should offer the possibility of screening ENaC for mutation to all clinicians in charge of patients putatively affected by one of these diseases. This is the next step in a better characterization of these diseases.

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