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Epithelial Sodium Channel and Salt-Sensitive Hypertension

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Abstract

The development of high blood pressure is influenced by genetic and environmental factors, with high salt intake being a known environmental contributor. Humans display a spectrum of sodium-sensitivity, with some individuals displaying a significant blood pressure rise in response to increased sodium intake while others experience almost no change. These differences are, in part, attributable to genetic variation in pathways involved in sodium handling and excretion. The epithelial sodium channel (ENaC) is one of the key transporters responsible for the reabsorption of sodium in the distal nephron. This channel has an important role in the regulation of extracellular fluid volume and consequently blood pressure. Herein we review the role of ENaC in the development of salt-sensitive hypertension, and present mechanistic insights into the regulation of ENaC activity and how it may accelerate sodium-induced damage and dysfunction. We discuss the traditional role of ENaC in renal sodium reabsorption and review work addressing ENaC expression and function in the brain, vasculature and immune cells, and how this has expanded the implications for its role in the initiation and progression of salt-sensitive hypertension.

Graphical Abstract

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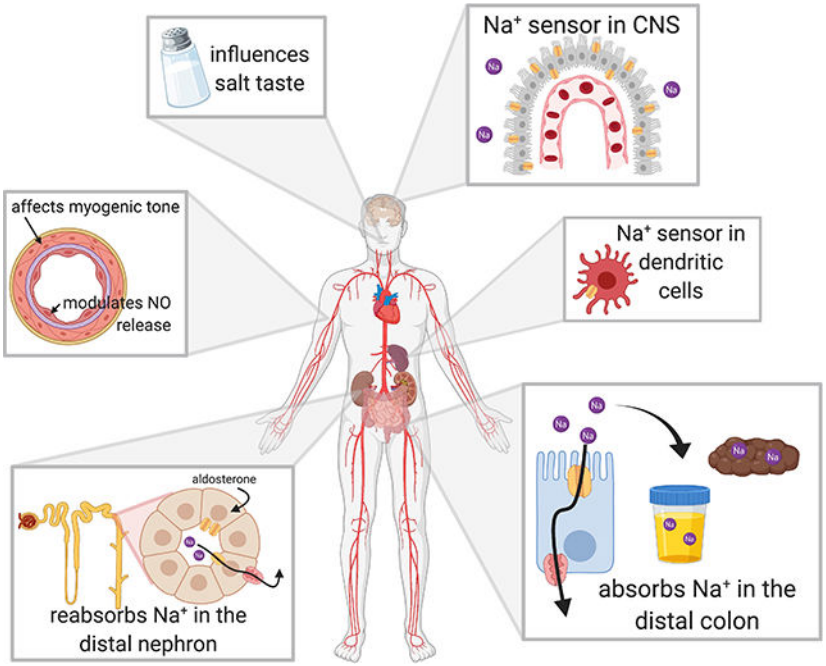
Disclosure

None



Keywords

ENaC; salt sensitivity; blood pressure; sodium

Introduction

Blood pressure (BP) is a multifactorial metric, dependent upon complex interactions between environmental, genetic, and demographic factors. One environmental factor known to influence BP is sodium (Na^+) intake. Studies have shown increased Na^+ raises BP, while a reduction can lower BP in hypertensives[1]. However, there is a continuum of BP sensitivity to Na^+ within the human population. Variability in Na^+ sensitivity reflects, in part, gene variances related to the renin-angiotensin-aldosterone system and renal Na^+ transporters[2]. Immune cells[3], vascular endothelium and smooth muscle[4], and specific regions of the central nervous system (CNS) also contribute to salt-sensing variability. In this review, we discuss how epithelial sodium channels (ENaCs) contribute to the salt-sensitivity of BP, both in the kidney and beyond.

ENaC's role in the kidney

The kidney is largely responsible for Na^+ homeostasis. While the majority of filtered Na^+ is reabsorbed in the proximal tubule, the distal nephron, comprised of the distal convoluted tubule, connecting tubule and collecting duct, absorbs 6–10% of filtered Na^+, and is where the nephron fine-tunes the amount excreted in urine[5]. This distal segment is a key site of hormone-regulated Na^+ absorption, as principal cells in the distal nephron respond to aldosterone by increasing ENaC apical membrane expression and open probability[6]. Other hormones such as vasopressin, angiotensin II and insulin also modulate ENaC activity, with

their contributions being implicated in Na^+ retention in pathophysiological states[6]. ENaCs are composed of three structurally-related subunits termed α, β, and γ[7]. While a δ-subunit can replace the α-subunit to form functional channels in non-renal tissues[8], it is not expressed in rodents and its functional role is not well understood.

