REVIEW

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Lessons learned about epithelial sodium channels from transgenic mouse models

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Purpose of review
This review provides an up-to-date understanding about the regulation of epithelial sodium channel (ENaC) expression and function. In particular, we will focus on its implication in renal Na^+ and K^+ handling and control of blood pressure using transgenic animal models.

Recent findings
In kidney, the highly amiloride-sensitive ENaC maintains whole body Na^+ homeostasis by modulating Na^+ transport via epithelia. This classical role is mostly confirmed using genetically engineered animal models. Recently identified key signaling pathways that regulate ENaC expression and function unveiled some nonclassical and unexpected channel regulatory processes. If aberrant, these dysregulated mechanisms may also result in the development of salt-dependent hypertension.
The purpose of this review is to highlight the most recent findings in renal ENaC regulation and function, in considering data obtained from animal models.

Summary
Increased ENaC-mediated Na^+ transport is a prerequisite for salt-dependent forms of hypertension. To treat salt-sensitive hypertension it is crucial to fully understand the function and regulation of ENaC.

Keywords
epithelial sodium channel, hyperkalemia, salt wasting, sodium retention, transgenic mice

INTRODUCTION

Epithelial sodium channel, salt-sensitivity and hypertension

The epithelial sodium channel (ENaC) is a membrane bound ion channel that is selectively permeable to Na^+ ions, thereby controlling fluid balance across tight epithelial membranes. ENaC is assembled principally at apical membranes of polarized cells as heterotrimer and is composed of three subunits, α or δ, β and γ, each encoded by different genes (SCNN1A, SCNN1D, SCNN1B, SCCN1G) (see, for review, [1,2]). Present in epithelial tissues/organs such as the skin, lungs, colon, intestine and kidney, ENaC allows the transport of fluid and the regulation of Na^+ and K^+ homeostasis. Several external factors such as hormones like aldosterone, vasopressin or insulin, Na^+ and K^+, proteases, but also internal factors like kinases, ubiquitin-ligases, phospholipids (PIP_2, PIP_3) or direct posttranslational modification regulate ENaC activity (see, for review, [3]). Any dysregulation of its activity might lead to tissue/organ-specific diseases including salt-sensitive hypertension (see, for review, [4]).

Hypertension or increased blood pressure is a global crisis that affects over one billion people worldwide. It is strongly associated with increased risk of cardiovascular events, stroke and kidney disease [5]. In most developed and developing countries, it presents the leading cause of death [6]. Since any increase in Na^+ and water retention is required for the development of most forms of hypertension, this places the renal highly amiloride-sensitive ENaC in a central position to influence the risk of hypertension [7]. In human, ENaC variants leading aberrant ENaC activity are associated with hypo- and hypertension [3]. Several mouse models altering ENaC expression and function confirm these findings and allowed us to determine ENaC function in specific organs or cells under physiological and/or pathophysiological conditions.

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KEY POINTS

- Epithelial sodium channel (ENaC) plays a central role in whole body sodium homeostasis.
- ENaC is expressed in epithelial as well as nonepithelial tissues.
- ENaC regulation is dependent on a complex network of signaling pathways.
- Modulators of ENaC expression and activity are found in renal as well as extrarenal tissues, and may thus be involved in the development of hypertension.

EFFECT OF CONSTITUTIVE SINGLE EPITHELIAL SODIUM CHANNEL SUBUNIT DELETION ON RENAL Na^+ AND K^+ HANDLING

ENaC is the limiting factor *in vivo* in the control of ionic composition of the extracellular fluid, regulation of blood volume and blood pressure [8]. Various transgenic and knockout mouse lines have been generated and phenotypically analyzed in which the ENaC activity ranged from zero channel activity with lung liquid failure [9] to ~20% salt-wasting syndrome pseudohypoaldosteronism (PHA-1) [10] and to hyperactive ENaC resulting in a salt-retaining phenotype and hypertension [11,12]. Constitutive deletion of each single subunit of α-, β-, γ-ENaC resulted in perinatal lethality [9,13,14] (Fig. 1). Mice with global partial loss of function of the αENaC subunit showed an intact capacity to maintain blood pressure and sodium balance when kept under different sodium diets [15]. These mice showed increased vascular responsiveness to exogenous angiotensin II and compensated for the reduced ENaC channels by increasing the number of renal AT1 receptors. Interestingly, plasma renin activity was blunted on low sodium diet and did not follow the changes of the AT1 receptor [15]. A transgene expression was sufficient to rescue the perinatal lethal pulmonary phenotype

