JGP Journal of General Physiology

PERSPECTIVE

Unsolved problems with ENaC: From molecules to mammals

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The epithelial Na channel (ENaC) mediates transport of Na^+ in the kidney, the colon, the lung, and the sweat and salivary ducts. The activity of these channels is highly regulated and contributes to the maintenance of total body Na^+, extracellular fluid volume, and plasma K^+. Defects in the channel itself or in its regulation are implicated in hypertension and in other diseases. Despite progress in revealing many aspects of ENaC biology, many important questions remain unanswered. These include the following: What determines the remarkable ion selectivity of the channel? How is the channel directed to the apical plasma membrane? How does aldosterone regulate the channel? What is its role in producing urine with minimal Na^+? And to what extent does the channel contribute to essential hypertension, cystic fibrosis, nephrotic syndrome, and liver failure? Here, I will discuss these questions and how they relate to our current understanding of the roles of ENaC in health and disease.

Historical introduction

In Koefoed-Johnsen and Ussing (1958) recognized that Na^+ crossed the apical plasma membrane of the frog skin through a conductive mechanism. This was based on the simple but profound observation that changes in transepithelial voltage changed when the Na^+ concentration on the apical, but not the basolateral, side was varied. The nature of this mechanism remained only vaguely understood until 1977, when Lindemann and Van-Driessche (1977) undertook noise analysis of transepithelial currents and concluded that they involved electrodiffusion through pores, based on the high rate of transit through individual conducting units. A more detailed description of single-channel properties followed with the application of the patch-clamp technique to epithelial cells (Hamilton and Eaton, 1986; Palmer and Frindt, 1986). Cloning of the channel subunits in 1993 and 1994 (Canessa et al., 1993; Canessa et al., 1994b) led to a molecular understanding of the channel protein and gave rise to the nomenclature of epithelial Na channel (ENaC). Most recently, application of crystallographic and cryo-electron microscopy (cryo-EM) revealed the atomic structure of the closely related ASIC (Jasti et al., 2007) and finally ENaC itself (Noreng et al., 2018).

We have gathered a lot of information regarding channel structure, function, and regulation, as well as its role in homeostasis and in disease, which has been summarized in recent reviews (Kellenberger and Schild, 2015; Rossier et al., 2015; Kleyman and Eaton, 2020; Pearce et al., 2022). In this Perspective, I will not attempt a comprehensive coverage of the literature but will focus on several topics that are particularly interesting and highlight what we still do not know about ENaC. The choices reflect some personal biases. Some reveal places in which the field has been stuck for some time and will require new approaches and ideas to move forward.

Selectivity

ENaC has remarkable ion selectivity. The ability of the channel to conduct Na^+ and not K^+ ensures that Na^+ can be transported into the cell without loss of K^+, as electrochemical driving forces will move the ions in opposite directions. This is especially important in organs like the frog skin, where K^+ moving out of cell would be lost into a large volume of pond water. Epithelia such as the distal tubule of the kidney and the colon use this driving force to secrete K^+. Here, the high selectivities of both ENaC and apical K^+ channels allow separate regulation of the two ions.

Na^+ and Li^+ are the only ions that can pass through ENaC at readily measurable rates (Palmer, 1987). Although H^+ ions appear to have a high permeability through the channels, currents are small due to the low proton concentrations in relevant biological fluids. Currents carried by K^+ are difficult to measure, and the Na^+/K^+ conductance ratio was estimated to be at least 500. Furthermore, ions such as NH_4OH, hydrazinium, and guanidinium, which can pass through voltage-gated channels (Hille, 1971), are excluded from the ENaC pore.

Based on these findings, we proposed that ENaC could act as a molecular sieve, excluding ions with an unhydrated diameter of >2 Å (Palmer, 1987) (Fig. 1). An alternative scheme that would involve strong, selective binding of Na^+ within the pore would predict a high affinity for Na^+ ions, whereas the measured K_m

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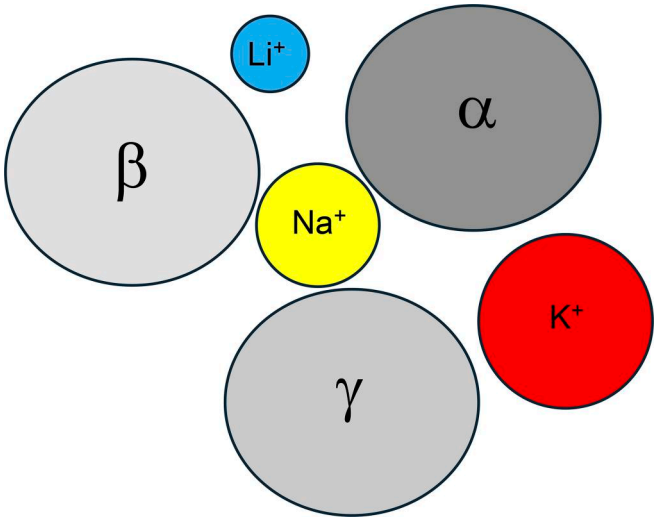


Figure 1. **Schematic view of the putative selectivity filter of ENaC.** The transmembrane segments of the α, β, and γ subunits form a constriction in the pore that allows passage of small, dehydrated ions (Na^+, Li^+) but is too small for K^+ or larger cations.

values for single-channel currents are 30 mM or higher (Van-Driessche and Lindemann, 1979; Palmer and Frindt, 1988). The tight-binding model also predicts substantial K^+ currents in the absence of Na^+, which were not observed.

Studies of the cloned channel in *Xenopus* oocytes supported the sieving idea (Kellenberger et al., 1999). A putative selectivity-filter region containing three critical consecutive amino acids in the predicted C-terminal transmembrane helices of the subunits (α = GSS, β = GGS, γ = SCS) was identified through site-directed mutations in the αENaC sequence. So far, there is no direct evidence that these residues line the narrowest part of the pore; although cryo-EM showed the extracellular aspects of ENaC at high resolution, the transmembrane aspects were not well resolved (Noreng et al., 2018).

In the related ASIC1 channels, higher resolution crystal structures have been obtained (Jasti et al., 2007; Baconguis et al., 2014). The second transmembrane helix (TM2) of ASIC1 exhibits a break at the GAS sequence corresponding to the proposed ENaC selectivity filter; the outer segment of the helix of one subunit joins the inner segment of an adjacent subunit in the closed state but joins its own inner segment in the open state (Cahill et al., 2026). This rearrangement is intriguing although its relevance to ENaC selectivity is unclear. Lynagh et al. used mutagenesis and molecular dynamics (MD) simulations to argue that selectivity in ASIC is determined at a more cytoplasmic part of TM2 (Lynagh et al., 2017). However, ASICs are much less selective than ENaC, and the two channels may use different selectivity filters (Yang and Palmer, 2018).

Assuming this part of the protein does form the filter, Na^+ would need to interact with these residues to replace the hydration shell surrounding the ion in bulk solution. The absence of negatively charged side chains eliminates the types of cation–carboxyl interactions known to participate in selectivity in voltage-gated Na^+ (Payandeh et al., 2011) and Ca^{2+} (Sather and McCleskey, 2003) channels and in ASIC channels (Lynagh et al., 2017). The polar side chains of serines found in the selectivity-filter motif of all three subunits could be involved, although the critical αS589 can be substituted with some nonpolar amino acids without disrupting selectivity. It is also possible that carbonyl oxygens could selectively stabilize Na^+ ions in the pore similar to the architecture of K^+ channels (Doyle et al., 1998).

ENaC selectivity is a fundamental unsolved problem in membrane biology and ion transport. To address it, we will need structures that include atomic-level resolution of the transmembrane pore, preferably in both open and closed states. The required biochemical studies with ENaC are challenging since three different subunits need to be combined, and it is unclear how the state of the protein can be controlled or recognized. MD simulations will also be required for a deep understanding of Na^+ permeation and selectivity. The combination of experimental and theoretical information may be just a start; even with high-resolution structures of the K^+ channel selectivity filter and extensive MD calculations, aspects of K^+ selectivity remain uncertain (Roux, 2017).

Apical sorting

ENaC is a prototypical apical membrane protein. As first described by Koefoed-Johnsen and Ussing (1958), the apical membrane of the frog skin contains a Na^+-selective conductance, while the basolateral membrane is selective for K^+. This asymmetry is essential for the Na^+ transport function of the epithelium. Despite now knowing the molecular underpinning of this conductance, we still do not have a clear picture of why ENaC itself is targeted selectively, if not exclusively, to the apical and not the basolateral surface of the cell.

This gap in understanding is not specific to ENaC. Although "sorting signals" based on amino acid sequences such as the tyrosine-based (NPxY) or dileucine motifs can target proteins to the basolateral membrane, equivalent sequences for apical localization have not been identified and apical sorting uses other mechanisms (Schuck and Simons, 2004; Rodriguez-Boulan and Macara, 2014; Stoops and Caplan, 2014). Glycolipid-anchored proteins are predominantly found in apical membranes, but this GPI-anchored targeting mechanism is not directly relevant to intrinsic membrane proteins such as ENaC.

Glycosylation has also been linked to apical localization of some membrane proteins (Rodriguez-Boulan and Macara, 2014; Stoops and Caplan, 2014). This mechanism involves the binding of lectins such as galectin-3 to sugars on secreted proteins or in the ectodomain of transmembrane proteins in the trans-Golgi network (TGN) and endosomal compartments (Fig. 2). How this leads to selective apical trafficking is not well understood, but lectin-mediated clustering of cargo has been implicated in apical sorting (Weisz and Rodriguez-Boulan, 2009). ENaC is a good candidate for using this scheme because its subunits are extensively glycosylated in patterns that switch from EndoH-sensitive to EndoH-resistant as the channels move through the Golgi apparatus (Hughey et al., 2003; Ergonul et al., 2006).

Membrane polarity can also be established in part by the plasma membrane lipid composition (Rodriguez-Boulan and Macara, 2014; Stoops and Caplan, 2014). Lipid "rafts" with a high

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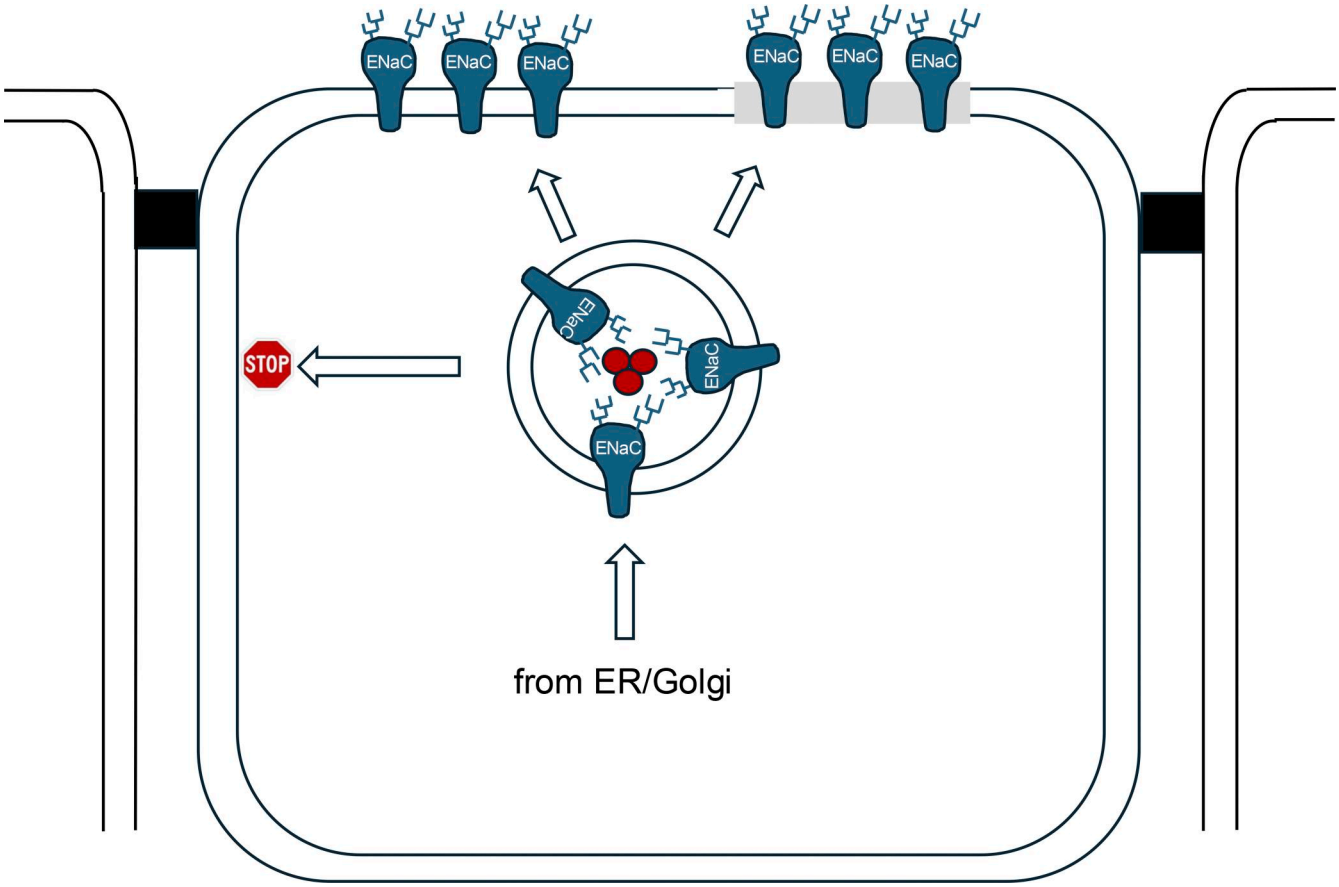