ENaC subunits have large extracellular domains that sense external environmental factors that modulate channel activity[7]. Each extracellular domain is connected to two transmembrane domains that form the channel pore and gate, and are in turn connected to short intracellular termini[7]. A number of factors influence channel gating and open probability (summarized in fig. 1A), including α− and γ-subunit proteolytic processing, β− and γ-subunit palmitoylation, intracellular and extracellular Na^+, extracellular protons and chloride, acidic phospholipids, and shear stress[9]. A His-Gly (HG) motif on the cytoplasmic N-termini also influences gating[10]. In addition to gating and open probability, ENaC is modulated by the number of channels at the membrane. Ubiquitination of cytoplasmic Lys residues on surface-resident channels targets them for internalization and degradation[11,12]. This process is regulated by the ubiquitin ligase Nedd4–2 that binds to the cytoplasmic C-termini of subunits at a Pro-Tyr (PY) motif[12]. Recent *in vivo* work has shown that only the α- and γ-subunits are ubiquitinated, and that the modification favors the cleaved, mature subunits[11]. A number of protein kinases, including PKA, PKC, ERK1/2, Sgk1, CK2 and MTS3 regulate ENaC and their dysregulation may contribute to hypertension[6,13].

While ENaC does not directly couple Na^+ movement to other ions, it indirectly influences renal K^+ and H^+ transport. Na^+ reabsorption by ENaC increases the driving force for K^+ secretion. These effects explain why the ENaC inhibitors amiloride and triamterene, which work by blocking the channel pore[14], also inhibit renal K^+ secretion and can lead to hyperkalemia. The activity of other transporters also affects ENaC activity. The Na^+-Cl^- cotransporter (NCC) is located in the distal convoluted tubule, both upstream of ENaC and in cells where ENaC and NCC are co-expressed. Increased NCC-mediated Na^+ absorption reduces Na^+ delivery to ENaC[15]. Direct interaction between the two proteins exists and is increased by aldosterone[16]. The Cl^--HCO_3^- exchanger pendrin also influences ENaC activity[17].

Mutations in genes encoding ENaC subunits (*SCNN1A, SCNN1B,* and *SCNN1G*) are associated with monogenic hyper- or hypotensive disorders, underscoring the importance of ENaC in BP regulation. Liddle syndrome is an autosomal dominant disorder associated with ENaC gain-of-function mutations, characterized by early-onset hypertension, hypokalemia and metabolic alkalosis. ENaC mutations associated with Liddle syndrome are found in the genes encoding the β− or γ-subunits[12,18]. These mutations disrupt the PY motif and impair channel ubiquitination, internalization from the cell surface, and degradation, resulting in increased surface expression and activity[6,12]. Conversely, pseudohypoaldosteronism type 1 (PHA-1) is an autosomal recessive disorder associated with ENaC loss-of-function mutations, resulting in severe neonatal salt wasting, volume depletion and hyperkalemia. Both frameshift mutations resulting in premature stop codons and missense mutations resulting in a reduction or loss of channel activity have been associated with this disorder, including mutations in the HG motif[10,18].

Mutations at other sites affect channel activity as well. Numerous mutations within the α− or γ-subunits modulate activity by changing the Na^+ self-inhibition response, where Na^+ binds to defined extracellular sites within ENaC and dampens channel open probability[9]. In this regard, a gain-of-function α-subunit mutation (C479R) was noted in siblings with a mild Liddle syndrome phenotype that exhibited decreased Na^+ self-inhibition[19,20].

While extreme phenotypes are seen with variants associated with large changes in ENaC activity, the contributions of other channel variants to more subtle BP phenotypes, including salt-sensitive hypertension, is still being unraveled. Inhibitors of renal Na^+ transporters are used to treat specific ENaC and NCC gain-of-function disorders. The fact that dietary intervention can partially alleviate specific severe monogenic BP disorders strengthens the idea of a genetic-dietary interaction[21]. Understanding ENaC's role in modulating BP sensitivity to environmental factors is critical for identifying individuals who should respond to ENaC-blocking therapies and dietary modulation. A growing body of evidence suggests that ENaC has a role in BP control beyond the kidney, including in other epithelia (lingual epithelium (salt taste) and distal colon (absorption of ingested Na^+)), the vasculature, brain and immune system.

Genetic studies linking ENaC to salt-sensitivity and BP

The expanding number of human whole-genome and whole-exome sequences have resulted in identification of thousands of variants within the four genes encoding the ENaC subunits. A few common non-synonymous mutations (e.g., αT663A and αA334T) were identified that alter channel function in heterologous expression systems but generally fail to associate with BP differences in humans[22,23]. However, the majority of variants are present at low frequency (mean allele frequency <0.05), making analysis of their contribution to specific phenotypes difficult. A β-subunit variant (T549M) in individuals of African ancestry was noted to occur more frequently in hypertensives[24], although this is not a consistent finding[25]. Another variant, βR563Q, was associated with hypertension in black and mixed-ancestry South Africans[26]. However, its presence in ~20% of the San people who maintain a rural lifestyle with low dietary Na^+ intake was not associated with hypertension[27]. These observations suggest that some ENaC mutations may drive salt-sensitive hypertension, but are tolerated when dietary salt is scarce.

