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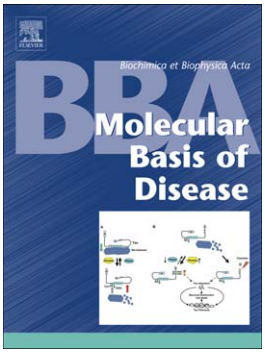

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The Epithelial Sodium Channel and the Control of Sodium Balance.

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Abstract

Studies aiming at the elucidation of the genetic basis of rare monogenic forms of hypertension have identified mutations in genes coding for the epithelial sodium channel ENaC, for the mineralocorticoid receptor, or for enzymes crucial for the synthesis of aldosterone. These genetic studies clearly demonstrate the importance of the regulation of Na^+ absorption in the aldosterone-sensitive distal nephron (ASDN), for the maintenance of the extracellular fluid volume and blood pressure.

Recent studies aiming at a better understanding of the cellular and molecular basis of ENaC-mediated Na^+ absorption in the distal part of nephron, have essentially focused on the regulation ENaC activity and on the aldosterone signaling cascade. ENaC is a constitutively open channel, and factors controlling the number of active channels at the cell surface are likely to have profound effects on Na^+ absorption in the ASDN, and in the amount of Na^+ that is excreted in the final urine.

A number of membrane bound proteases, kinases, have recently been identified that increase ENaC activity at the cell surface in heterologous expressions systems. Ubiquitylation is a general process that regulates the stability of a variety of target proteins that include ENaC. Recently, deubiquitylating enzymes have been shown to increase ENaC activity in heterologous expressions systems.

These regulatory mechanisms are likely to be nephron specific, since *in vivo* studies indicate that the adaptation of the renal excretion of Na^+ in response to Na^+ diet occurs predominantly in the early part (the connecting tubule) of the ASDN.

An important work is presently done to determine *in vivo* the physiological relevance of these cellular and molecular mechanisms in regulation of ENaC activity. The contribution of the proteases-dependent ENaC regulation in mediating Na^+ absorption in the ASDN is still not clearly understood. The signaling pathway that involves ubiquitylation of ENaC does not seem to be absolutely required for the aldosterone-mediated control of ENaC. These *in vivo* physiological studies presently constitute a major challenge for our understanding of the regulation of ENaC to maintain the Na^+ balance.

Introduction

A major challenge for the kidney is to maintain the osmolality and the composition of extracellular fluid volume within a very narrow margin; indeed to keep the extracellular volume constant, the kidney has to balance precisely the urinary excretion of electrolytes and water with the daily intake of salt and fluid. To this end, the urinary electrolyte and water excretion is under the tight control of aldosterone and vasopressin. These hormones act on target cells in the distal part of the nephron and on the collecting tubules of the kidney; these cells share the expression of the epithelial sodium channel (ENaC), the mineralocorticoid receptor (MR), the aquaporin-2 (AQP-2) and the vasopressin receptor (V_2R). These channels and receptors are central to sustain a hormonally controlled Na^+ and water reabsorption.

The cellular expression of ENaC is not uniquely restricted to the distal nephron and the collecting tubules; ENaC is expressed in the colon where, under the control of glucocorticoids, it mediates Na^+ absorption from the intestinal lumen [1]. The contribution of ENaC in the colon to the maintenance of the whole body Na^+ homeostasis still needs to be carefully evaluated. In addition, ENaC is expressed in the lung to maintain the composition of the air-surface liquid, as well as in exocrine glands where it sets the ionic composition of the exocrine secretions (sweat); a major contribution of ENaC in those latter epithelia in the control of the whole body Na^+ balance is however unlikely.

The importance of the aldosterone-sensitive distal nephron in maintaining extracellular electrolyte and water balance is nicely demonstrated by the elucidation of the genetic basis of rare monogenic forms of hypertension, due to mutations in the genes coding for ENaC, the mineralocorticoid receptor, or enzymes crucial for the synthesis of aldosterone [2].

The purpose of this review is to bridge our knowledge about the structural and functional charateristics of ENaC observed *in vitro*, with more physiological aspects of ENaC regulation *in vivo* in the kidney, as evidenced in recently available animal models.

Structural and functional aspects of ENaC.