Figure 2. **Possible signals for the selective apical expression of ENaC.** Glycosylation of the extracellular domains of the subunits may interact with galectin-3 (red shapes) in apical sorting endosomes, clustering channel proteins, and guiding them to the apical membrane but not the basolateral membrane (stop signal). Lipid rafts (gray area) in the apical membrane may also serve to sequester ENaC in the apical bilayer.

cholesterol content and low lipid mobility are preferentially found in the apical membrane. Any membrane protein that preferentially partitions into these rafts will tend to be sequestered apically. There is evidence that ENaC does associate at least to some extent with lipid rafts in a collecting duct cell line (Hill et al., 2007). Furthermore, ENaC activity in several systems correlates positively with changes in membrane cholesterol (Hill et al., 2007; Awayda et al., 2011; Zhai et al., 2019). Whether sequestration in rafts is necessary or sufficient for apical sorting of ENaC is unknown.

Defining the principles guiding the apical sorting of ENaC would be a breakthrough in understanding not only of ENaC but also of epithelial Na^+ transport and epithelial organization and function. This will be a difficult, given that the sorting signals could reside in any of three different subunits and be situated in the cytoplasmic, transmembrane, or external aspects of the protein. The role of glycosylation in establishing the apical location of ENaC is in principle testable by mutating asparagines at consensus N-linked glycosylation sites in the extracellular domain, but the task is daunting as there are 23 of these collectively within the three subunits (Canessa et al., 1994a). Testing for possible sequestration of the channels in lipid rafts requires a better understanding of the lipid composition of the apical membrane of ENaC-expressing cells. Techniques for isolating these membranes would help to address this issue.

Better *in vitro* models would also be useful. There are mammalian cell lines that express ENaC, but there are quantitative and qualitative differences with the distal nephron segments from which they are derived. The most successful of these is the mCCDcl1 line (Gaeggeler et al., 2005). Transepithelial Na^+ currents in these cells are on the order of 10 $\mu A/cm^2$ (Gaeggeler et al., 2005; Spirli et al., 2019; Sure et al., 2025), while in a well-stimulated connecting tubule (CNT) or cortical collecting duct (CCD) cell, they can be one to two orders of magnitude higher (Frindt et al., 2022). In addition, while the cell line has been successfully used for RNA expression studies (Loughlin et al., 2023), little information on protein expression is available. Alternatively, more sophisticated *in vivo* models with better labeling of ENaC (Bharadwaj et al., 2026, *Preprint*) could be employed.

Regulation

ENaC is probably the most highly regulated ion-transport protein in the kidney; along with aquaporin-2, it is perhaps the most highly regulated renal transporter of anything. It controls the movement of two ions between tubular and extracellular

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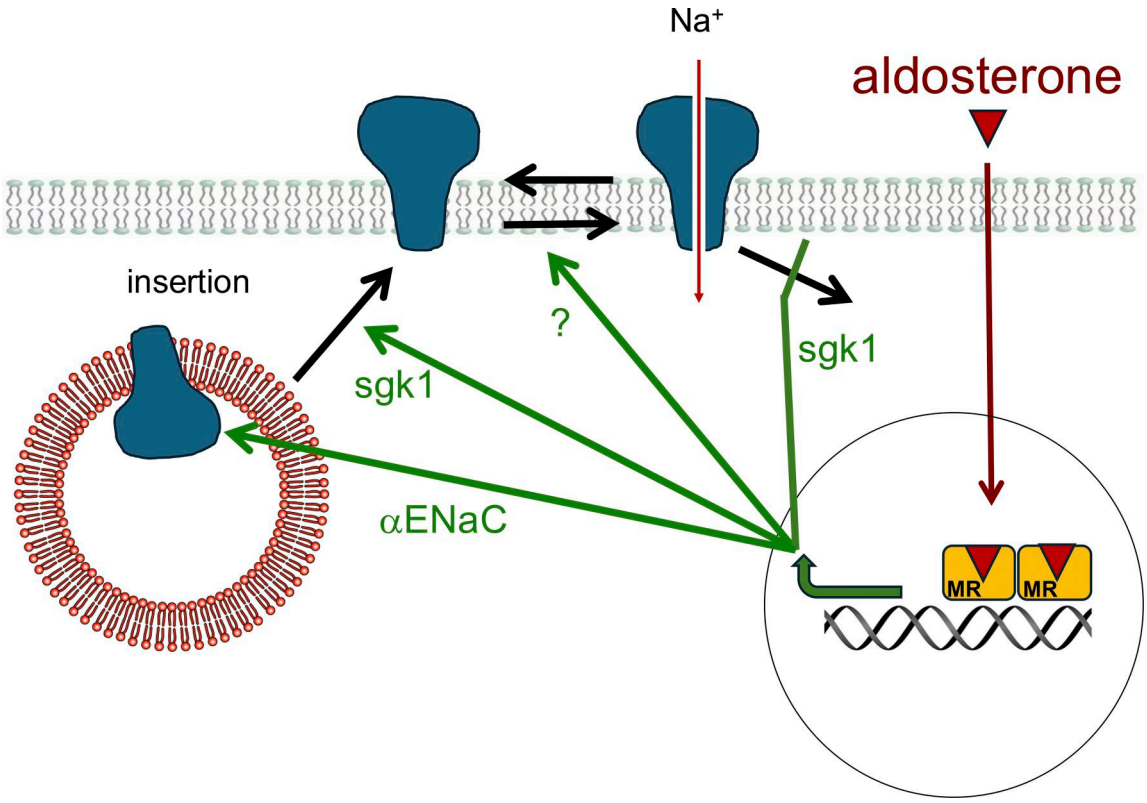


Figure 3. **Regulation of ENaC by aldosterone.** Aldosterone binds to and activates MRs, leading to altered gene transcription. This increases (1) the overall abundance of channel subunits, particularly that of αENaC, (2) the surface expression of ENaC through SGK1-dependent stimulation of forward trafficking of the assembled protein to the apical membrane and inhibition of its retrieval and degradation, and (3) the P_o of channels at the cell surface.

fluids—Na^+ reabsorption directly and K^+ secretion indirectly via increased electrical driving forces across the apical membrane. These processes are physiologically important for maintaining extracellular fluid volume, blood pressure, and extracellular K^+ concentrations.

Aldosterone is the most important factor regulating ENaC. This hormone participates in the control of both extracellular fluid volume and plasma K^+, as both hypovolemia and hyperkalemia promote its secretion. The pathways involved are complex; they entail changes in the processes of transcription and translation of channel subunits, protein trafficking, and channel activation (Fig. 3).

Transcription

Aldosterone increases the abundance of mRNA for αENaC in the kidney, while that for βENaC and γENaC is not strongly affected (Asher et al., 1996; Farman et al., 1997; Stokes and Sigmund, 1998). How the hormone stimulates αENaC transcription is uncertain. Thomas and colleagues reported evidence for a glucocorticoid response element in the cis-regulatory region of the gene that mediates effects of both aldosterone and glucocorticoids (Mick et al., 2001). Zhang et al. proposed an alternative mechanism in which αENaC transcription is derepressed by aldosterone-dependent repression of chromatin-associated proteins Dot1a and Af9, and by inactivation of Af9 through phosphorylation of the aldosterone-induced protein SGK1 (Zhang et al., 2007). These actions are not mutually exclusive.

The regulation of ENaC subunit transcription is remarkably tissue-dependent. In the lung, the pattern is similar to that of the kidney, with steroid regulation of αENaC but not βENaC or γENaC, but the lung response is specific to glucocorticoid administration (Stokes and Sigmund, 1998). This is presumably because the lung has glucocorticoid receptors and not mineralocorticoid receptors (MR) and lacks 11β-HSD, an enzyme that metabolizes cortisol to cortisone in the kidney, preventing occupancy of MR by the more abundant glucocorticoids. However, in the colon, another epithelium that responds to aldosterone, the situation is reversed with αENaC expressed constitutively while βENaC and γENaC are induced by the hormone (Asher et al., 1996; Stokes and Sigmund, 1998). This difference was reported decades ago, and yet, we still do not have an explanation for how the same hormone binding to the same receptor stimulates transcription of the same channel subunits so differently. The mechanism presumably involves interactions with additional unknown transcription and/or coregulatory factors.

Trafficking

In 2000, Loffing et al. used immunocytochemistry to determine that the location of ENaC within the cell depends on dietary Na^+ (Loffing et al., 2000). When Na^+ intake is high, βENaC and γENaC are expressed in a diffuse pattern throughout the cytoplasm, and αENaC is undetectable. When Na^+ intake is low, the same subunits, together with the now visible αENaC, move toward the apical pole of the cell and presumably into the apical

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membrane. The redistribution was also documented in animals treated with exogenous aldosterone (Loffing et al., 2001).

The compartments where the channels reside under these different conditions have not been identified. However, Western blots showed that apical migration of channel protein correlated with proteolytic cleavage of αENaC and γENaC (Masilamani et al., 1999; Ergonul et al., 2006), a process that depends in part on furin, presumably in the TGN (Kleyman et al., 2009). It also correlated with the acquisition of EndoH-resistant glycosylation (Ergonul et al., 2006), which occurs in the median Golgi. Thus, under Na^+-replete (low-aldosterone) conditions the channels likely reside in the ER and/or cis-Golgi, while under Na^+-depleted (high-aldosterone) circumstances, they are probably in post-Golgi compartments including apical sorting and recycling endosomes and the apical plasma membrane itself. Measurements in membrane vesicles isolated using immunoaffinity and in plasma membrane fractions isolated using biotinylation support this picture (Frindt et al., 2016). Both kinetic and steady-state assays suggested that the transition from cytoplasmic to apical distributions results from stimulation of the rate of movement of channels from the former to the latter compartments (Frindt et al., 2016; Frindt et al., 2025).

The immediate signals controlling this trafficking event are unknown. It could involve a posttranslational modification of the channel protein, association with different chaperone proteins, or both. A more detailed understanding will require better methods for identifying binding partners for ENaC under different physiological conditions. So far, antibodies that interact with the subunits have not been useful in immunoprecipitation assays.

Retrieval of ENaC from the apical membrane is thought to involve ubiquitination, particularly of the α and γ subunits (Staub et al., 1997; Staub et al., 2000), offering another point for hormonal control. Aldosterone induces the serine/threonine kinase SGK1, which can phosphorylate and deactivate the ubiquitin ligase Nedd4-2 (Debonneville et al., 2001). This mechanism would operate by slowing the degradation of the channels rather than speeding up the maturation process.

To what extent ENaC is recycled back to the apical membrane after retrieval is also an open question. Work with a heterologous expression system (Lu et al., 2007) and with a cell line derived from the collecting duct (Butterworth et al., 2005) revealed recycling under conditions of elevated cAMP. However, there is little information on this process in native epithelial cells or on its potential regulation by aldosterone.