The GenSalt study provided additional evidence of the association of ENaC variants with salt-sensitivity. Carried out in rural northern China, study participants received a 7-day low Na^+ intervention (3g of salt/day) followed by a 7-day high Na^+ intervention (18g of salt/day) while monitoring BP[28]. Multiple ENaC single nucleotide polymorphisms were associated with BP variation in response to Na^+ manipulation[29]. Additional non-synonymous single nucleotide variants were identified that increased or decreased ENaC activity in an oocyte expression system, although these functional variants did not significantly correlate with salt-sensitivity in humans[30]. As these were primarily low frequency variants, a larger population may be necessary to demonstrate significant association. Compensatory mechanisms might also mask the effects of ENaC variants in these individuals. Resequencing of the 300 most salt-sensitive and salt-resistant participants identified carriers of *SCNN1A* rare variants as having a 0.52 decreased odds of salt-sensitivity as compared to

noncarriers, while *SCNN1B* and *SCNN1G* rare variants were not associated with salt-sensitivity[31].

As outlined above, genetic data support ENaC as a contributor to salt-sensitivity. However, the question remains whether therapeutically targeting ENaC could lower BP in salt-sensitive individuals. ENaC-blockers are rarely prescribed as first-line antihypertensive medications, and relatively few trials have systematically addressed their efficacy[32]. Amiloride might be most beneficial when administered in a targeted manner. Indeed, when black hypertensives expressing the βT594M variant were taken off their antihypertensive regimen and administered only amiloride, BP was controlled effectively to the same level[33]. A recent study showed amiloride could blunt the BP response to Na^+ loading in a Nigerian population[34]. Furthermore, an individual with an αC479R variant displaying a modest Liddle phenotype had BP reduction with an ENaC blocker[19]. Together, these studies provide evidence that ENaC inhibitors lower BP in specific populations and individuals bearing specific ENaC variants.

A number of human gain-of-function variants increase channel activity by suppressing Na^+ self-inhibition, and it is likely that additional human gain-of-function variants will be identified[9]. Future studies will determine whether hypertensive individuals with gain-of-function ENaC variants, identified through genetic and functional screens, respond to ENaC blockers with improved BP.

ENaC's role in salt-sensitive rodent models

While genetic studies have shown ENaC's association with salt-sensitive hypertension in certain groups, animal studies and cell culture data provide mechanistic insight into why ENaC may facilitate salt-sensitive hypertension. In a comparison of salt-resistant versus salt-sensitive rat strains, renal ENaC showed dysregulation with high salt diet (HSD). While Na^+-loaded salt-resistant strains displayed decreased ENaC in accordance with lower aldosterone, Dahl salt-sensitive rats (Dahl-SS) had a paradoxical increase in ENaC mRNA and protein as well as increased ENaC activity[35,36]. Dahl-SS rats experienced increased BP within the first week of HS. This was attenuated by amiloride or benzamil, suggesting the increased ENaC expression was, in part, responsible for the salt-induced hypertension[35, 36]. Additionally, while Dahl-SS rats had low renin levels with HSD, there was a paradoxical activation of the mineralocorticoid receptor (MR), demonstrated by increased expression of the downstream target Sgk1[37]. This hormone-independent activation was shown to be mediated by the small GTPase Rac1, allowing for increased ENaC expression without higher aldosterone[38]. These observations suggest that ENaC activation through dysregulation of MR signaling contributes to salt-sensitive hypertension in Dahl-SS rats[39]. Rac1 also serves as a structural unit of NADPH oxidases (NOX), protein complexes responsible for generation of reactive oxygen species (ROS)[39]. ROS levels have been shown to be elevated in salt-sensitive rats and hypertensive patients, and its production, specifically by NOX4, can positively regulate ENaC activity in the kidney, potentially through mechanisms dependent on PI3-kinase or prostaglandins[40] (fig. 1B).

ENaC α- and γ-subunits are activated by cleavage[9]. The serine protease furin cleaves the α-subunit twice as it traffics through the *trans*-Golgi network, releasing an inhibitory tract and transitioning channels from a low- to moderate-activity state. The γ-subunit is cleaved once by furin, and cleavage by another protease, such as prostasin, matriptase, kallikrein, plasmin, or elastase, releases a second inhibitory tract, inducing a high-activity state[9]. While it is still unclear which additional proteases are responsible for cleaving the γ-subunit in the kidney, ENaC proteolysis likely has a role in channel activation in Dahl-SS rats. Dahl-SS rats fed a HSD have enhanced γ-subunit proteolysis, and the serine protease inhibitor camostat mesylate attenuated the rise in BP[35,36].