ENaC belongs to a family of ion channels that includes the neuronal acid-sensing ion channels (ASIC1, ASIC2, ASIC3) in mammals, touch-activated ion channels called degenerins in *C. elegans*, and a variety of homologs in drosophila, mollusks; so far no homologous proteins has been identified in prokaryotes [1].

ENaC is a highly Na^+ selective channel made of three α,β, and γ homologous subunits [3]. The α subunit is absolutely required for channel activity, whereas the β and γ are necessary for maximal channel expression and activity at the cell surface. Several biochemical and functional evidences are consistent with a heterotetrameric structure of the channel, likely comprising 2α, 1β and 1γ subunit [4-6]. However this subunit architecture of ENaC needs to be revisited since the recently reported crystal structure of an ASIC channel isoform ($ASIC1_A$) that shows a trimeric oligomerization[7]. The tridimensional structure of the individual ASIC1A subunit is consistent with early studies on the membrane topology of ENaC α, β, and γ subunits, that proposed two transmembrane segments, intracellular N- and C-termini, and a large extracellular loop that makes of more than half of the channel protein [8] (figure 1). The large extracellular domain is essential for channel function, but its role in supporting Na^+ ion flow through the channel, or in channel gating remains unclear. Mutagenesis studies of ENaC predicted that the second transmembrane (TM2) α helix of each subunit is arranged pseudosymetrically around a central axis forming an ion-conducting channel[9]. The cytosolic N-terminus contains domains that regulate channel gating and likely as well the pore opening on the cytosolic side[10]. The C-terminus of each subunit contains a proline-rich domain involved in specific interactions with regulatory proteins.

ENaC is a constitutively open channel localized at the apical membrane of principal cells and displaying a short half-life of residency (less than 1 hour) [11, 12]. ENaC activity can be inhibited by pharmacological blockers such as triamterene or amiloride that act from the extracellular side and bind a common site on the TM2 segment lining the channel pore [13].

In the kidney, ENaC is expressed in the aldosterone-sensitive distal nephron (ASDN), a region that includes successively, the late portion of the distal convoluted tubule (DCT), the connecting tubule (CNT) and the collecting duct (CD)[14]. In spite of their different embryonic origin, all three nephron segments share in common the expression of ENaC, the mineralocorticoid receptor (MR) and the 11-β hydroxysteroid dehydrogenase type-2, the enzyme that confers to the MR receptor, a functional mineralocorticoid specificity [15, 16]. The subcellular location and the level of expression of ENaC subunits change drastically upon the elevation of plasma aldosterone levels in response to sodium diet [17, 18]. In rodents under standard Na^+ diet (the "european diet") associated with moderate plasma aldosterone levels, ENaC subunits are detectable by immunohistochemistry in the cytosol of principal cells, with a discrete labeling of the apical membrane of the late DCT and the CNT. Under low Na^+ diet leading to high plasma levels of aldosterone in these animals, ENaC subunits decorate the apical membrane of principal cells of the DCT, CNT and CD, with a prominent expression in the early ASDN compared with the late ASDN.

This axial distribution of ENaC along the ASDN, which has also been observed by immunohistochemical experiments, was corroborated by elegant patch-clamp experiments on microdissected ASDN segments of rats kept under different Na^+ diets: under normal Na^+ diet there were no measurable amiloride-sensitive whole cell currents in principal cells of the CNT or CCD . In contrast, under low Na^+ diet with high plasma levels of aldosterone, a significant amiloride-sensitive current could be detected which was more prominent in the CNT, and decreased along the CCD [19]. These results indicate that the adaptation of the renal excretion of Na^+ in response to Na^+ diet occurs predominantly in the early ASDN.

ENaC in the apical membrane of principal cells is the major entry pathway for Na^+ ions from the tubule lumen into the cell (Figure 2): Na^+ ions enter the cell across the apical membrane along a favorable electrochemical gradient [20]. The vectorial transport of Na^+ across the epithelial cell is ensured by the Na^+/K^+-ATPase located at the basolateral membrane that pumps the Na^+ ions out of the cell into the interstitium. Since ENaC is a constitutively open channel

with an open probability averaging 0.4, all the factors that control the number of active channels at the cell surface will have a more profound effect on Na^+ absorption in the ASDN and in the amount of Na^+ that is excreted in the final urine, than factors affecting essentially channel gating [21].