Open probability

The initial characterization of single-channel currents through ENaC in the kidney showed a remarkable range of channel open probability (P_o) from ∼0.05 to ∼0.95 for ostensibly the same conditions (Palmer and Frindt, 1986). The physical basis for these variations remains entirely unknown; current cryo-EM structures lack resolution of the ion conduction pathway that might reveal sites at which the pore geometry might be regulated (Noreng et al., 2018). It is clear, however, that there is ample opportunity for channel regulation through changes in gating.

Many factors can affect P_o. These include internal signals such as pH (high pH activates P_o [Palmer and Frindt, 1987]), ang II signaling (activates P_o [Mamenko et al., 2012]), and purinergic signaling (inhibits P_o [Pochynyuk et al., 2008]); physical factors such as shear stress (activates P_o [Morimoto et al., 2006]) and membrane hyperpolarization (activates P_o [Palmer and Frindt, 1996]); and external factors including extracellular Na^+ (inhibits P_o [Sheng et al., 2007]) and bile acids (activate P_o [Wiemuth et al., 2014; Ilyaskin et al., 2016; Wang et al., 2022b]). The roles of extracellular factors have been recently reviewed (Kleyman and Eaton, 2020; Kashlan et al., 2024). How these signals are transferred to the pore of the channel to modulate the open and closed states is unknown and will require higher resolution structures for a fuller understanding.

We did not observe any consistent changes in P_o in rat CCDs from rats with different concentrations of plasma aldosterone (Pácha et al., 1993), despite large effects on the number of conducting channels. A caveat was that at the lowest hormone levels, channel activity was undetectable, making it impossible to distinguish very low channel density from very low P_o. A subsequent study of mouse CCD revealed basal channel activity with low P_o (∼0.1), which increased to ∼0.4 after treatment with DOCA, a MR agonist (Mamenko et al., 2012). Aldosterone also increased P_o in the amphibian A6 cell line (Kemendy et al., 1992). It therefore seems likely that aldosterone can upregulate ENaC in part through increasing P_o, from low or near-zero starting points.

Several pathways have been proposed to mediate effects of aldosterone that could impact gating:

a *Methylation.* Eaton and colleagues showed that aldosterone increases methylation of proteins in amphibian epithelial cells (A6) and that methylase inhibitors reduce ENaC activity and P_o (Becchetti et al., 1994; Stockand et al., 1999). These observations have not been extended to mammalian cells.

b *PIP3.* PIP2K activity was shown to be essential for aldosterone-dependent Na^+ transport in amphibian epithelia (Blazer-Yost et al., 1999), and application of PIP3 to inside-out patches prevented ENaC "rundown" (Ma et al., 2002). Infusion of PIP3 to mpkCCD cells through whole-cell patch-clamp pipettes increased ENaC currents through effects that were not additive with that of aldosterone (Staruschenko et al., 2007). However, these actions may affect channel trafficking rather than or in addition to P_o (Helms et al., 2005).

c *K-Ras.* Aldosterone increased the abundance and activity of the small G-protein K-Ras2A in amphibian epithelial cells, and decreasing its expression levels inhibited aldosterone-dependent Na^+ transport (Tong et al., 2004). The study did not distinguish effects on channel density from those on P_o.

d *Subunit cleavage.* As mentioned in the section on regulation of trafficking, the α and γ subunits of ENaC both undergo proteolytic cleavage at two sites in their extracellular domains, enabling the release of inhibitory peptides (Kleyman et al., 2009). αENaC can be cleaved at both sites by furin in the TGN, while γENaC is cut once by furin and once by an extracellular protease, probably Tmprss2 (Hughey et al., 2004; Sure et al., 2025). *In vivo*, cleavage of both subunits is

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enhanced by aldosterone (Masilamani et al., 1999; Ergonul et al., 2006), although the effects of the hormone may reflect translocation of ENaC through the *Golgi* apparatus to the plasma membrane, rather than a direct activation of the proteolytic process itself (Frindt et al., 2016). In expression systems, exogenous proteases increased P_o of channels with low basal activity (Caldwell et al., 2004).

The physiological significance of cleavage is unclear. Mice lacking one of the furin cleavage sites in either or both the α and γ subunits can produce fully active channels, albeit with elevated aldosterone levels in the latter case (Nickerson et al., 2024; Ray et al., 2024). Furthermore, mice lacking renal kallikrein-1 have reduced γENaC cleavage but normal Na^+ and K^+ handling by the kidney (Curry et al., 2026). Sequence analysis suggested that cleavage sites evolved with terrestrial migration of vertebrates (Wang et al., 2022a), but we lack a clear idea of why the system developed in this way. Cleavage could be part of a signaling pathway that has yet to be identified or could be an evolutionary accident.

Which of these pathways, if any, contribute to the increased activity of ENaC in the apical membrane in response to aldosterone is unknown.

Aldosterone-induced genes

We have known for some time that the regulation of epithelial Na^+ transport by aldosterone works primarily through binding to MRs and activation of gene transcription (Edelman et al., 1963). The search for aldosterone-induced (or repressed) proteins that are key to regulating ENaC has made some progress but is far from complete. A few candidates are listed below.

αENaC

As mentioned, ENaC mRNA and protein abundance can be increased by aldosterone, but which subunits are involved and the mechanisms of enhanced transcription depend on the target cell. A quantitative analysis of the ENaC protein in the kidney indicated that under basal conditions, αENaC protein content is lower than that for βENaC and γENaC (Frindt et al., 2022), suggesting that αENaC might be rate-limiting for channel assembly. However, increases in αENaC content alone are not sufficient to increase channel surface expression or activity (Frindt and Palmer, 2012). At least in the kidney cortex, transcriptional regulation may serve only an auxiliary function. Whether increased transport in the colon can be explained by higher levels of β and γENaC is unknown.

Sgk1

The serum- and glucocorticoid-activated kinase *Sgk1* was identified as an aldosterone-induced gene in cultured kidney cells (Chen et al., 1999; Náray-Fejes-Tóth and Fejes-Tóth, 2000). This finding has been confirmed in native kidney tissue using microarrays and RNAseq technology (Fakitsas et al., 2007; Poulsen et al., 2018; Swanson et al., 2019; Loughlin et al., 2023). The kinase increases channel activity when co-expressed with ENaC subunits (Chen et al., 1999; Náray-Fejes-Tóth et al., 1999), and has been implicated in both the upregulation of αENaC transcription (Zhang et al., 2007) and ENaC trafficking (Debonneville et al., 2001; Yang et al., 2017). Mice lacking *Sgk1* have defects in electrolyte homeostasis. They can achieve Na^+ balance on a low-Na^+ diet, and K^+ balance on a high-K^+ diet, but only with higher levels of aldosterone and, in the latter case, plasma K^+ (Wulff et al., 2002; Huang et al., 2004).

Sgk1-null mice exhibit strongly blunted aldosterone-dependent cleavage and surface expression of ENaC (Yang et al., 2017). However, increases in ENaC activity measured in CCDs *ex vivo* were not significantly different from those of WT animals (Fejes-Tóth et al., 2008; Yang et al., 2017). The kinase appears to be an important player in response to aldosterone, but it is not indispensable.

Gilz

The glucocorticoid-induced leucine zipper protein GILZ is another product of a gene that can be upregulated by either glucocorticoids or mineralocorticoids (Soundararajan et al., 2005). In expression systems, it can increase ENaC activity, likely through suppression of extracellular signal–regulated kinase signaling (Soundararajan et al., 2005). However, *Gilz*-knockout mice have a phenotype suggesting that it has more important effects on NCC activity in the distal convoluted tubule (DCT) than on that of ENaC (Rashmi et al., 2017). Its role in regulating ENaC through aldosterone *in vivo* is still unclear.

Usp2

ENaC can be ubiquitinated by the E3 ubiquitin protein ligase Nedd4-2 and targeted to proteasomes (Staub and Verrey, 2005). The ubiquitination can be reversed by the enzyme USP2-45, whose mRNA levels increase with aldosterone (Fakitsas et al., 2007). This suggests a mechanism in which the hormone upregulates ENaC protein levels by inhibiting its degradation. However, knockout of this gene in mice does not produce measurable defects in electrolyte balance (Pouly et al., 2013). It therefore does not appear to be an essential aldosterone-induced protein.

Other candidates

Microarrays and RNAseq technology have identified many genes that are regulated by aldosterone and/or physiological perturbations such as a low-Na^+ diet. For example, 22 genes were affected in mouse CNT by acute administration of aldosterone (Fakitsas et al., 2007). 18 genes were affected in mouse CNT/CCD by both dietary Na^+ restriction and chronic aldosterone administration (Poulsen et al., 2018; Swanson et al., 2019). In the mCCDcl1 cell line that retains responsiveness to steroids, nine transcripts were regulated by aldosterone (Loughlin et al., 2023). These are likely to be minimum estimates of aldosterone-dependent genes. The lists have some members in common but many that are not. Most of these genes have no obvious connection with ENaC or electrolyte balance; in a few cases such as Zbtb16 (Náray-Fejes-Tóth et al., 2008) and microRNAs (Butterworth, 2021), the induced genes correlate with a decrease in Na^+ transport.

No gene has been identified as essential for aldosterone regulation of ENaC. It is possible that we have missed key targets

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and that improved screening techniques may reveal them. Another explanation, however, is that many genes make small contributions to the overall response to the hormone. A small increment in transport could be difficult to detect in functional assays, but many of them could have additive or synergistic effects that combine to form a robust response.

Better *in vitro* models would also be helpful in sorting out these issues. The mCCDcl1 line discussed above responds to aldosterone and has been used for gene expression studies (Loughlin et al., 2023). One significant difference with the kidney *in vivo* is that the cells also increase transport in response to glucocorticoids (Gaeggeler et al., 2005; Loughlin et al., 2023). This recapitulates the properties of the mammalian colon (Will et al., 1985) but not the kidney (Frindt and Palmer, 2012).

Role in homeostasis

In model epithelia such as frog skin and toad urinary bladder, the function of ENaC is to assimilate and preserve Na^+. Frogs use the channels to take up Na^+ from pond water with concentrations of 1 mM or less (García Romeu et al., 1969). In toads, the urinary bladder stores water and ENaC can both limit amounts of Na^+ lost to excretion and dilute the urine to enable water reabsorption under the control of ADH orthologs (Bentley, 1958).

Textbooks generally assign ENaC and aldosterone the roles of fine-tuning of urinary Na^+ and K^+ excretion and maintaining overall balance of both these electrolytes in terrestrial mammals. ENaC promotes Na^+ conservation directly by mediating reabsorption in the distal parts of the renal tubule. At the same time, the voltage in the tubular lumen becomes more negative, enhancing K^+ secretion. Regulating both ions independently involves in part the separate regulation of secretory apical K^+ channels (Palmer, 1999). Furthermore, inhibition of reabsorption in more proximal segments such as the DCT (Castañeda-Bueno et al., 2022) or proximal tubule (Wang et al., 2023) shifts Na^+ transport from electroneutral transport with simultaneous reabsorption of anions to maintain electroneutrality, to those that effectively exchange Na^+ for K^+ exchange process. Ultimately, the regulated excretion of K^+ depends on Na^+ movement through ENaC; activators (e.g., aldosterone) and blockers (e.g., amiloride) of ENaC produce hypokalemia and hyperkalemia, respectively.

The role of ENaC as the final arbiter of Na^+ excretion is less clear. The channels provide the main pathway for Na^+ reabsorption in the CNT and the CCD. In the inner medulla, however, *in vivo* measurements using micropuncture and microcatheterization recorded robust rates of Na^+ reabsorption (Weinstein, 1998) that were only partially inhibited by amiloride (Rocha and Kudo, 1990). Classical micropuncture experiments in rats on a low-Na^+ diet indicated that minimal urine Na^+ concentrations are achieved in the collecting ducts (Malnic et al., 1966). However, knockout of ENaC specifically in the ducts does not prevent the kidneys form minimizing Na^+ excretion (Rubera et al., 2003).