ENaC proteolysis is seen in other models of Na^+ retention and hypertension, strengthening the idea this process plays a role in salt-sensitivity. Aldosterone administration is associated with ENaC proteolytic processing, channel activation, and renal Na^+ retention[41]. Proteolytic activation of ENaC is also observed in nephrotic syndrome[42]. Altogether, while ENaC proteolytic activation is an important channel regulator, further study is needed to understand how it contributes to salt-sensitivity. Other ENaC regulatory mechanisms, such as palmitoylation, phosphorylation and sensitivity to intracellular and extracellular Na^+ (discussed above) may also influence salt-sensitivity[9]. Additionally, while ENaC has a role in the development of salt-sensitive hypertension in specific settings, other transporters and channels involved in Na^+ or K^+ homeostasis have also been implicated in this phenotype[15], as well as signaling pathways ranging from immune activation, nitric oxide (NO) and ROS production, and mTOR-mediated signaling[3,4]. More work is needed to understand how these signaling pathways implicated in salt-sensitive hypertension may affect ENaC function.

ENaCs contribute to salt-sensitive vascular dysfunction

Salt-mediated dysfunction extends beyond the kidney. Endothelial cells (ECs) are well-known BP regulators, sensing and responding to mechanical stretch and chemical alterations in the blood. ECs translate a mechanical stimulus (i.e., changes in shear stress) to a chemical signal by regulating production of the gaseous vasodilator NO[43]. Numerous studies have observed that high salt intake or increased aldosterone levels lead to vascular dysfunction, defined by decreased NO production, cellular stiffening, and pressure-independent vascular remodeling[44,45]. A growing body of evidence suggests endothelial ENaC (EnENaC) has a role in promoting this dysfunction.

EnENaC expression is regulated by aldosterone[46]. ECs or *ex vivo* vessel preparations cultured with aldosterone and a high [Na^+] developed resistance to mechanical deformation that correlated with increased ENaC surface expression. This was attenuated with the MR antagonist spironolactone or amiloride[47,48]. The cortical actin cytoskeleton, a meshwork of filaments located within 50–200nm of the cell membrane, appears to mediate this stiffening[49]. ENaC influences this cytoskeletal architecture by increasing local [Na^+], which stabilizes polymerized actin and leads to a denser mesh[50]. ENaC may also interact with actin directly to stabilize the network[51]. This stiffening impairs NO production by rendering the cell less sensitive to shear stress[49]. An increase in the filamentous (F) to globular (G) actin ratio also facilitates interactions between actin filaments and endothelial nitric oxide synthase (eNOS), limiting its ability to convert L-arginine to NO[52] (Fig. 2). ENaC inhibition

in isolated rat mesenteric vessels led to higher NO production and increased eNOS phosphorylation at Ser1177 (an activating event), providing further proof that this pathway modulates NO availability and vasodilation[53].

While renal ENaC is inhibited by increases in intracellular or extracellular [Na^+][9], studies in cultured ECs and *ex vivo* vessel preparations suggest that EnENaC is activated by high extracellular (and presumably intracellular) [Na^+][54], a feed-forward activation process. In contrast, *in vivo* studies where rats were fed a HSD showed a decrease in EnENaC activity over the first 7–14 days but a return to normal after 21 days[55]. These results suggest that EnENaC regulation varies with acute versus chronic HSD, and that whole animal studies may have circulating regulatory factors not matched in *ex vivo* studies.

While Na^+ may affect ENaC levels directly, increased Na^+ may also damage the endothelial glycocalyx, a negatively charged layer of glycoprotein polysaccharides that protects ECs and mediates shear stress responsiveness[56]. The heparan sulfate component of the glycocalyx was significantly reduced in the presence of high Na^+ [57], and removal of this in isolated ECs with heparinase resulted in increased intracellular [Na^+] and transendothelial resistance[58]. These observations suggest increased extracellular Na^+ may enhance both EnENaC surface expression and accessibility to extracellular Na^+ (Fig. 2).