Regulation of ENaC activity.

From functional investigations in heterologous expression systems, ENaC was found to be stimulated, by soluble proteases such as trypsin, chymotrypsin, elastase, thrombin or kallikrein [22, 23]. In addition, membrane bound proteases increase ENaC activity at the cell surface; they were identified by functional cloning in Xenopus oocytes, and subsequently by homology cloning. Among these proteases, the first to be identified was named CAP-1 (channel activating protease-1) and is an ortholog of the human prostasin [24, 25]. Subsequently, mouse CAP-2, an ortholog of the human transmembrane protease serine 4 (TRPMSS4), and another mouse CAP-3 (ortholog of the matriptase or epithin) were shown to stimulate ENaC to a similar extend [26]. Finally the identification of a cleavage sequence for the furin-like protein-convertase in the extracellular domain of the α and γ ENaC subunits, led to the demonstration of the role of furin in ENaC cleavage, ENaC sorting and activity at the cell surface [27-29]. A number of cleavage sites for these membrane-bound proteases in the extracellular loop of α, β, and γ subunits have been identified, indicating that ENaC is the likely substrate of these proteases [30].

The physiological relevance of the ENaC regulation mediated by proteases is still not clearly understood, but indirect evidence suggests the presence of endogenously expressed membrane-bound proteases in epithelia and their possible role in ENaC activation. Indeed, soluble proteases are inefficient to further increase ENaC activity in kidney epithelial cells, although serine protease inhibitors such as aprotinin have been shown to reduce baseline ENaC activity [25]. Assessing the abundance of ENaC channels at the cell surface by surface biotinylation in intact kidneys of rats under different salt diet, Frindt & Palmer found that the expression and the cleavage of γENaC increased under low Na^+ diet, and that γENaC cleavage represents the parameter that best correlated with the increase in ENaC activity [31].

However, the precise molecular and cellular mechanisms responsible for ENaC activation remain largely unknown. Whether these proteases are able to directly interact with ENaC, or whether they act sequentially on the channel activity remains to be determined.

When active at the cell surface, ENaC gating (i.e. channel open probability) is relatively poorly controlled under physiological conditions: cellular stress linked to unphysiological conditions such as the increase of extracellular or intracellular Na^+ concentrations, or a drop in intracellular pH, a rise in intracellular Ca^{2+}, and intracellular oxidative stress, are all factors that tend to decrease the channel open probability; the closure of the channel is likely important to preserve the cell from a massive entry of Na^+ ions that would overcome the pumping capacity of the Na^+/K^+-ATPase [32-35]. Luminal ATP and UTP nucleotides have been shown to reduce the open probability of ENaC; this effect is likely mediated by an apical G-protein coupled $P2Y_2$ receptor. Indeed the inhibitory effect of ATP of ENaC activity in the cortical collecting duct *in vivo* could not be observed in mice lacking the $P2Y_2$ receptor, with an altered salt handling by the kidney [36]. This mechanism together with aldosterone may contribute to a decreased Na^+ absorption under a high sodium diet.

The regulation of Na^+ absorption in the ASDN relies more on the number of active ENaC channels at the apical membrane of principal cells than on the modulation of channel gating itself. The number of active ENaC channels at the cell surface is tightly controlled by aldosterone and vasopressin. The main determinants of the stability of ENaC at the cell surface are now quite well understood. Ubiquitylation is a general process that regulates the stability of a variety of target proteins; mono or multi-ubiquitylation can serve as signal for endocytosis of transmembrane proteins, a process that usually results in their degradation by the lysosome and/or the proteasome [37]. Nedd4 E3 protein-ubiquitin ligases that belong to the HECT family, contain 3-4 WW domains responsible for binding to canonical PY motifs of the target substrates; such PY motifs are found at the C termini of the β and the γ subunits of ENaC [38] and are essential for efficient mono-ubiquitylation of specific lysine residues in the N-termini of β or γ ENaC by the HECT domain of Nedd4-2. In Xenopus oocytes,

Nedd4-2 was shown to efficiently suppress ENaC activity by ubiquitylation of the functional channel complex at the cell surface, leading to clathrin-mediated endocytosis and degradation [39]. Enzymes that are able to deubiquitylate ENaC such as UCH-L3 or Usp2-45, have been shown to increase ENaC-mediated Na^+ current *in vitro*, further supporting the role of ubiquitylation in regulating ENaC stability at the cell surface [40].