	Diets	Salt wasting	Hyponatremia	Hyperkalemia	Reference
αENaC KO	SD	+	-	+	(9)
Hypomorphic αENaC	SD	+	-	-	(15)
$\alpha ENaC^{(-/-)}Tg$ (CMV-αENaC)	SD,LNa	-	-	-	(10)
βENaC KO	SD	+	+	+	(13)
Hypomorphic βENaC	LNa [a]KCl SD LNa	+ - - -	- - - Not reported	+ ++ - ++	(16) (16) (17) (18)
βENaC – Liddle knockin	SD HNa	- -	- -	- -*	(12)
γENaC KO	SD	+	-	+	(14)
Hypomorphic γENaC	SD HNa HK	- - -	- - -	- - -	(19)

FIGURE 1. Renal phenotype of mice with global altered ENaC expression. Global knockouts of α-, β-, and γ-ENaC subunits and their major renal phenotype under various diet conditions (SD, standard, 0.17–0.25% Na^+; LNa, low sodium, 0.01% Na^+; HNa, high sodium, 4% Na^+; HK, high potassium diet, 5–6% K^+). KO, knockout mouse; + to ++, presence and severity of the trait; –, absence of trait, not different from control mice. * Mice with hypokalemia. [a]130 mM KCl infusion. ENaC, epithelial sodium channel.

but only partially restored Na^+ transport in renal, colonic, and pulmonary epithelia leading to a compensated PHA-1 with normalized electrolyte and acid/base in the presence of higher aldosterone levels [10]. Mice heterozygous for βENaC deletion exhibited a lower potassium excretion although they conserved sodium overall [16]. Mice expressing low levels of the βENaC subunit showed elevated plasma aldosterone levels on standard diet that became only evident on sodium restriction [17]. These mice with reduced levels of βENaC showed signs of renal inflammation and injury, and elevated blood pressure likely due to loss of βENaC-mediated myogenic constriction [18]. Mice with globally reduced γENaC subunit expression showed salt-sensitivity of volume and blood pressure, mildly elevated plasma aldosterone levels, and reduced blood pressure on high dietary sodium [19▪]. Unexpectedly, these mice exhibited no hyperkalemia, and only a transient fluid gain and reduction in diurnal blood pressure variation. The authors discussed that extrarenal ENaC expression might be partly responsible for this phenotype [19▪].

To summarize, the analysis of systemic effect of ENaC subunit function is hampered by the lethality of any of these ENaC subunits when deleted. The detailed phenotyping of these models further suggested that each ENaC subunit might have its specific role and some of the phenotypes described might be the consequence of loss of ENaC function in tissues beyond renal tubular epithelia.

PHYSIOLOGICAL FUNCTION OF THE THREE EPITHELIAL SODIUM CHANNEL SUBUNITS ALONG THE NEPHRON

To overcome the early lethality in constitutive ENaC mutant mice, conditional alleles for the αENaC (*Scnn1a*) [20], βENaC (*Scnn1b*) and γENaC (*Scnn1g*) gene loci [21] were generated to selectively inactivate these protein subunits in specific tissues to dissect their function (for review, see [1]). The ENaC subunit-mediated sodium transport along the nephron in adulthood was achieved using induced nephron tubular- and nephron segment-specific knockouts of α-, β-, γ-ENaC, and surprisingly revealed a varying degree in the severity of salt wasting and potassium retention (Fig. 2). Whereas the nephron tubular-specific knockout of αENaC showed the classical