Two different schemes could resolve this dilemma (Fig. 4). First, minimal urine Na^+ could be achieved in the CNTs between the most distal micropuncture site and the beginning of the collecting duct. This scenario requires that very low (1 mM or less) luminal Na^+ concentrations be maintained along the length of the collecting duct. This in turn mandates that leak pathways through paracellular tight junctions have resistances much higher than those that have been measured (Palmer et al., 2026). A second possibility is that in the collecting duct, Na^+ transporters other than ENaC ultimately determine how much Na^+ is reabsorbed from rather than excreted in the urine. The identity of these transporters, if they exist, remains a mystery. Development of imaging techniques to monitor luminal Na^+ along the nephron might help to resolve this dilemma.

Role in diseases

ENaC has been implicated in the etiology of several diseases. In particular, hyperactivity of the channels may contribute to high blood pressure and to the symptoms in nephrotic syndrome liver failure and cystic fibrosis.

Hypertension

Hypertension is an important health issue, affecting over 1 billion people worldwide (Farrar and Frieden, 2025). It contributes significantly to both heart and kidney failure. The widespread use of diuretics and renin–angiotensin–aldosterone system (RAAS) inhibitors to control the disease points to Na^+ retention by the kidneys as a key contributor.

ENaC figures prominently in the rare cases where the cause of hypertension is well understood. The clearest example of this is Liddle syndrome, a condition involving gain-of-function mutations in ENaC itself. These mutations lead to severe hypertension with hypokalemia and metabolic alkalosis that is transmitted in an autosomal-dominant pattern (Hansson et al., 1995). Conversely, loss-of-function mutations in ENaC give rise to pseudohypoaldosteronism type 1b, characterized by salt wasting, hyperkalemia, and acidosis (Chang et al., 1996).

High blood pressure also results from hyperaldosteronism. This can result from an aldosterone-secreting adenoma, renal artery stenosis activating renin secretion, or somatic mutations in which aldosterone production is controlled by ACTH rather than Ang II (glucocorticoid-remediable aldosteronism) (Lifton et al., 2001). Overactivation of ENaC is likely to be a key part of the pathophysiology of these conditions.

We do not know to what extent less-active variants of ENaC contribute to essential hypertension, a much more common condition that cannot be mapped to a single genetic mutation. In this case, changes in ENaC activity might increase blood pressure by a small amount but would combine with alterations in other genes and with environmental factors to have more significant effects. Identifying such variants has been difficult (Ambrosius et al., 1999; Hannila-Handelberg et al., 2005; Wang et al., 2008; Liu et al., 2015; Blobner et al., 2022).

Channel activity can be readily assessed using heterologous expression systems, comparing variants with normal genotypes. For example, the αA663T mutant, expressed in CHO cells together with βENaC and γENaC, produced currents 60% higher than those with a WT subunit, but this variant has not been associated with hypertension (Wang et al., 2008). Also, simple

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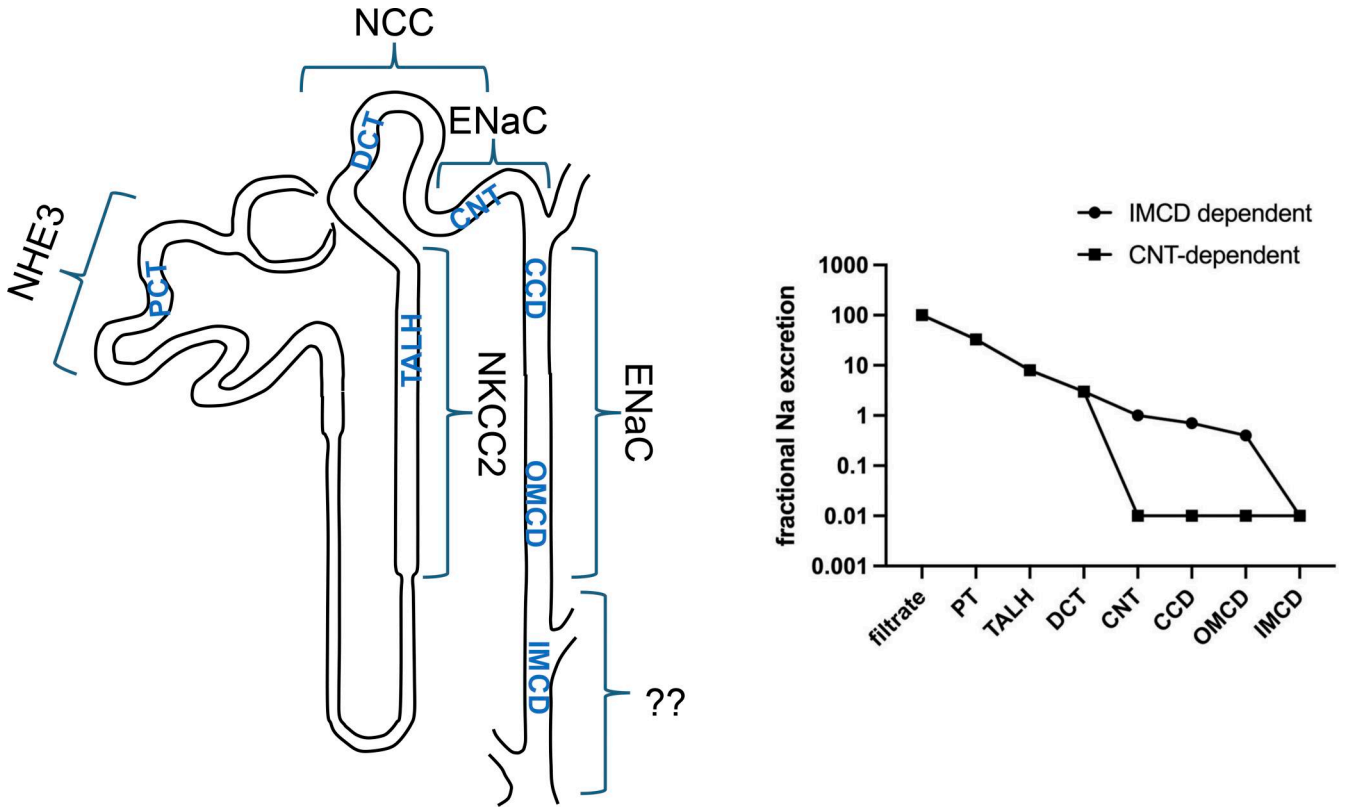


Figure 4. **Role of ENaC in minimizing urinary Na^+ excretion.** The nephron contains segments with different apical transporters mediating Na^+ reabsorption including the Na^+-H^+ exchanger NHE3 in the proximal tubule, the Na^+-K^+-$2Cl^-$ cotransporter NKCC2 in the thick ascending limb of Henle's loop, and the Na^+-Cl^- cotransporter NCC in the distal convoluted tubule. The graph shows the percentage of filtered Na^+ remaining at the end of each segment under two scenarios. In the CNT-dependent scenario, minimal urine Na^+ is established by ENaC in the CNT segment just proximal to the CCD. This minimal Na^+ is maintained along the CCD, OMCD, and IMCD, and in the final urine. In the IMCD-dependent scenario, minimal Na^+ is not achieved until it passes through the IMCD, in which Na^+ is reabsorbed by as yet unidentified transporters in addition to ENaC. OMCD, outer medullary collecting duct; IMCD, inner medullary collecting duct.

measurements of activity may not tell the whole story. Contributions of ENaC to hypertension will likely be salt-sensitive, becoming more severe when Na^+ intake is high. Thus, the defect with a hyperactive ENaC may result from a failure of the channels to appropriately downregulate under these conditions. Assessing this phenotype in heterologous systems is not straightforward.

ENaC in nonepithelial cells, including endothelia, vascular smooth muscle, and immune cells, has also been implicated in hypertension. This topic is beyond the scope of this Perspective but has been recently reviewed (Mutchler et al., 2021).

Nephrotic syndrome and liver failure

ENaC likely plays a role in the production of peripheral edema in conditions that include nephrotic syndrome and liver failure. In both cases, retention of fluid may be caused or exacerbated by overactivation of the channels either through the RAAS or through more direct pathways.

In nephrotic syndrome, damage to the renal glomeruli results in filtration and excretion of large amounts of plasma proteins. This is accompanied by edema, indicating accumulation of fluid in the interstitial spaces. One explanation for the edema is based on the loss of protein from blood plasma, lowering the oncotic pressure in the capillary fluid. The resulting imbalance of Starling forces redistributes fluid from the plasma to the interstitial fluid, reducing the effective circulating volume of the blood, leading to stimulation of the RAAS, with aldosterone activating ENaC and promoting fluid retention. This is consistent with observations on a rat model of nephrotic syndrome in which Na^+ reabsorption in nephron segments beyond the DCT was increased (Ichikawa et al., 1983).

However, not all patients with nephrotic syndrome have elevated plasma aldosterone (Meltzer et al., 1979). This observation led to the hypothesis that ENaC might be hyperactivated not by aldosterone but by proteolytic enzymes that appear in the urine (Svenningsen et al., 2009; Artunc et al., 2019). These include tissue plasminogen, which can be converted to plasmin in the urine. Plasmin, like other serine proteases, has been shown to activate ENaC in heterologous expression systems (Bohnert et al., 2019). Increased cleavage of γENaC was observed in a mouse model of nephrotic syndrome (Bohnert et al., 2021), although the effects of increased urinary protease activity are difficult to dissociate from those of increased aldosterone, which also increases cleavage. Tests for the requirement of urokinase plasminogen activator—presumably required for generating active plasmin—for Na^+ retention have led to conflicting results in mouse models of nephrotic syndrome (Bohnert et al., 2019; Hinrichs et al., 2019).

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One difficulty with this scheme is that at least in rat kidneys, most of the ENaC at the surface is in the cleaved form even under physiological conditions, as shown by Western blots of surface proteins and a lack of response of CCD principal cells to exogenous trypsin *in vitro* (Frindt et al., 2008). It is therefore unclear whether additional proteolytic activity could enhance ENaC activity.

Extracellular fluid is also retained in chronic liver failure, and this has been correlated with increased plasma aldosterone (Wolff et al., 1958). This may again reflect reduced plasma oncotic pressure if albumin production is reduced but also involves increased venous hydrostatic pressure secondary to vasodilation (Schrier, 2011). Both events will activate RAAS and promote ENaC activity.

However, as with nephrotic syndrome, increased aldosterone levels are not always observed (Wilkinson and Williams, 1980). In a mouse model of liver failure, increased Na^+ transport through ENaC was observed even with MR blockade (Wang et al., 2024). Bile acids accumulate in blood and urine in liver failure, and these compounds can stimulate ENaC activity (Wiemuth et al., 2014). This appears to involve direct interactions with the extracellular domain of the channel (Ilyaskin et al., 2016; Wang et al., 2022b). The structural rearrangements of the protein involved in the process are unknown.

It should be emphasized, however, that activation of ENaC is not sufficient for producing these edematous states. Primary increases in function as in Liddle syndrome are not associated with peripheral edema. How the channels interact with other factors in these diseases is an open question.

Cystic fibrosis

ENaC can contribute to the pulmonary issues in cystic fibrosis. Here, the primary problem is reduced secretion of salt and water in the airways due to a loss of function of CFTR Cl^- channels (Quinton, 1990; Welsh, 1990; Frizzell, 1995). This shrinks the mucous layer at the airway surface, increasing its viscosity and decreasing its clearance along with airway pathogens, increasing the risk and severity of infections. However, the fluid layer is also affected by the rate of Na^+ absorption by airway epithelia, and this in turn is dependent on ENaC. Loss of ENaC function results in overaccumulation of lung fluid, impairing gas exchange and in a mouse model causing early postnatal death (Hummler et al., 1996).

Boucher and colleagues put forward evidence that CFTR downregulates ENaC and that cystic fibrosis results in ENaC hyperactivity (Knowles et al., 1981; Boucher et al., 1986). This implies that the hydration of the airway surface layer is reduced by both decreasing Cl^- secretion (via CFTR) and increasing Na^+ absorption (via ENaC). The underlying mechanism has not been elucidated, and it does not appear to apply to the sweat ducts, an absorptive epithelium expressing both ENaC and CFTR (Reddy and Quinton, 2003).