As mentioned before, aldosterone essentially increases the number of active ENaC channels at the cell surface [18]. Different approaches have been used to to identify aldosterone-induced transcripts, in order to decipher the aldosterone-signaling cascade. Aldosterone induces the phosphatidylinositide 3'-kinase(PI3K) -dependent kinase SGK-1 (serum- and glucocorticoid-regulated kinase 1), a Ser/Thr kinase that elevates ENaC level and activity at the cell surface in heterologous expression systems [41]. Subsequently, SGK-1 was found to phosphorylate Nedd4-2, leading to a decreased interaction and ubiquitylation of ENaC subunits, finally resulting in an increased ENaC activity at the cell surface (figure 2) [42]. Other aldosterone-induced transcripts have been identified, in particular the de-ubiquitylating enzyme Usp2-45, which also increases ENaC activity at the cell surface *in vitro* [43]. These observations suggest that the regulation of ENaC activity by aldosterone is mediated at least in part by inhibition of Nedd4-2-dependent ubiquitylation of ENaC, that ultimately tends to increase the number of active channel at the cell surface[44].

In addition to aldosterone, vasopressin also increases ENaC activity in the ASDN [45]. Vasopressin binds the V2 receptors, stimulating the release of cAMP, which in turn increases the density of ENaC channel at the cell surface[46, 47]. In vitro studies indicate that PKA, much like SGK-1, also phosphorylates Nedd4-2, providing a cellular mechanism for the positive regulation of ENaC by vasopressin [48]. PKA and SGK1 phosphorylate the same residues of Nedd4-2. However, the converging phosphorylation of Nedd4-2 by these two kinases does not explain the additive effects of vasopressin and aldosterone on ENaC-mediated Na^+ absorption in the ASDN [45]; therefore, other mechanisms are most likely involved in the regulation of ENaC by PKA.

Still, the regulation of Nedd4-2 remains poorly understood. The phosphorylation of Nedd4-2 by SGK1 and PKA stabilizes the interaction of Nedd4-2 with 14-3-3 scafolding proteins, preventing the Nedd4-2 dependent ubiquitylation of ENaC [49]. Interstingly 2 isoforms of the 14-3-3 proteins are induced by aldosterone, as SGK1. Another 14-3-3 binding protein have recently been identified that controls the trafficking of ENaC from an intracellular compartment to the cell surface: this binding partner, AS160, is a Rab GTPase-activating protein (Rab-GAP) known to play a role in GLUT4 transporter to the plasma membrane in response to insulin [50]. In CCD cells, AS160 phosphorylation, possibly by SGK1, allows ENaC trafficking to the cell surface, providing a mechanism for endocytosed ENaC to recycle to the cell surface.

In parallel to the SGK1/ Nedd4-2 pathway that stabilizes ENaC at the cell surface, a signaling cascade involving the Raf-1-MAPK / ERK kinases acts to inhibit the cell surface expression of ENaC by stimulating the interaction between Nedd4-2 and ENaC [51, 52]. Interestingly, the activation of ERK could be inhibited by another aldosterone-induced protein in a cortical collecting cell line, the glucocorticoïd-induced leucine zipper protein (GILZ).

These studies essentially performed *in vitro* on heterologous expression systems or in cortical collecting duct cell lines, identifiy Nedd4-2 as a critical convergence point for the regulation of ENaC at the cell surface.