Long-term administration of low-dose amiloride suggested that EnENaC is involved in high fat diet induced vascular dysfunction in female mice. Amiloride protected against high fat-induced aortic stiffening and small vessel dysfunction, without altering BP[59]. Studies with endothelial-specific αENaC knockout mice (EC-αENaC KO) support EnENaC's contribution to vascular stiffening, as these mice had a blunted rise in pulse wave velocity with aldosterone when compared to controls[60]. In addition, EC-αENaC KO lacked an aldosterone-dependent reduction in acetylcholine-induced vasodilation seen in controls. While EC-αENaC KOs did show a resistance to vascular stiffening consistent with pharmacological studies, discrepancy exists on the effects of pharmacological versus genetic inhibition of ENaC on acetylcholine-induced NO production. Blocking ENaC with benzamil in mesenteric arteries caused a decrease in acetylcholine-induced NO production, whereas genetic deletion of the α-subunit did not affect NO production[61]. These results were surprising, as previous experiments, discussed above, suggested inhibition of EnENaC should increase NO. Despite this discrepancy, both pharmacological treatment and genetic deletion caused a decrease in flow-mediated vasodilation, suggesting ENaC functions as a mechanosensor in vessels[61,62].

ENaCs are also expressed in vascular smooth muscle (VSM) where they participate in regulating the myogenic response, a flow-induced reaction intrinsic to VSM that controls perfusion and blood flow characterized by vasoconstriction in response to increasing perfusion pressure. In isolated, perfused mouse renal arteries, ENaC inhibition through multiple means abolished pressure-induced vasoconstriction[63,64]. This was also observed in cerebral vessels[65]. However, there is discrepancy regarding which subunits are expressed across these vessels. In mouse renal interlobar arteries, mRNA and protein expression for only the β- and γ-subunits was observed within freshly dissociated cells[63]. All three subunits were found in freshly dissociated rat mesenteric VSM cells, and HSD lowered the

expression of the α- and γ-subunits without affecting overall expression of the β-subunit[66]. While some studies have suggested β- and γ-subunits can form functional channels[67], an intriguing alternative is that β- and γ-subunits are heteromultimerizing with structurally related polypeptides, such as acid-sensing ion channel (ASIC) subunits. Further work is needed to understand if this occurs, and if these heteromultimeric channels are functional. Overall, while ENaC has a role in the myogenic response, additional studies are needed to define the role of VSM ENaC in the development of salt-sensitive hypertension.

Does ENaC in the brain sense Na^+ and contribute to hypertension?

Na^+ levels in the CNS influence BP. In rats, BP increases are observed when cerebrospinal fluid (CSF) [Na^+] is increased. Furthermore, rats given a HSD experience increases in CSF [Na^+] in salt-sensitive strains, but not resistant strains, and this increase precedes BP changes by several days[68]. Central aldosterone infusion also elicits a BP increase[69], and Dahl-SS rats have higher central aldosterone synthesis compared to Sprague-Dawley rats[70]. Moreover, the increase in BP with a HSD can be prevented via intracerobroventricular (ICV) infusion of spironolactone or benzamil, suggesting the MR and ENaC have roles in Na^+-sensing[71].

ENaC is expressed in areas of the brain related to fluid and electrolyte balance, including areas that participate in the control of thirst, Na^+ excretion, blood volume regulation, and vasopressin secretion[72]. These sites also express MR[72] and proteins that modulate ENaC activity (e.g., Sgk1 and Nedd4–2)[73, 74]. ICV infusion of Na^+-enriched artificial CSF produces elevations in hypothalamic aldosterone levels, attributable to local production[75].

Increases in CSF [Na^+] by as little as 2mM lead to increased neuronal firing rates, sympathetic activity, and hypertension[68]. Interestingly, these changes are observed mainly in salt-sensitive versus salt-resistant rats[76]. In magnocellular neurosecretory cells of the paraventricular and supraoptic nuclei, ENaC appears to mediate a Na^+ leak current that affects steady-state cell membrane potential[77]. Baseline ENaC expression within this region was higher in Dahl-SS as compared to Sprague-Dawley rats and increased further with HSD. Additionally, ENaC appeared in dendrites of these neurons only in Dahl-SS, suggesting ENaC could provide the means for abnormally depolarized local membrane potentials that enhance firing and vasopressin release, contributing to salt-sensitivity[78].

Choroid plexus cells uniquely express ENaC on both the basolateral and apical membranes, with the latter expressing channels on small microvilli which extend into the CSF[79]. In stroke prone versus Wistar Kyoto rats, the choroid plexus displays higher levels of both Sgk1 and ENaC under unstimulated conditions, suggesting baseline MR activation. This increase in expression is enhanced with HSD and presumably allows for greater Na^+ transport into CSF, as [Na^+] increased only in the stroke prone rats[80]. Choroid plexus ENaC activity may determine whether increased dietary Na^+ affects overall CSF [Na^+], influencing whether salt leads to an increase in sympathetic activity.

Studies using ENaC inhibitors provide evidence of ENaC's role in the central response to HSD, and that the channel can perhaps sense small increases in the extracellular [Na^+]. How ENaC affects signaling pathways in response to small increases in CSF [Na^+] is unclear.