Pharmacology

These diseases potentially involve inappropriate elevations in ENaC activity that can lead, depending on the context, to increased extracellular fluid volume, edema, or decreased airway hydration. Drugs that inhibit the channel (amiloride and triamterene) have been available for decades, although their use has been limited by the ancillary effects of decreased renal K^+ secretion, leading to hyperkalemia. This is a direct result of inhibiting ENaC and is inherent to the use of this type of blocker. Amiloride is effective in treating hypertension associated with Liddle syndrome (Warnock, 1998) and primary aldosteronism (Reincke et al., 2021) where ENaC is hyperactive and baseline plasma K^+ levels are low.

Recently, both amiloride and the MR antagonist spironolactone have been proposed as an option to treat resistant hypertension, which is not well controlled by a combination of three different medications (Williams et al., 2015; Lee et al., 2025). Amiloride has also been suggested as an appropriate therapy for treating the edema of nephrotic syndrome (Schork et al., 2024) although it is not more effective than furosemide, a diuretic that works upstream of the ENaC-expressing segments. ENaC also provides a target for treating the pulmonary sequelae of cystic fibrosis (Knowles et al., 1990). This potential therapy has led to the development of a new generation of drugs with high affinity for the channel and which can be delivered in aerosols with low rates of absorption into the circulation (O'Riordan et al., 2014; Mall, 2020; Betzemeier et al., 2024). Even then, however, hyperkalemia may limit their use unless their effects on renal K^+ secretion can be mitigated.

Conclusions

ENaC is a channel that still presents many unsolved problems in ion transport. These include the following: (1) how Na^+ selectivity can be achieved without strong ion binding, (2) how multipass membrane proteins can be selectively expressed in apical membranes, (3) how the adrenocorticoid steroids can regulate transport, and (4) how abnormalities in this regulation produce various disease states. Resolution of these questions will require new experimental approaches and new ideas.

Acknowledgments

Joseph A. Mindell served as editor.

I thank Drs. David Ellision, Henry Sackin, and Ryan Schreiner for critical reading of the manuscript and helpful suggestions.

Writing of this Perspective was supported by National Institutes of Health grant R01 DK111380.

Author contributions: Lawrence G. Palmer: conceptualization, funding acquisition, project administration, and writing—original draft, review, and editing.

Disclosures: The author declares no competing interests exist.

Submitted: 25 June 2026
Revised: 10 August 2026
Accepted: 22 October 2026

References

Ambrosius, W.T., L.J. Bloem, L. Zhou, J.F. Rebhun, P.M. Snyder, M.A. Wagner, C. Guo, and J.H. Pratt. 1999. Genetic variants in the epithelial sodium channel in relation to aldosterone and potassium excretion and risk for hypertension. *Hypertension*. 34:631–637. https://doi.org/10.1161/01.hyp.34.4.631

Artunc, F., M. Worn, A. Schork, and B.N. Bohnert. 2019. Proteasuria-the impact of active urinary proteases on sodium retention in nephrotic

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syndrome. *Acta Physiol. (Oxf).* 225:e13249. https://doi.org/10.1111/apha.13249

Asher, C., H. Wald, B.C. Rossier, and H. Garty. 1996. Aldosterone-induced increase in the abundance of Na^+ channel subunits. *Am. J. Physiol.* 271: C605–C611. https://doi.org/10.1152/ajpcell.1996.271.2.C605

Awayda, M.S., K.L. Awayda, O. Pochynyuk, V. Bugaj, J.D. Stockand, and R.M. Ortiz. 2011. Acute cholesterol-induced anti-natriuretic effects: Role of epithelial Na+ channel activity, protein levels, and processing. *J. Biol. Chem.* 286:1683–1695. https://doi.org/10.1074/jbc.M110.159194

Baconguis, I., C.J. Bohlen, A. Goehring, D. Julius, and E. Gouaux. 2014. X-ray structure of Acid-sensing ion channel 1-snake toxin complex reveals open state of a Na^+-selective channel. *Cell.* 156:717–729. https://doi.org/10.1016/j.cell.2014.01.011

Becchetti, A., S. Sariban-Sohraby, and D.C. Eaton. 1994. Transmethylation increases activity of highly selective apical Na+ channel in A6 epithelia. *FASEB J.* 8:A339.

Bentley, P.J. 1958. The effects of neurohypophysial extracts on the water transfer across the wall of the isolated urinary bladder of the toad Bufo marinus. *J. Endocrinol.* 17:201–209. https://doi.org/10.1677/joe.0.0170201

Betzemeier, B., C. Braun, P. Sieger, A. Heckel, G. Linz, B. Linehan, T. Veser, D. Wiedenmayer, and J.T. Kley. 2024. Discovery and development of BI 1265162, an ENaC inhibitor for the treatment of cystic fibrosis. *Eur. J. Med. Chem.* 265:116038. https://doi.org/10.1016/j.ejmech.2023.116038

Bharadwaj, A., J. Curry, X.-T. Su, R.B. Maturana, J.A. McCormick, D.H. Ellison, and I. Baconguis. 2026. Differential assembly of native ENaC complexes across mouse epithelial tissues. *bioRxiv*. https://doi.org/10.64898/2026.01.23.701393 (Preprint posted January 25, 2026).

Blazer-Yost, B.L., T.G. Păunescu, S.I. Helman, K.D. Lee, and C.J. Vlahos. 1999. Phosphoinositide 3-kinase is required for aldosterone-regulated sodium reabsorption. *Am. J. Physiol.* 277:C531–C536. https://doi.org/10.1152/ajpcell.1999.277.3.C531

Blobner, B.M., A. Kirabo, O.B. Kashlan, S. Sheng, D.K. Arnett, L.C. Becker, E. Boerwinkle, J.C. Carlson, Y. Gao, R.A. Gibbs, et al. 2022. Rare variants in genes encoding subunits of the epithelial Na^+ channel are associated with blood pressure and kidney function. *Hypertension*. 79:2573–2582. https://doi.org/10.1161/HYPERTENSIONAHA.121.18513

Bohnert, B.N., S. Daiminger, M. Wörn, F. Sure, T. Staudner, A.V. Ilyaskin, F. Batbouta, A. Janessa, J.C. Schneider, D. Essigke, et al. 2019. Urokinase-type plasminogen activator (uPA) is not essential for epithelial sodium channel (ENaC)-mediated sodium retention in experimental nephrotic syndrome. *Acta Physiol. (Oxf).* 227:e13286. https://doi.org/10.1111/apha.13286

Bohnert, B.N., D. Essigke, A. Janessa, J.C. Schneider, M. Wörn, M.Z. Kalo, M. Xiao, L. Kong, K. Omage, J. Hennenlotter, et al. 2021. Experimental nephrotic syndrome leads to proteolytic activation of the epithelial Na^+ channel in the mouse kidney. *Am. J. Physiol. Ren. Physiol.* 321:F480–F493. https://doi.org/10.1152/ajprenal.00199.2021

Boucher, R.C., M.J. Stutts, M.R. Knowles, L. Cantley, and J.T. Gatzy. 1986. Na+ transport in cystic fibrosis respiratory epithelia. Abnormal basal rate and response to adenylate cyclase activation. *J. Clin. Invest.* 78:1245–1252. https://doi.org/10.1172/JCI112708

Butterworth, M.B. 2021. Non-coding RNAs and the mineralocorticoid receptor in the kidney. *Mol. Cell Endocrinol.* 521:111115. https://doi.org/10.1016/j.mce.2020.111115

Butterworth, M.B., R.S. Edinger, J.P. Johnson, and R.A. Frizzell. 2005. Acute ENaC stinulation by cAMP in a kidney cell line is mediated by exocytotic insertion from a recycling channel pool. *J. Gen. Physiol.* 125:81–101. https://doi.org/10.1085/jgp.200409124

Cahill, J., K.A. Hartfield, S.A. Heusser, N. Ritter, M.H. Poulsen, C. Yoshioka, S.A. Pless, and I. Baconguis. 2026. Conformational plasticity of human acid-sensing ion channel 1a. *Nat. Struct. Mol. Biol.* 33:1171–1182. https://doi.org/10.1038/s41594-026-01845-0

Caldwell, R.A., R.C. Boucher, and M.J. Stutts. 2004. Serine protease activation of near-silent epithelial Na+ channels. *Am. J. Physiol. Cell Physiol.* 286: C190–C194. https://doi.org/10.1152/ajpcell.00342.2003

Canessa, C.M., J.-D. Horisberger, and B.C. Rossier. 1993. Epithelial sodium channel related to proteins involved in neurodegeneration. *Nature*. 361: 467–470. https://doi.org/10.1038/361467a0

Canessa, C.M., A.-M. Mérillat, and B.C. Rossier. 1994a. Membrane topology of the epithelial sodium channel. *Am. J. Physiol.* 267:C1682–C1690. https://doi.org/10.1152/ajpcell.1994.267.6.C1682

Canessa, C.M., L. Schild, G. Buell, B. Thorens, I. Gautschi, J.-D. Horisberger, and B.C. Rossier. 1994b. Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits. *Nature*. 367:463–467. https://doi.org/10.1038/367463a0

Castañeda-Bueno, M., D.H. Ellison, and G. Gamba. 2022. Molecular mechanisms for the modulation of blood pressure and potassium homeostasis by the distal convoluted tubule. *EMBO Mol. Med.* 14:e14273. https://doi.org/10.15252/emmm.202114273

Chang, S.S., S. Grunder, A. Hanukoglu, A. Rosler, P.M. Mathew, I. Hanukoglu, L. Schild, Y. Lu, R.A. Shimkets, C. Nelson-Williams, et al. 1996. Mutations in subunits of the epithelial sodium channel cause salt wasting with hyperkalaemic acidosis, pseudohypoaldosteronism type 1. *Nat. Genet.* 12:248–253. https://doi.org/10.1038/ng0396-248

Chen, S.Y., A. Bhargava, L. Mastroberardino, O.C. Meijer, J. Wang, P. Buse, G.L. Firestone, F. Verrey, and D. Pearce. 1999. Epithelial sodium channel regulated by aldosterone-induced protein sgk. *Proc. Natl. Acad. Sci. USA*. 96:2514–2519. https://doi.org/10.1073/pnas.96.5.2514

Curry, J.N., X.T. Su, Q. Wu, Y. Maeoka, C.L. Yang, E. Delpire, R.A. Fenton, P.A. Welling, and D.H. Ellison. 2026. Kidney kallikrein-1 contributes to cleavage of gamma-ENaC in vivo. *Am. J. Physiol. Ren. Physiol.* 330: F422–F432. https://doi.org/10.1152/ajprenal.00358.2025

Debonneville, C., S. Flores, E. Kamynina, P.J. Plant, C. Tauxe, M.A. Thomas, C. Münster, A. Chraïbi, J.-H. Pratt, J.D. Horisberger, et al. 2001. Phosphorylation of Nedd4-2 by Sgk1 regulates epithelial Na(+) channel cell surface expression. *EMBO J.* 20:7052–7059. https://doi.org/10.1093/emboj/20.24.7052

Doyle, D.A., J. Morais Cabral, R.A. Pfuetzerl, A. Kuo, J.M. Gulbis, S.L. Cohen, B.T. Chait, and R. MacKinnon. 1998. The structure of the potassium channel: Molecular basis of K^+ conduction and selectivity. *Science*. 280: 69–77. https://doi.org/10.1126/science.280.5360.69

Edelman, I.S., R. Bogoroch, and G.A. Porter. 1963. On the mechanism of action of aldosterone on sodium transport: The role of protein synthesis. *Proc. Natl. Acad. Sci. USA*. 50:1169–1177. https://doi.org/10.1073/pnas.50.6.1169

Ergonul, Z., G. Frindt, and L.G. Palmer. 2006. Regulation of maturation and processing of ENaC subunits in the rat kidney. *Am. J. Physiol. Ren. Physiol.* 291:F683–F693. https://doi.org/10.1152/ajprenal.00422.2005

Fakitsas, P., G. Adam, D. Daidié, M.X. van Bemmelen, F. Fouladkou, A. Patrignani, U. Wagner, R. Warth, S.M. Camargo, O. Staub, and F. Verrey. 2007. Early aldosterone-induced gene product regulates the epithelial sodium channel by deubiquitylation. *J. Am. Soc. Nephrol.* 18:1084–1092. https://doi.org/10.1681/ASN.2006080902

Farman, N., C.R. Talbot, R. Boucher, M. Fay, C. Canessa, B.C. Rossier, and J.P. Bonvalet. 1997. Non-coordinated expression of a, b, and g, subunit mRNAs of the epithelial sodium channel along the rat respiratory tract. *Am. J. Physiol.* 272:C131–C141.