ENaC and human diseases

Liddle's syndrome is an autosomal dominant form of salt-sensitive hypertension with early onset of elevated blood pressure during adolescence; this hypertension is usually associated with hypokalemia, metabolic alkalosis, low plasma aldosterone, and suppressed plasma renin activity [53]. The elevated blood pressure in Liddle syndrome can be normalized under salt restriction and amiloride treatment. Based on these clinical evidences, G.W. Liddle postulated that the syndrome was "a disorder in which the renal tubules transport ions with such abnormal facility that the end result simulates that of a mineralocorticoid excess "[54]. Notably, the antinatriuretic

response to aldosterone was maintained in those patients, despite the low plasma aldosterone levels. The mutation identified in the index case, is a 45 amino acid deletion in the cytosolic C-terminus of the β ENaC subunit. Despite the fact that Liddle's syndrome is a very rare condition, subsequent genetic analysis of other Liddle's syndrome families revealed corresponding deletion mutations in the C-terminus of the γ ENaC subunit, but none in the α subunit [55]. Furthermore, missense mutations causing Liddle's syndrome identified the target amino acid sequence in the β and γ C-termini that involves the conserved proline-rich motif (the canonical PY motif) [56-59].

Important for our understanding of Liddle's syndrome, ENaC channels expressed from synthetic RNA and carrying the mutations associated with Liddle's syndrome exhibit an increased channel activity in Xenopus oocytes and in a cortical collecting duct cell line[46, 60]. This gain of function of the mutated channels results from both, an increased number of active channels at the cell surface, and a higher probability for the channel to remain open[61]. The gain of function of ENaC mutant channels is consistent with Liddle's hypothesis; the understanding of the cellular and molecular mechanisms underlying these gain of function mutations essentially came from further studies in the *Xenopus* oocyte expression system.

As already mentioned, the PY motifs of β and γ ENaC bind the ubiquitin ligase Nedd4-2 which is responsible for the ubiquitylation of the active channel complex at the cell surface, and the subsequent targeting of the channel for endocytosis and lysosomal degradation. In Liddle's syndrome, deletion or missense mutations of PY motifs in the β or γ impair the ability of Nedd4-2 to bind and thus ubiquitylate the channel, leading to the accumulation of active ENaC channels at the cell surface[62].

Pseudohypoaldosteronism type 1 (PHA-1) is characterized in the first week of life by severe dehydration, hyponatremia, and hyperkalemia [63]. There are two clinically distinct forms of (PHA-1), an autosomal recessive form affecting multiple organs (systemic form), including kidneys, colon, salivary glands, and sweat ducts (but not the skin, or inner ear, for instance) and an autosomal dominant form restricted to the kidney. Patients with a systemic form of PHA-1 have mutations in α, β and γ ENaC subunits [63]. Mutations in the α β or γ

ENaC genes include nonsense, frameshift or missense mutations leading to channel loss of function, and the clinical severity of the syndrome correlates with the extent to which ENaC activity is reduced. The autosomal dominant form of PHA-1 is caused by mutations of the mineralocorticoid receptors [64]. Thus, both Liddle's and PHA-1 syndromes clearly make evident the critical role of ENaC in the ASDN for the maintenance of the whole body sodium homeostasis.

Mouse models

A variety of genetic mouse models have been generated to study *in vivo* the physiological and pathophysiological roles of ENaC in the maintenance of whole body salt balance, and in the control of blood pressure. Constitutive inactivation of all three ENaC subunits in mice clearly showed the importance of ENaC in Na^+ and K^+ handling by the kidney: mice lacking the α subunit of ENaC died soon after birth from hyperkalemia, metabolic and respiratory acidosis, and pulmonary edema due to impaired lung fluid clearance[65]. The constitutive β and γ ENaC null-mice exhibit a milder pulmonary phenotype and essentially renal features that involve increased Na^+ excretion, K^+ retention, and elevated plasma aldosterone [66, 67]. Using a gene targeting approach, α ENaC expression was selectively abolished in the CCD segment of the ASDN; interestingly these CCD-specific KO mice do not exhibit any impairment in Na^+ nor in K^+ handling by the kidney to maintain electrolyte balance, as made evident by plasma aldosterone levels similar to those in wt mice, suggesting that the absence of ENaC-mediated Na^+ absorption and K^+ secretion in the CCD can be compensated in other parts of the nephron[68]. These observations also suggest that the late DCT and CNT are the main regulatory sites of Na^+ and K^+ excretion in the ASDN and confirm the axial gradient of ENaC expression along the distal nephron. Obviously, these experiments do not address the role of the ENaC-mediated Na^+ absorption in the colon in the maintenance of Na balance; the answer to this question awaits the study of additional tissue-specific ENaC KO mouse models to investigate the ability of the colon to retain salt under salt deprivation.