ENaC currents mediated by αβγ channels saturate at a [Na^+] well below 140mM[81]. As ENaCs with non-traditional subunit compositions lack this saturation, it will be important to define the subunit composition and behavior of ENaCs at different CNS sites. Inhibitors can have off-target effects, and rodent models with genetic manipulation of ENaC in specific neural regions are needed to confirm ENaC's role in CNS Na^+-sensing.

ENaCs expressed in immune cells sense Na^+ and contribute to inflammation and hypertension

Dendritic cells (DCs) classically process and present antigens within the grooves of major histocompatibility complexes (MHCs) classes I and II. A novel activation of DCs by high salt has recently been described, where Na^+ enters DCs through ENaC. An increase in the intracellular [Na^+] facilitates calcium influx via the Na^+-Ca^{2+} exchanger, leading to activation of protein kinase C and NADPH oxidase, an increase in superoxide, and protein modification by ketoaldehydes, termed isolevuglandin (isoLG)-protein adducts. These isoLGs incite an autoimmune-like reaction with release of cytokines and an increase in BP[82, 83] (Fig. 3).

The initial Na^+ influx appears to be mediated by ENaC formed by α- and γ-subunits, as β-subunits are not expressed in DCs[82], but δ-subunits may contribute to functional channels in human antigen presenting cells. Sgk1 is expressed in DCs and promotes subunit assembly under conditions of high Na^+ [84]. When DCs primed with high salt are transferred to a naïve animal, hypertension in response to suppressor doses of angiotensin II develops, suggesting that the inflammatory state produced by high salt activation of DCs is sufficient to promote a hypertensive phenotype, and that immune cell ENaC activation has a role in the development of salt-sensitive hypertension.

Exposure of B and T lymphocytes to a high extracellular [Na^+] also drives a pro-inflammatory phenotype[85, 86], characterized by increased IL-17A that promotes renal and vascular dysfunction[87]. While ENaC expression has been shown in these cells, the roles of ENaC and other Na^+ transporters in mediating Na^+ entry have not been determined. Therefore, further work is needed to understand ENaC's function across different lymphocyte populations.

Conclusions

Guyton's seminal works described hypertension as a disease of disturbed volume balance and altered pressure-natriuresis response. In this model, ENaC was a perfect culprit for promoting hypertension, given that increased Na^+ reabsorption through the channel would lead to increased extracellular volume and ultimately require an increased BP set point to restore volume homeostasis. However, our view of blood pressure control has evolved to include a role for multiple organ systems, in parallel with a growing knowledge of ENaC's expression in non-epithelial tissues. In addition to its role in the kidney, ENaC acts as a Na^+ transporter and a Na^+-sensor and/or mechanotransducer in neurons, ECs, VSM and DCs. This positions ENaC as a player in multiple aspects of salt-sensitive hypertension, including

inflammatory cytokine release, vascular stiffening, and central activation of sympathetic tone.

Given its involvement in promoting Na^+-induced dysfunction across a variety of cell types, ENaC is an attractive pharmacological target for combatting the adverse BP effects of a HSD. While amiloride is an ENaC inhibitor used as a diuretic in the clinical setting, only low doses are needed to reach sufficient concentrations in the lumen of the nephron, preventing therapeutic levels from being achieved in the circulation. Further work is needed to develop ENaC inhibitors that selectively target the channel in non-epithelial cells. The adverse anti-kaliuretic effects of ENaC blockers that limit their therapeutic use must be overcome. While more work is needed to understand the channel's role in normal and pathophysiologic states in non-epithelial tissues, the combined results of the studies detailed here strongly support ENaC's role as a mediator of Na^+-sensitive hypertension.