Farrar, J., and T. Frieden. 2025. WHO global report on hypertension 2025. *Lancet*. 406:2318–2319. https://doi.org/10.1016/S0140-6736(25)02208-1

Fejes-Tóth, G., G. Frindt, A. Náray-Fejes-Tóth, and L.G. Palmer. 2008. Epithelial Na^+ channel activation and processing in mice lacking SGK1. *Am. J. Physiol.* 294:F1298–F1305. https://doi.org/10.1152/ajprenal.00579.2007

Frindt, G., Z. Ergonul, and L.G. Palmer. 2008. Surface expression of epithelial Na channel protein in rat kidney. *J. Gen. Physiol.* 131:617–627. https://doi.org/10.1085/jgp.200809989

Frindt, G., D. Gravotta, and L.G. Palmer. 2016. Regulation of ENaC trafficking in rat kidney. *J. Gen. Physiol.* 147:217–227. https://doi.org/10.1085/jgp.201511533

Frindt, G., J.R. Meyerson, A. Satty, J.M. Scandura, and L.G. Palmer. 2022. Expression of ENaC subunits in epithelia. *J. Gen. Physiol.* 154:e202213124. https://doi.org/10.1085/jgp.202213124

Frindt, G., and L.G. Palmer. 2012. Regulation of epithelial Na+ channels by adrenal steroids: Mineralocorticoid and glucocorticoid effects. *Am. J. Physiol. Ren. Physiol.* 302:F20–F26. https://doi.org/10.1152/ajprenal.00480.2011

Frindt, G., S. Shi, T. Krogh-Madsen, T. Rohan, S.C.M. Whelan, and L.G. Palmer. 2025. Kinetics of aldosterone-dependent ENaC trafficking in the kidney. *J. Gen. Physiol.* 157:e202513821. https://doi.org/10.1085/jgp.202513821

Frizzell, R.A. 1995. Functions of the cystic fibrosis transmembrane conductance regulator protein. *Am. J. Respir. Crit. Care Med.* 151:S54–S58. https://doi.org/10.1164/ajrccm/151.3_Pt_2.S54

Gaeggeler, H.P., E. Gonzalez-Rodriguez, N.F. Jaeger, D. Loffing-Cueni, R. Norregaard, J. Loffing, J.D. Horisberger, and B.C. Rossier. 2005. Mineralocorticoid versus glucocorticoid receptor occupancy mediating aldosterone-stimulated sodium transport in a novel renal cell line. *J. Am. Soc. Nephrol.* 16:878–891. https://doi.org/10.1681/ASN.2004121110

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García Romeu, F., A. Salibián, and S. Pezzani-Hernádez. 1969. The nature of the in vivo sodium and chloride uptake mechanisms through the epithelium against sodium and of bicarbonate against chloride. *J. Gen. Physiol.* 53:816–835. https://doi.org/10.1085/jgp.53.6.816

Hamilton, K., and D.C. Eaton. 1986. Regulation of single sodium channels in renal tissue: A role in sodium homeostasis. *Fed. Proc.* 45:2713–2717.

Hannila-Handelberg, T., K. Kontula, I. Tikkanen, T. Tikkanen, F. Fyhrquist, K. Helin, H. Fodstad, K. Piippo, H.E. Miettinen, J. Virtamo, et al. 2005. Common variants of the beta and gamma subunits of the epithelial sodium channel and their relation to plasma renin and aldosterone levels in essential hypertension. *BMC Med. Genet.* 6:4. https://doi.org/10.1186/1471-2350-6-4

Hansson, J.H., L. Schild, Y. Lu, T.A. Wilson, I. Gautschi, R. Shimkets, C. Nelson-Williams, B.C. Rossier, and R.P. Lifton. 1995. A de novo missense mutation of the beta subunit of the epithelial sodium channel causes hypertension and Liddle syndrome, identifying a proline-rich segment critical for regulation of channel activity. *Proc. Natl. Acad. Sci. USA*. 92:11495–11499. https://doi.org/10.1073/pnas.92.25.11495

Helms, M.N., L. Liu, Y.Y. Liang, O. Al-Khalili, A. Vandewalle, S. Saxena, D.C. Eaton, and H.P. Ma. 2005. Phosphatidylinositol 3,4,5-trisphosphate mediates aldosterone stimulation of epithelial sodium channel (ENaC) and interacts with gamma-ENaC. *J. Biol. Chem.* 280:40885–40891. https://doi.org/10.1074/jbc.M509646200

Hill, W.G., M.B. Butterworth, H. Wang, R.S. Edinger, J. Lebowitz, K.W. Peters, R.A. Frizzell, and J.P. Johnson. 2007. The epithelial sodium channel (ENaC) traffics to apical membrane in lipid rafts in mouse cortical collecting duct cells. *J. Biol. Chem.* 282:37402–37411. https://doi.org/10.1074/jbc.m704084200

Hille, B. 1971. The permeability of the sodium channel to organic cations in myelinated nerve. *J. Gen. Physiol.* 58:599–619. https://doi.org/10.1085/jgp.58.6.599

Hinrichs, G.R., K. Weyer, U.G. Friis, P. Svenningsen, I.K. Lund, R. Nielsen, G. Mollet, C. Antignac, C. Bistrup, B.L. Jensen, and H. Birn. 2019. Urokinase-type plasminogen activator contributes to amiloride-sensitive sodium retention in nephrotic range glomerular proteinuria in mice. *Acta Physiol. (Oxf).* 227:e13362. https://doi.org/10.1111/apha.13362

Huang, D.Y., P. Wulff, H. Volkl, J. Loffing, K. Richter, D. Kuhl, F. Lang, and V. Vallon. 2004. Impaired regulation of renal K+ elimination in the sgk1-knockout mouse. *J. Am. Soc. Nephrol.* 15:885–891. https://doi.org/10.1097/01.asn.0000120368.59693.a8

Hughey, R.P., J.B. Bruns, C.L. Kinlough, K.L. Harkleroad, Q. Tong, M.D. Carattino, J.P. Johnson, J.D. Stockand, and T.R. Kleyman. 2004. Epithelial sodium channels are activated by furin-dependent proteolysis. *J. Biol. Chem.* 279:18111–18114. https://doi.org/10.1074/jbc.C400080200

Hughey, R.P., G.M. Mueller, J.B. Bruns, C.L. Kinlough, P.A. Poland, K.L. Harkleroad, M.D. Carattino, and T.R. Kleyman. 2003. Maturation of the epithelial Na^+ channel involves proteolytic processing of the alpha- and gamma-subunits. *J. Biol. Chem.* 278:37073–37082. https://doi.org/10.1074/jbc.M307003200

Hummler, E., P. Barker, J. Gatzy, F. Beermann, C. Verdumo, A. Schmidt, R. Boucher, and B.C. Rossier. 1996. Early death due to defective neonatal lung liquid clearance in alpha ENaC-deficient mice. *Nat. Genet.* 12:325–328. https://doi.org/10.1038/ng0396-325

Ichikawa, I., H.G. Rennke, J.R. Hoyer, K.F. Badr, N. Schor, J.L. Troy, C.P. Lechene, and B.M. Brenner. 1983. Role for intrarenal mechanisms in the impaired salt excretion of experimental nephrotic syndrome. *J. Clin. Invest.* 71:91–103. https://doi.org/10.1172/jci110756

Ilyaskin, A.V., A. Diakov, C. Korbmacher, and S. Haerteis. 2016. Activation of the human epithelial sodium channel (ENaC) by bile acids involves the degenerin site. *J. Biol. Chem.* 291:19835–19847. https://doi.org/10.1074/jbc.M116.726471

Jasti, J., H. Furukawa, E.B. Gonzales, and E. Gouaux. 2007. Structure of acid-sensing ion channel 1 at 1.9 A resolution and low pH. *Nature*. 449:316–323. https://doi.org/10.1038/nature06163

Kashlan, O.B., X.P. Wang, S. Sheng, and T.R. Kleyman. 2024. Epithelial Na^+ channels function as extracellular sensors. *Compr. Physiol.* 14:1–41. https://doi.org/10.1002/cphy.c230015

Kellenberger, S., N. Hoffmann-Pochon, I. Gautschi, E. Schneeberger, and L. Schild. 1999. On the molecular basis of ion permeation in the epithelial Na+ channel. *J. Gen. Physiol.* 114:13–30. https://doi.org/10.1085/jgp.114.1.13

Kellenberger, S., and L. Schild. 2015. International Union of Basic and Clinical Pharmacology. XCI. structure, function, and pharmacology of acid-sensing ion channels and the epithelial Na+ channel. *Pharmacol. Rev.* 67:1–35. https://doi.org/10.1124/pr.114.009225

Kemendy, A.E., T.R. Kleyman, and D.C. Eaton. 1992. Aldosterone alters the open probability of amiloride-blockable sodium channels in A6 epithelia. *Am. J. Physiol.* 263:C825–C837. https://doi.org/10.1152/ajpcell.1992.263.4.C825

Kleyman, T.R., M.D. Carattino, and R.P. Hughey. 2009. ENaC at the cutting edge: Regulation of epithelial sodium channels by proteases. *J. Biol. Chem.* 284:20447–20451. https://doi.org/10.1074/jbc.R800083200

Kleyman, T.R., and D.C. Eaton. 2020. Regulating ENaC's gate. *Am. J. Physiol. Cell Physiol.* 318:C150–C162. https://doi.org/10.1152/ajpcell.00418.2019

Knowles, M.R., N.L. Church, W.E. Waltner, J.R. Yankaskas, P. Gilligan, M. King, L.J. Edwards, R.W. Helms, and R.C. Boucher. 1990. A pilot study of aerosolized amiloride for the treatment of lung disease in cystic fibrosis. *New Engl. J. Med.* 322:1189–1194. https://doi.org/10.1056/NEJM199004263221704

Knowles, M., J. Gatzy, and R. Boucher. 1981. Increased bioelectric potential difference across respiratory epithelia in cystic fibrosis. *N. Eng. J. Med.* 305:1489–1495. https://doi.org/10.1056/NEJM198112173052502

Koefoed-Johnsen, V., and H.H. Ussing. 1958. The nature of the frog skin potential. *Acta Physiol. Scand.* 42:298–308. https://doi.org/10.1111/j.1748-1716.1958.tb01563.x

Lee, C.J., S.H. Ihm, D.H. Shin, J.O. Jeong, J.H. Kim, K.H. Chun, J. Ryu, H.Y. Lee, S. Choi, E.M. Lee, et al. 2025. Spironolactone vs amiloride for resistant hypertension: A randomized clinical trial. *JAMA*. 333:2073–2082. https://doi.org/10.1001/jama.2025.5129

Lifton, R.P., A.G. Gharavi, and D.S. Geller. 2001. Molecular mechanisms of human hypertension. *Cell*. 104:545–556. https://doi.org/10.1016/s0092-8674(01)00241-0

Lindemann, B., and W. Van-Driessche. 1977. Sodium specific membrane channels in frog skin are pores: Current fluctuations reveal high turnover. *Science*. 195:292–294. https://doi.org/10.1126/science.299785

Liu, F., X. Yang, X. Mo, J. Huang, J. Chen, T.N. Kelly, J.E. Hixson, D.C. Rao, C.C. Gu, L.C. Shimmin, et al. 2015. Associations of epithelial sodium channel genes with blood pressure: The GenSalt study. *J. Human Hypertension*. 29:224–228. https://doi.org/10.1038/jhh.2014.78

Loffing, J., L. Pietri, F. Aregger, M. Bloch-Faure, U. Ziegler, P. Meneton, B.C. Rossier, and B. Kaissling. 2000. Differential subcellular localization of ENaC subunits in mouse kidney in response to high- and low-Na diets. *Am. J. Physiol. Ren. Physiol.* 279:F252–F258. https://doi.org/10.1152/ajprenal.2000.279.2.F252