To obtain new insights into the pathophysiology of salt sensitive hypertension, a mouse model of Liddle's syndrome has been developed, with the deletion of the C-terminal end of the β ENaC subunit [69]. Under normal salt diet, these mice develop normally with a blood pressure similar to their wild type counterparts of identical genetic background. However, under high salt diet the Liddle mice develop high blood pressure, hypokalemia, metabolic alkalosis, low plasma aldosterone, a profile that recapitulates Liddle's syndrome.

The mouse model of Liddle's syndrome with the mutation in the PY motif of β ENaC, together with the Nedd4-2 KO and SGK1 KO mouse models allow a genetic dissection of some of the important converging points of the aldosterone-signaling pathway.

Measurements of ENaC-mediated currents by the whole-cell patch clamp technique on microdissected collecting tubules revealed that, under normal salt diet, the currents measured in both wt and Liddle principal cells were undetectable [70]; the interpretation of these data is not clear. A possibility is that, although ENaC activity is undetectable under these experimental conditions, it might be however sufficient to drive enough Na^+ to generate hypervolemia and hypertension. However under low salt diet with high plasma aldosterone, Liddle mice had a much larger ENaC-mediated current compared to wt. This demonstrates that ENaC lacking the PY motif in the C-terminus of β ENaC and thus displaying defective interactions with Nedd4-2, are still highly sensitive to stimulation by aldosterone. These data obtained *in vivo* indicate that the Nedd4-2 signaling cascade does not constitute the predominant pathway for aldosterone-mediated upregulation of ENaC. Rather they suggest that Liddle's syndrome constitute a state of hypersensitivity to aldosterone, with an exacerbated response to physiological plasma levels of the hormone. In the colon of Liddle mice, ENaC channels also retain their sensitivity to aldosterone [71].

The Nedd4-2 knock-out mice (Nedd4-2 $^{-/-}$) have a slight elevation of BP under normal salt diet that was exacerbated under high Na^+ diet [72]. A tendency to lower plasma aldosterone levels was observed under normal salt diet that became significant under low salt diet. At the protein level, the

expression of all three ENaC subunits in the whole kidney was increased together with that of the NaCl cotransporter (NCCT). ENaC activity directly measured in the colon by recordings of the rectal transepithelial and amiloride-sensitive potential difference, remains sensitive to stimulation by aldosterone. Interestingly, and by contrast to 'Liddle' mice, no hypokalemia was detected, but rather a significant hyperkalemia under normal Na^+ diet was observed in the Nedd4-2 $^{-/-}$ mice; this suggests an increased activity of both ENaC, and likely the NCC cotransporter that might play a role in the hypervolemia and hypertension in the Nedd4-2 $^{-/-}$ mice.

Two independent SGK1 knockout mice $SGK^{-/-}$ have been generated [73, 74]: both mice models are viable and do not exhibit any major phenotype, and no evidence for a salt loosing nephropathy under normal salt diet. This contrasts with the severity of the renal phenotype of both the ENaC knockout and the mineralocorticoïd receptor (MR) knockout mice [65, 75]. When put on low Na^+ diet, both $SGK^{+/+}$ and $SGK^{-/-}$ mice reduced their urinary Na^+ excretion, but the $SGK^{-/-}$ showed a higher natriuresis under this condition. In one $SGK^{-/-}$ model, the natriuresis under chronic aldosterone treatment (1wk) or low Na^+ diet was correlated with ENaC activity in the CCD recorded by whole cell patch clamp technique [74]. As reported in other mouse models, under normal Na^+ diet, no detectable amiloride-sensitive current could be measured in either mice $SGK^{+/+}$ or $SGK^{-/-}$; however, under chronic aldosterone treatment a robust amiloride-sensitive current was measured that was undistinguishable between $SGK^{-/-}$ and $SGK^{+/+}$ mice. Furthermore, after 2-3 days of Na^+ restriction, $SGK^{-/-}$ mice displaying a higher natriuresis compared to wt, showed a higher ENaC activity as measured by the amiloride-sensitive Na^+ current, together with a higher plasma aldosterone level [74]. This again suggests that Na^+ transporters other than ENaC in upstream tubular segments, are defective in the $SGK^{-/-}$.