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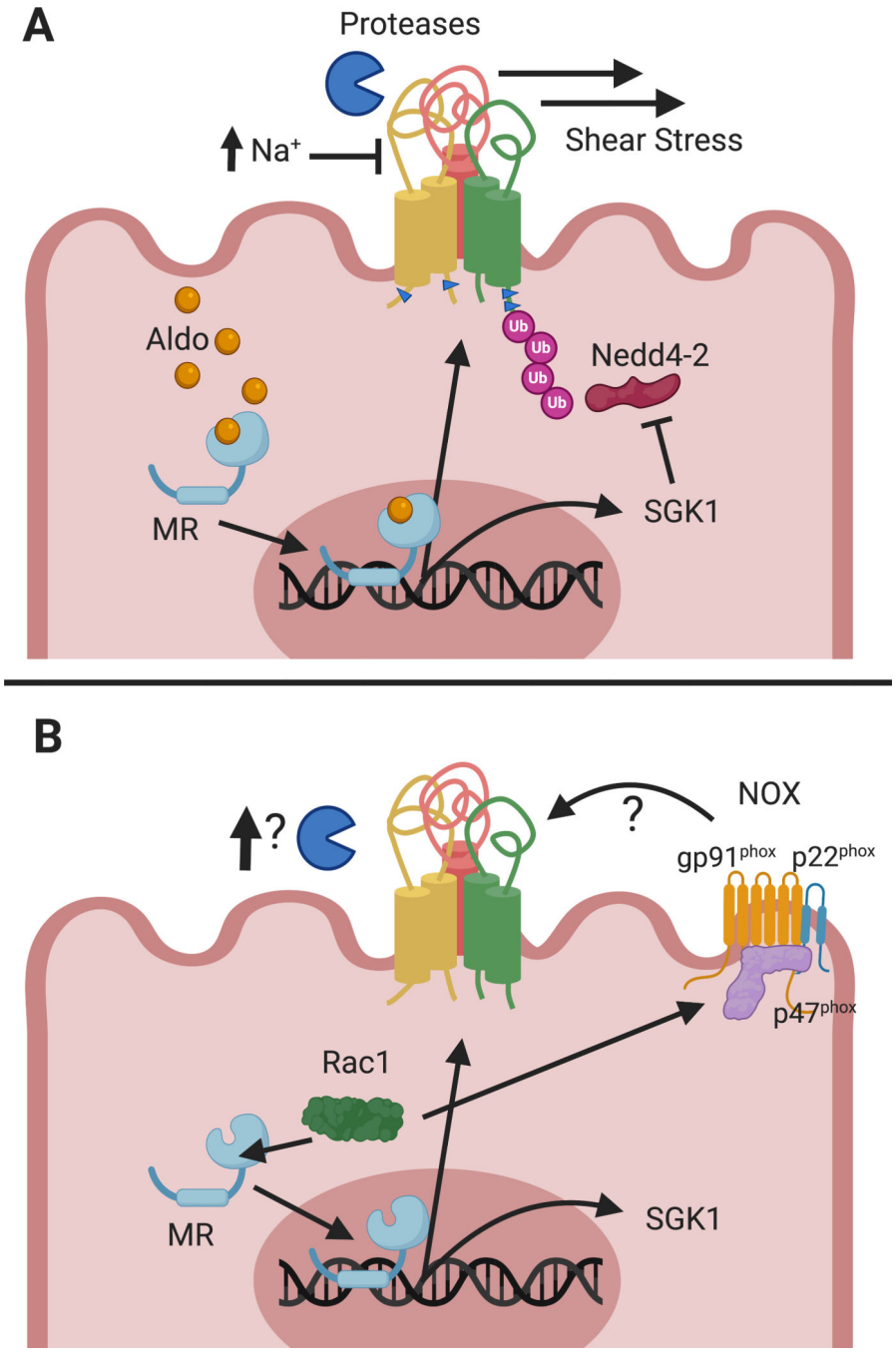

Figure 1. Regulation of ENaC in the normal and salt-sensitive kidney

The top panel, A, illustrates several mechanisms responsible for ENaC regulation in the kidney. ENaC open probability and activity is regulated by shear stress, proteolytic cleavage, palmitoylation (indicated by blue cytoplasmic arrowheads), and extracellular [Na^+]. Nedd4–2 facilitates ENaC ubiquitination and endocytosis. Aldosterone activates MR, increasing transcription of αENaC and the regulatory kinase Sgk1. However, in the salt sensitive kidney, panel B, MR is activated by the GTPase Rac1, independent of aldosterone. Rac1 also increases ROS production via NOX, which activates ENaC. Increased proteolytic activation

of ENaC has also been reported, however, further work is needed to establish which proteases may be responsible.