Loffing, J., M. Zecevic, E. Feraille, C. Asher, B.C. Rossier, G.L. Firestone, D. Pearce, and F. Verrey. 2001. Aldosterone induces rapid apical translocation of ENaC in early portion of renal collecting system: Possible role of SGK. *Am. J. Physiol.* 280:F675–F682. https://doi.org/10.1152/ajprenal.2001.280.4.F675

Loughlin, S., H.M. Costello, A.J. Roe, C. Buckley, S.M. Wilson, M.A. Bailey, and M.K. Mansley. 2023. Mapping the transcriptome underpinning acute corticosteroid action within the cortical collecting duct. *Kidney360*. 4:226–240. https://doi.org/10.34067/KID.0003582022

Lu, C., S. Pribanic, A. Debonneville, C. Jiang, and D. Rotin. 2007. The PY motif of ENaC, mutated in Liddle syndrome, regulates channel internalization, sorting and mobilization from subapical pool. *Traffic*. 8:1246–1264. https://doi.org/10.1111/j.1600-0854.2007.00602.x

Lynagh, T., E. Flood, C. Boiteux, M. Wulf, V.V. Komnatnyy, J.M. Colding, T.W. Allen, and S.A. Pless. 2017. A selectivity filter at the intracellular end of the acid-sensing ion channel pore. *Elife*. 6:e24630. https://doi.org/10.7554/eLife.24630

Ma, H.P., S. Saxena, and D.G. Warnock. 2002. Anionic phospholipids regulate native and expressed epithelial sodium channel (ENaC). *J. Biol. Chem.* 277:7641–7644. https://doi.org/10.1074/jbc.C100737200

Mall, M.A. 2020. ENaC inhibition in cystic fibrosis: Potential role in the new era of CFTR modulator therapies. *Eur. Respir. J.* 56:2000946. https://doi.org/10.1183/13993003.00946-2020

Malnic, G., R.M. Klose, and G. Giebisch. 1966. Micropuncture study of distal tubular potassium and sodium transport in rat nephron. *Am. J. Physiol.* 211:529–547. https://doi.org/10.1152/ajplegacy.1966.211.3.529

Mamenko, M., O. Zaika, D.V. Ilatovskaya, A. Staruschenko, and O. Pochynyuk. 2012. Angiotensin II increases activity of the epithelial Na+ channel (ENaC) in distal nephron additively to aldosterone. *J. Biol. Chem.* 287:660–671. https://doi.org/10.1074/jbc.M111.298919

Masilamani, S., G.H. Kim, C. Mitchell, J.B. Wade, and M.A. Knepper. 1999. Aldosterone-mediated regulation of ENaC alpha, beta, and gamma subunit proteins in rat kidney. *J. Clin. Invest.* 104:R19–R23. https://doi.org/10.1172/JCI7840

Meltzer, J.I., H.J. Keim, J.H. Laragh, J.E. Sealey, K.M. Jan, and S. Chien. 1979. Nephrotic syndrome: Vasoconstriction and hypervolemic types

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indicated by renin-sodium profiling. *Ann. Intern. Med.* 91:688–696. https://doi.org/10.7326/0003-4819-91-5-688

Mick, V.E., O.A. Itani, R.W. Loftus, R.F. Husted, T.J. Schmidt, and C.P. Thomas. 2001. The alpha-subunit of the epithelial sodium channel is an aldosterone-induced transcript in mammalian collecting ducts, and this transcriptional response is mediated via distinct cis-elements in the 5'-flanking region of the gene. *Mol. Endocrinol.* 15:575–588. https://doi.org/10.1210/mend.15.4.0620

Morimoto, T., W. Liu, C. Woda, M.D. Carattino, Y. Wei, R.P. Hughey, G. Apodaca, L.M. Satlin, and T.R. Kleyman. 2006. Mechanism underlying flow stimulation of sodium absorption in the mammalian collecting duct. *Am. J. Physiol. Ren. Physiol.* 291:F663–F669. https://doi.org/10.1152/ajprenal.00514.2005

Mutchler, S.M., A. Kirabo, and T.R. Kleyman. 2021. Epithelial sodium channel and salt-sensitive hypertension. *Hypertension.* 77:759–767. https://doi.org/10.1161/HYPERTENSIONAHA.120.14481

Náray-Fejes-Tóth, A., C. Boyd, and G. Fejes-Tóth. 2008. Regulation of epithelial sodium transport by promyelocytic leukemia zinc finger protein. *Am. J. Physiol. Ren. Physiol.* 295:F18–F26. https://doi.org/10.1152/ajprenal.00573.2007

Náray-Fejes-Tóth, A., C. Canessa, E.S. Cleaveland, G. Aldrich, and G. Fejes-Tóth. 1999. Sgk is an aldosterone-induced kinase in the renal collecting duct. Effects on epithelial Na^+ channels. *J. Biol. Chem.* 274:16973–16978. https://doi.org/10.1074/jbc.274.24.16973

Náray-Fejes-Tóth, A., and G. Fejes-Tóth. 2000. The sgk, an aldosterone-induced gene in mineralocorticoid target cells, regulates the epithelial sodium channel. *Kidney Int.* 57:1290–1294. https://doi.org/10.1046/j.1523-1755.2000.00964.x

Nickerson, A.J., S. Sheng, N.A. Cox, K.G. Szekely, A.L. Marciszyn, T. Lam, J. Chen, S. Gingras, O.B. Kashlan, A. Kirabo, et al. 2024. Loss of the alpha subunit distal furin cleavage site blunts ENaC activation following Na^+ restriction. *J. Physiol.* 602:4309–4326. https://doi.org/10.1113/JP286559

Noreng, S., A. Bharadwaj, R. Posert, C. Yoshioka, and I. Baconguis. 2018. Structure of the human epithelial sodium channel by cryo-electron microscopy. *Elife.* 7:e39340. https://doi.org/10.7554/eLife.39340

O'Riordan, T.G., K.H. Donn, P. Hodsman, J.H. Ansede, T. Newcomb, S.A. Lewis, W.D. Flitter, V.S. White, M.R. Johnson, A.B. Montgomery, et al. 2014. Acute hyperkalemia associated with inhalation of a potent ENaC antagonist: Phase 1 trial of GS-9411. *J. Aerosol Med. Pulm. Drug Deliv.* 27:200–208. https://doi.org/10.1089/jamp.2013.1037

Pácha, J., G. Frindt, L. Antonian, R.B. Silver, and L.G. Palmer. 1993. Regulation of Na channels of the rat cortical collecting tubule by aldosterone. *J. Gen. Physiol.* 102:25–42. https://doi.org/10.1085/jgp.102.1.25

Palmer, L.G. 1987. Ion selectivity of epithelial Na channels. *J. Membr. Biol.* 96:97–106. https://doi.org/10.1007/bf01869236

Palmer, L.G. 1999. Potassium secretion and the regulation of distal nephron K channels. *Am. J. Physiol.* 277:F821–F825. https://doi.org/10.1152/ajprenal.1999.277.6.f821

Palmer, L.G., and G. Frindt. 1986. Amiloride-sensitive Na channels from the apical membrane of the rat cortical collecting tubule. *Proc. Natl. Acad. Sci. USA.* 83:2767–2770. https://doi.org/10.1073/pnas.83.8.2767

Palmer, L.G., and G. Frindt. 1987. Effects of cell Ca^{2+} and pH on Na^+ channels from rat cortical collecting tubule. *Am. J. Physiol.* 253:F333–F339. https://doi.org/10.1152/ajprenal.1987.253.2.F333

Palmer, L.G., and G. Frindt. 1988. Conductance and gating of epithelial Na channels from rat cortical collecting tubule. Effects of Na and Li. *J. Gen. Physiol.* 92:121–138. https://doi.org/10.1085/jgp.92.1.121

Palmer, L.G., and G. Frindt. 1996. Gating of Na channels in the rat cortical collecting tubule: Effects of voltage and membrane stretch. *J. Gen. Physiol.* 107:35–45. https://doi.org/10.1085/jgp.107.1.35

Palmer, L.G., A.M. Weinstein, and G. Frindt. 2026. How does the kidney conserve Na^+ in a salt-scarce environment? *Am. J. Physiol. Ren. Physiol.* 30:F601–F606. https://doi.org/10.1152/ajprenal.00020.2026

Payandeh, J., T. Scheuer, N. Zheng, and W.A. Catterall. 2011. The crystal structure of a voltage-gated sodium channel. *Nature.* 475:353–358. https://doi.org/10.1038/nature10238

Pearce, D., A.D. Manis, V. Nesterov, and C. Korbmacher. 2022. Regulation of distal tubule sodium transport: Mechanisms and roles in homeostasis and pathophysiology. *Pflugers Arch.* 474:869–884. https://doi.org/10.1007/s00424-022-02732-5

Pochynyuk, O., V. Bugaj, A. Vandewalle, and J.D. Stockand. 2008. Purinergic control of apical plasma membrane PI(4,5)P2 levels sets ENaC activity in principal cells. *Am. J. Physiol. Ren. Physiol.* 294:F38–F46. https://doi.org/10.1152/ajprenal.00403.2007

Poulsen, S.B., K. Limbutara, R.A. Fenton, T. Pisitkun, and B.M. Christensen. 2018. RNA sequencing of kidney distal tubule cells reveals multiple mediators of chronic aldosterone action. *Physiol. Genomics.* 50:343–354. https://doi.org/10.1152/physiolgenomics.00084.2017

Pouly, D., A. Debonneville, D. Ruffieux-Daidié, M. Maillard, H. Abriel, J. Loffing, and O. Staub. 2013. Mice carrying ubiquitin-specific protease 2 (Usp2) gene inactivation maintain normal sodium balance and blood pressure. *Am. J. Physiol. Ren. Physiol.* 305:F21–F30. https://doi.org/10.1152/ajprenal.00012.2013

Quinton, P.M. 1990. Cystic fibrosis: A disease in electrolyte transport. *FASEB J.* 4:2709–2717. https://doi.org/10.1096/fasebj.4.10.2197151

Rashmi, P., G. Colussi, M. Ng, X. Wu, A. Kidwai, and D. Pearce. 2017. Glucocorticoid-induced leucine zipper protein regulates sodium and potassium balance in the distal nephron. *Kidney Int.* 91:1159–1177. https://doi.org/10.1016/j.kint.2016.10.038

Ray, E.C., A. Nickerson, S. Sheng, R. Carrisoza-Gaytan, T. Lam, A. Marciszyn, L. Zhang, A. Jordahl, C. Bi, A. Winfrey, et al. 2024. Influence of proteolytic cleavage of ENaC's γ subunit upon Na^+ and K^+ handling. *Am. J. Physiol. Ren. Physiol.* 326:F1066–F1077. https://doi.org/10.1152/ajprenal.00027.2024

Reddy, M.M., and P.M. Quinton. 2003. Functional interaction of CFTR and ENaC in sweat glands. *Pflugers Arch.* 445:499–503. https://doi.org/10.1007/s00424-002-0959-x

Reincke, M., I. Bancos, P. Mulatero, U.I. Scholl, M. Stowasser, and T.A. Williams. 2021. Diagnosis and treatment of primary aldosteronism. *Lancet Diabetes Endocrinol.* 9:876–892. https://doi.org/10.1016/s2213-8587(21)00210-2

Rocha, A.S., and L.H. Kudo. 1990. Factors governing sodium and choride transport across the inner medullary collecting duct. *Kidney Int.* 38:654–667. https://doi.org/10.1038/ki.1990.256

Rodriguez-Boulan, E., and I.G. Macara. 2014. Organization and execution of the epithelial polarity programme. *Nat. Rev. Mol. Cell Biol.* 15:225–242. https://doi.org/10.1038/nrm3775

Rossier, B.C., M.E. Baker, and R.A. Studer. 2015. Epithelial sodium transport and its control by aldosterone: The story of our internal environment revisited. *Physiol. Rev.* 95:297–340. https://doi.org/10.1152/physrev.00011.2014

Roux, B. 2017. Ion channels and ion selectivity. *Essays Biochem.* 61:201–209. https://doi.org/10.1042/ebc20160074