These mice models indicate that the signaling pathway that involves SGK1 and Nedd4-2 is not absolutely required for aldosterone-mediated control of ENaC, and if physiologically relevant, this pathway likely plays only a minor role in ENaC-mediated regulation of Na^+ balance. The other signaling pathways responsible for the modulation of ENaC activity remain to be

identified. Interestingly, the SGK1–Nedd4-2 pathway might play an important role in controlling aldosterone sensitive NCC activity and Na^+ absorption in the DCT.

Conclusions.

From genetic studies and mouse models it is now recognized that ENaC in the ASDN, under the control of aldosterone and vasopressin, plays a key role in the fine adaptation of urinary Na^+ excretion in order to precisely balance the daily intake of salt. The control of this Na^+ balance is essential for the maintenance of the extracellular fluid volume and blood pressure.

A number of uncertainties still persist in our understanding of regulation of ENaC activity. Two signaling pathways have been described in vitro and in cultured cell lines that regulate ENaC trafficking and activity; in addition, proteases are involved in the processing of ENaC activation. Studies on animal models allowing the genetic dissection of these ENaC regulatory pathways are now needed to establish their physiological relevance in the maintenance of sodium homeostasis, in the control of the extracellular fluid volume and blood pressure. The presently available models suggest the presence of alternative pathways than those involving Nedd4-2 and SGK1, in the hormonal regulation of ENaC, that need to be considered.