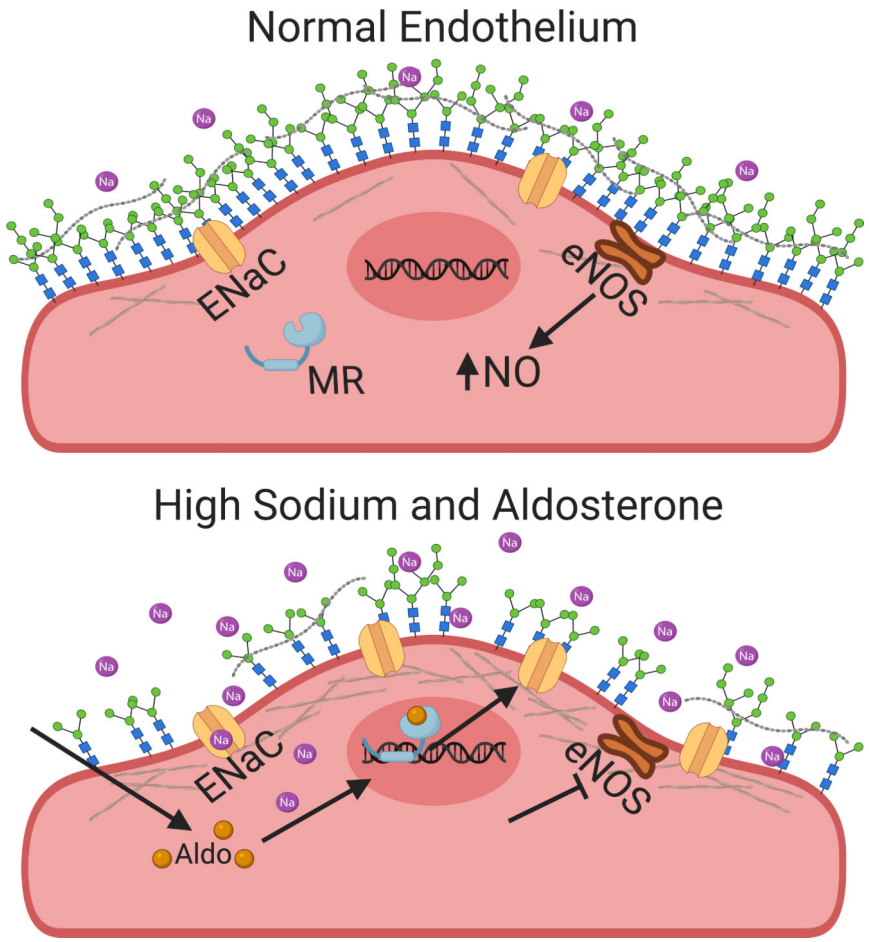

Figure 2. EnENaC limits NO production in the setting of high [Na^+] and aldosterone

ECs have a thick surface glycocalyx that limits the availability of Na^+ for ENaC-dependent Na^+ transport. The glycocalyx also enhances the endothelial response to shear stress, inducing an increase in intracellular Ca^{2+}, activation of eNOS, NO production and vasodilation. Increased extracellular [Na^+] leads to glycocalyx breakdown and enhanced Na^+ entry via ENaC. An increase in the intracellular [Na^+] stabilizes F-actin filaments, increasing the density of the cortical cytoskeleton and limiting cellular deformation. This events inhibit eNOS and NO production, leading to vascular dysfunction. Activation of MR by aldosterone in ECs increases ENaC expression, stabilizing the cytoskeleton and reducing deformability.

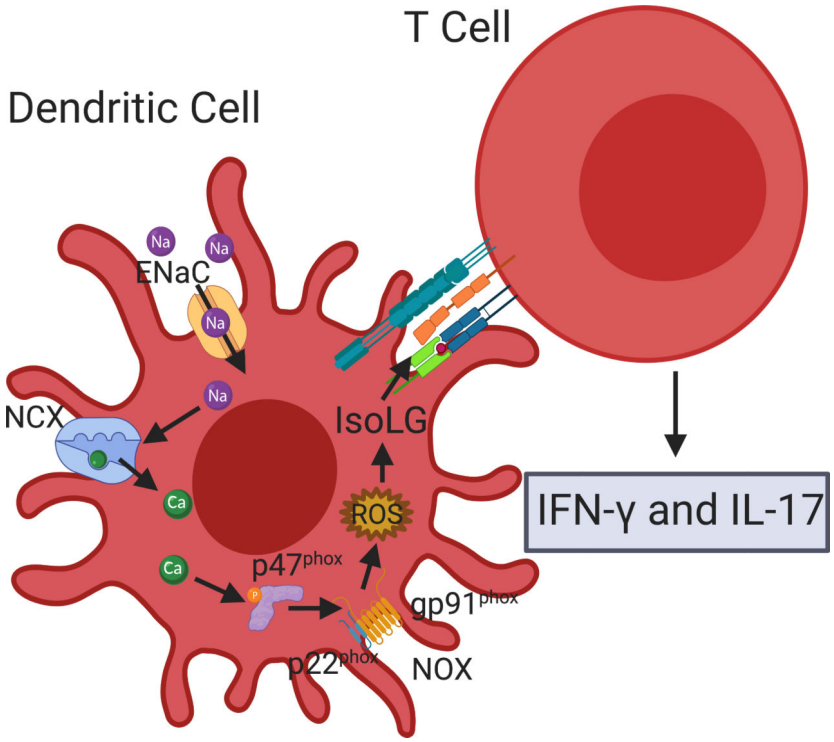

Figure 3. ENaC-dependent Na^+ transport promotes DC activation and inflammation

ENaC-dependent Na^+ transport in DCs triggers an influx of Ca^{2+} via the Na^+-Ca^{2+} exchanger (NCX), leading to phosphorylation of the p47phox subunit of NOX, facilitating NOX assembly and production of ROS, and creation of IsoLG adducts. DCs with IsoLG-modified surface proteins activate T cells, leading to release of pro-inflammatory cytokines interferon gamma, and IL-17[82].