Rubera, I., J. Loffing, L.G. Palmer, G. Frindt, N. Fowler-Jaeger, D. Sauter, T. Carroll, A. McMahon, E. Hummler, and B.C. Rossier. 2003. Collecting duct specific gene inactivation of aENaC in the mouse kidney does not impair sodium and potassium balance. *J. Clin. Invest.* 112:554–565. https://doi.org/10.1172/jci200316956

Sather, W.A., and E.W. McCleskey. 2003. Permeation and selectivity in calcium channels. *Annu. Rev. Physiol.* 65:133–159. https://doi.org/10.1146/annurev.physiol.65.092101.142345

Schork, A., E. Vogel, B.N. Bohnert, D. Essigke, M. Wörn, I. Fischer, N. Heyne, A.L. Birkenfeld, and F. Artunc. 2024. Amiloride versus furosemide for the treatment of edema in patients with nephrotic syndrome: A pilot study (AMILOR). *Acta Physiol. (Oxf).* 240:e14183. https://doi.org/10.1111/apha.14183

Schrier, R.W. 2011. Use of diuretics in heart failure and cirrhosis. *Semin. Nephrol.* 31:503–512. https://doi.org/10.1016/j.semnephrol.2011.09.005

Schuck, S., and K. Simons. 2004. Polarized sorting in epithelial cells: Raft clustering and the biogenesis of the apical membrane. *J. Cell Sci.* 117:5955–5964. https://doi.org/10.1242/jcs.01596

Sheng, S., A.B. Maarouf, J.B. Bruns, R.P. Hughey, and T.R. Kleyman. 2007. Functional role of extracellular loop cysteine residues of the epithelial Na+ channel in Na+ self-inhibition. *J. Biol. Chem.* 282:20180–20190. https://doi.org/10.1074/jbc.M611761200

Soundararajan, R., T.T. Zhang, J. Wang, A. Vandewalle, and D. Pearce. 2005. A novel role for glucocorticoid-induced leucine zipper protein in epithelial sodium channel-mediated sodium transport. *J. Biol. Chem.* 280:39970–39981. https://doi.org/10.1074/jbc.m508658200

Spirli, A., L. Cheval, A. Debonneville, D. Penton, C. Ronzaud, M. Maillard, A. Doucet, J. Loffing, and O. Staub. 2019. The serine-threonine kinase PIM3 is an aldosterone-regulated protein in the distal nephron. *Physiol. Rep.* 7:e14177. https://doi.org/10.14814/phy2.14177

Staruschenko, A., O. Pochynyuk, A. Vandewalle, V. Bugaj, and J.D. Stockand. 2007. Acute regulation of the epithelial Na+ channel by phosphatidylinositide 3-OH kinase signaling in native collecting duct principal cells. *J. Am. Soc. Nephrol.* 18:1652–1661. https://doi.org/10.1681/ASN.2007010020

Staub, O., H. Abriel, P. Plant, T. Ishikawa, V. Kanelis, R. Saleki, J.D. Horisberger, L. Schild, and D. Rotin. 2000. Regulation of the epithelial Na+

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channel by Nedd4 and ubiquitination. *Kidney Int.* 57:809–815. https://doi.org/10.1046/j.1523-1755.2000.00919.x

Staub, O., I. Gautschi, T. Ishikawa, K. Breitschopf, A. Ciechanover, L. Schild, and D. Rotin. 1997. Regulation of stability and function of the epithelial Na+ channel (ENaC) by ubiquitination. *EMBO J.* 16:6325–6336. https://doi.org/10.1093/emboj/16.21.6325

Staub, O., and F. Verrey. 2005. Impact of Nedd4 proteins and serum and glucocorticoid-induced kinases on epithelial Na^+ transport in the distal nephron. *J. Am. Soc. Nephrol.* 16:3167–3174. https://doi.org/10.1681/ASN.2005050454

Stockand, J.D., N.F. Al-Baldawi, O.K. Al-Khalili, R.T. Worrell, and D.C. Eaton. 1999. S-adenosyl-L-homocysteine hydrolase regulates aldosterone-induced Na+ transport. *J. Biol. Chem.* 274:3842–3850. https://doi.org/10.1074/jbc.274.6.3842

Stokes, J.B., and R.D. Sigmund. 1998. Regulation of rENaC mRNA by dietary NaCl and steroids: Organ, tissue, and steroid heterogeneity. *Am. J. Physiol.* 274:C1699–C1707. https://doi.org/10.1152/ajpcell.1998.274.6.C1699

Stoops, E.H., and M.J. Caplan. 2014. Trafficking to the apical and basolateral membranes in polarized epithelial cells. *J. Am. Soc. Nephrol.* 25:1375–1386. https://doi.org/10.1681/ASN.2013080883

Sure, F., S. Afonso, D. Essigke, P. Schmidt, M.Z. Kalo, V. Nesterov, A. Kißler, M. Bertog, R. Rinke, S. Wittmann, et al. 2025. Transmembrane serine protease 2 and proteolytic activation of the epithelial sodium channel in mouse kidney. *J. Am. Soc. Nephrol.* 36:420–434. https://doi.org/10.1681/ASN.0000000521

Svenningsen, P., C. Bistrup, U.G. Friis, M. Bertog, S. Haerteis, B. Krueger, J. Stubbe, O.N. Jensen, H.C. Thiesson, T.R. Uhrenholt, et al. 2009. Plasmin in nephrotic urine activates the epithelial sodium channel. *J. Am. Soc. Nephrol.* 20:299–310. https://doi.org/10.1681/ASN.2008040364

Swanson, E.A., J.W. Nelson, S. Jeng, K.J. Erspamer, C.L. Yang, S. McWeeney, and D.H. Ellison. 2019. Salt-sensitive transcriptome of isolated kidney distal tubule cells. *Physiol. Genomics*. 51:125–135. https://doi.org/10.1152/physiolgenomics.00119.2018

Tong, Q., R.E. Booth, R.T. Worrell, and J.D. Stockand. 2004. Regulation of Na+ transport by aldosterone: Signaling convergence and cross talk between the PI3-K and MAPK1/2 cascades. *Am. J. Physiol. Ren. Physiol.* 286: F1232–F1238. https://doi.org/10.1152/ajprenal.00345.2003

Van-Driessche, W., and B. Lindemann. 1979. Concentration dependence of currents through single sodium-selective pores in frog skin. *Nature*. 282:519–520. https://doi.org/10.1038/282519a0

Wang, T., T. Liu, S. Xu, G. Frindt, A.M. Weinstein, and L.G. Palmer. 2023. High dietary K^+ intake inhibits proximal tubule transport. *Am. J. Physiol. Ren. Physiol.* 325:F224–F234. https://doi.org/10.1152/ajprenal.00013.2023

Wang, X.-f., X.M. Lu, R.Y. Lin, S.Z. Wang, L.P. Zhang, J. Qian, D.R. Lu, H. Wen, and L. Jin. 2008. Lack of association of functional variants in alpha-ENaC gene and essential hypertension in two ethnic groups in China. *Kidney Blood Press. Res.* 31:268–273. https://doi.org/10.1159/000151286

Wang, X.P., D.M. Balchak, C. Gentilcore, N.L. Clark, and O.B. Kashlan. 2022a. Activation by cleavage of the epithelial Na^+ channel α and γ subunits independently coevolved with the vertebrate terrestrial migration. *Elife*. 11:e75796. https://doi.org/10.7554/eLife.75796

Wang, X.P., S.M. Mutchler, R. Carrisoza-Gaytan, A.J. Nickerson, C.J. Baty, M. Al-Bataineh, A. Vandevender, T. Morimoto, P. Srinivasan, R.J. Tan, et al. 2024. Epithelial Na^+ channel activation after bile duct ligation with mineralocorticoid receptor blockade. *J. Am. Soc. Nephrol.* 35:1466–1477. https://doi.org/10.1681/ASN.0000000000000442

Wang, X.P., V. Tomilin, A.J. Nickerson, R. Tian, M. Ertem, A. McKernan, X. Lei, O. Pochynyuk, and O.B. Kashlan. 2022b. Bile acids regulate the epithelial Na^+ channel in native tissues through direct binding at multiple sites. *J. Physiol.* 600:4695–4711. https://doi.org/10.1113/JP283318

Warnock, D.G. 1998. Liddle syndrome: An autosomal dominant form of human hypertension. *Kidney Int.* 53:18–24. https://doi.org/10.1046/j.1523-1755.1998.00728.x

Weinstein, A.M. 1998. A mathematical model of the inner medullary collecting duct of the rat: Pathways for Na and K transport. *Am. J. Physiol.* 274:F841–F855. https://doi.org/10.1152/ajprenal.1998.274.5.F841

Weisz, O.A., and E. Rodriguez-Boulan. 2009. Apical trafficking in epithelial cells: Signals, clusters and motors. *J. Cell Sci.* 122:4253–4266. https://doi.org/10.1242/jcs.032615

Welsh, M.J. 1990. Abnormal regulation of ion channels in cystic fibrosis. *FASEB J.* 4:2718–2725. https://doi.org/10.1096/fasebj.4.10.1695593

Wiemuth, D., C.M. Lefèvre, H. Heidtmann, and S. Gründer. 2014. Bile acids increase the activity of the epithelial Na+ channel. *Pflugers Arch.* 466: 1725–1733. https://doi.org/10.1007/s00424-013-1403-0

Wilkinson, S.P., and R. Williams. 1980. Renin-angiotensin-aldosterone system in cirrhosis. *Gut*. 21:545–554. https://doi.org/10.1136/gut.21.6.545

Will, P.C., R.N. Cortright, R.C. DeLisle, J.G. Douglas, and U. Hopfer. 1985. Regulation of amiloride-sensitive electrogenic sodium transport in the rat colon by steroid hormones. *Am. J. Physiol.* 248:G124–G132. https://doi.org/10.1152/ajpgi.1985.248.1.G124

Williams, B., T.M. MacDonald, S. Morant, D.J. Webb, P. Sever, G. McInnes, I. Ford, J.K. Cruickshank, M.J. Caulfield, J. Salsbury, et al. 2015. Spironolactone versus placebo, bisoprolol, and doxazosin to determine the optimal treatment for drug-resistant hypertension (PATHWAY-2): A randomised, double-blind, crossover trial. *Lancet*. 386:2059–2068. https://doi.org/10.1016/S0140-6736(15)00257-3

Wolff, H.P., K.R. Koczorek, and E. Buchborn. 1958. Aldosterone and antidiuretic hormone (adiuretin) in liver disease. *Acta Endocrinol. (Copenh)*. 27: 45–58. https://doi.org/10.1530/acta.0.0270045

Wulff, P., V. Vallon, D.Y. Huang, H. Volkl, F. Yu, K. Richter, M. Jansen, M. Schlunz, K. Klingel, J. Loffing, et al. 2002. Impaired renal Na(+) retention in the sgk1-knockout mouse. *J. Clin. Invest.* 110:1263–1268. https://doi.org/10.1172/JCI15696

Yang, L., G. Frindt, F. Lang, D. Kuhl, V. Vallon, and L.G. Palmer. 2017. SGK1-dependent ENaC processing and trafficking in mice with high dietary K intake and elevated aldosterone. *Am. J. Physiol. Ren. Physiol.* 312:F65–F76. https://doi.org/10.1152/ajprenal.00257.2016

Yang, L., and L.G. Palmer. 2018. Determinants of selective ion permeation in the epithelial Na^+ channel. *J. Gen. Physiol.* 150:1397–1407. https://doi.org/10.1085/jgp.201812164

Zhai, Y.J., M.M. Wu, V.A. Linck, L. Zou, Q. Yue, S.P. Wei, C. Song, S. Zhang, C.R. Williams, B.L. Song, et al. 2019. Intracellular cholesterol stimulates ENaC by interacting with phosphatidylinositol-4,5-bisphosphate and mediates cyclosporine A-induced hypertension. *Biochim. Biophys. Acta Mol. Basis Dis.* 1865:1915–1924. https://doi.org/10.1016/j.bbadis.2018.08.027

Zhang, W., X. Xia, M.R. Reisenauer, T. Rieg, F. Lang, D. Kuhl, V. Vallon, and B.C. Kone. 2007. Aldosterone-induced Sgk1 relieves Dot1a-AF9-mediated transcriptional repression of epithelial Na^+ channel alpha. *J. Clin. Invest.* 117:773–783. https://doi.org/10.1172/JCI29850

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