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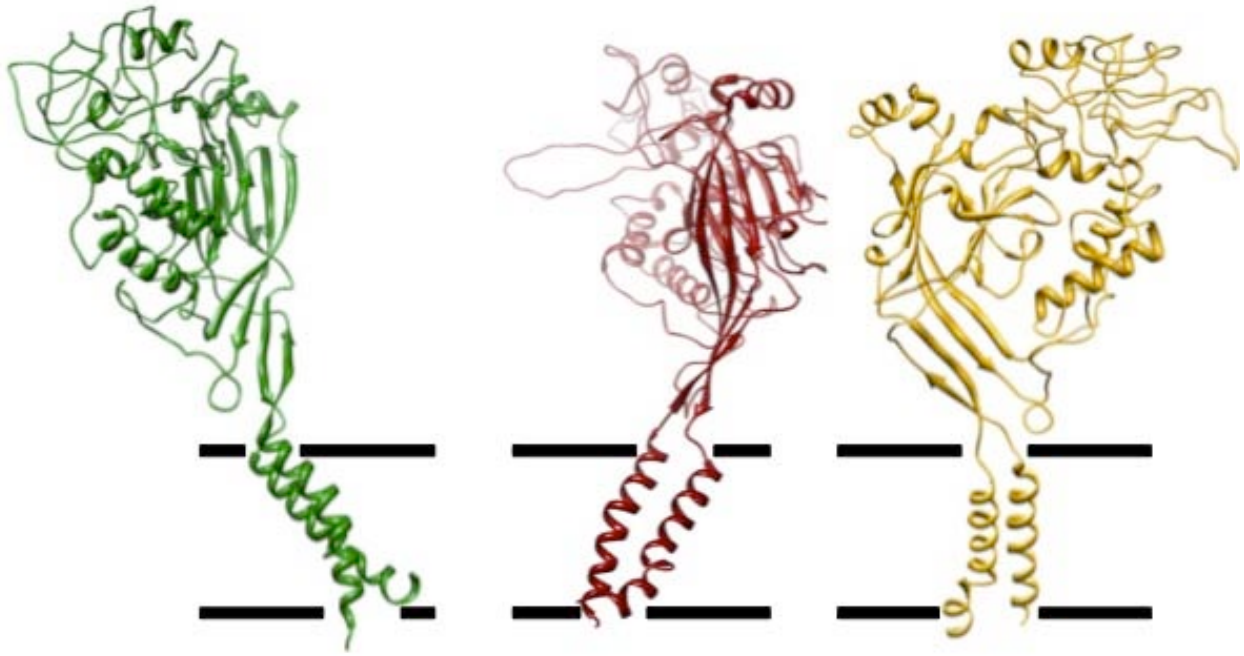
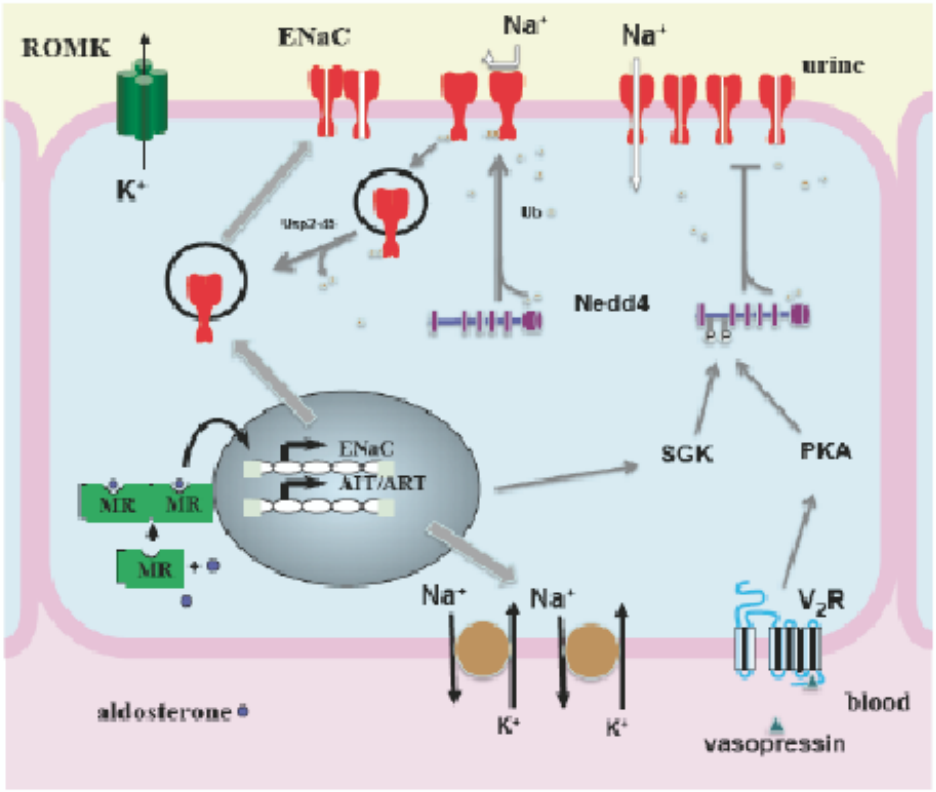
FIGURES

Figure 1: Homology models of ENaC subunits based on the crystal structure of ASIC channel: α ENaC (green) βENaC (red), γENaC (yellow)



This homology model shows the predicted structures of the α (green) β (red) γ (yellow) ENaC subunits based on the 3D structure obtained for the ASIC1a channel. These homologous subunits show a large extracellular domain that represents more than half of the mass of the protein. The two transmembrane domains (TM1 and TM2) consist of α helices, and the TM2 is involved in lining the pore. The crystal obtained for ASIC1a did not allow the resolution of the structure of the amino and the carboxy termini, and therefore are absent as well in the present homology models of α β and γ subunits.

Figure 2 : Model of ENaC regulation by aldosterone and vasopressin



Upon binding of aldosterone to its receptor (MR), the complex ligand receptor dimerizes, and subsequently it translocates into the nucleus where it binds to specific DNA responsive elements. This leads both to the induction of a specific set of AIT transcripts (AIT = aldosterone-induced transcripts) such as ENaC, the Na/K-ATPase, or SGK1 and, alternatively, to the repression of a number of transcripts (ART = aldosterone-repressed transcripts). Vasopressin binds to the G-protein coupled receptor V2R, which leads to activation of a downstream protein kinase A (PKA).

Both SGK1 and PKA inactivate Nedd4 upon phosphorylation, leading to retention of active ENaC at the cell surface. Ubiquitylated ENaC channels at the cell surface are internalized; deubiquitylation by USP2-45 allows ENaC to recycle from endocytic vesicles back to the cell surface.

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