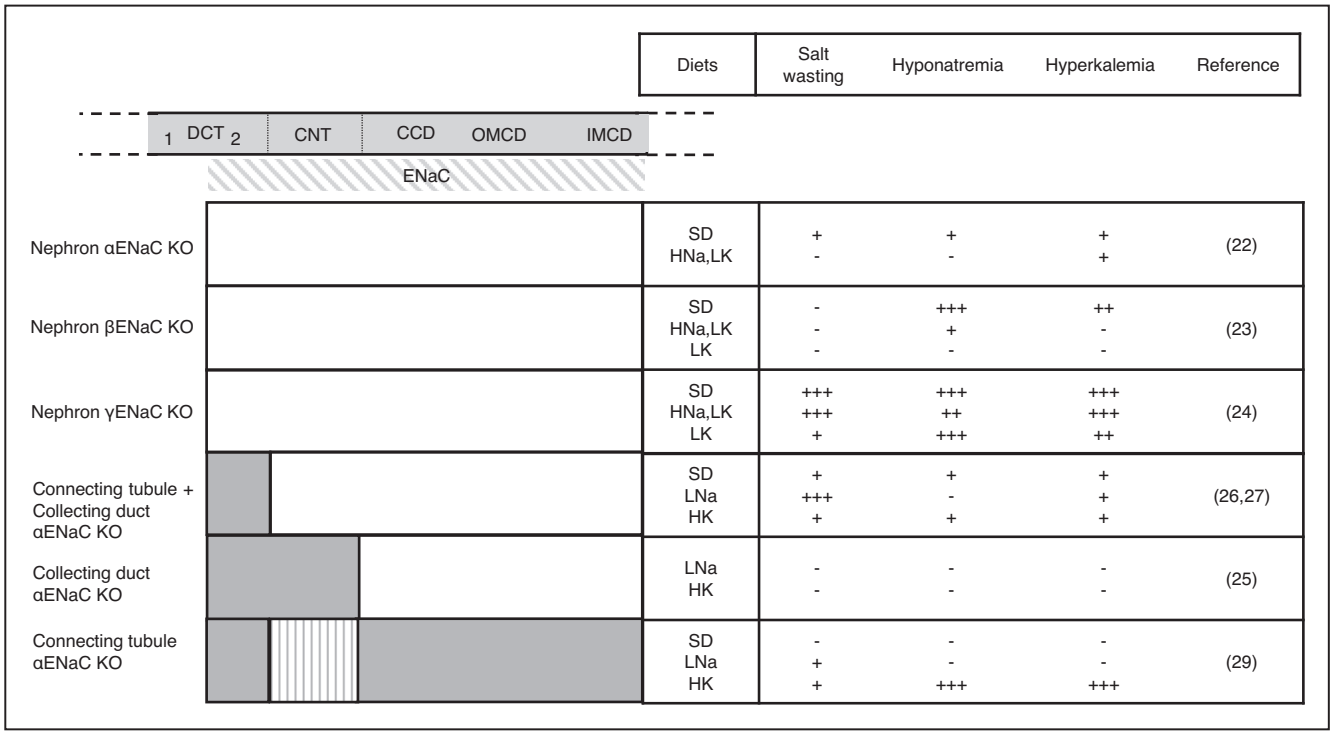

FIGURE 2. Renal phenotype of mice with nephron segment specific altered ENaC expression. Constitutive and/or inducible knockouts of α-, β-, and γ-ENaC subunits and their major renal phenotype under various diet conditions (SD, standard diet, 0.17–0.25% Na^+; LNa, low sodium, 0.01Na^+; HNa, high sodium 4% Na^+; HNa, LK, high sodium, low potassium 3.5% Na^+, 0.1% K^+; LK, low potassium, 0.1% K^+; HK, high potassium, 5–6% K^+). KO, knockout mouse; + to +++, presence and severity of the trait; –, absence of trait, not different from control mice. CCD, collecting duct; CNT, connecting tubule; DCT, distal convoluted tubule; ENaC, epithelial sodium channel; IMCD, inner medullary collecting duct; OMCD, outer medullary collecting duct.

Diets	Nephron αENaC KO	Nephron βENaC KO	Nephron γENaC KO
SD	X	X	X
HNa,LK	✓	✓	X
LK	n.d	✓	X
SD,LK + Kayexalate	n.d	n.d	✓
Reference	(22)	(23)	(24)

FIGURE 3. Rescue of global ENaC subunit knockout mice on various diet conditions. Supplementation with sodium and potassium-containing diet rescues the lethal phenotype of global α-, β-, and γ-ENaC knockout mice. SD, standard diet 0.17Na^+; HNa, LK, high sodium, low potassium, 3.5% Na^+,0.2% K^+; LK, low potassium, 0.1% K^+; kayexalate 0.96 µl/day/g of body weight. n.d., not determined. ENaC, epithelial sodium channel; KO, knockout mouse.

pseudohypoaldosteronism type 1 with loss of body weight, increased urinary Na^+ excretion and highly activated aldosterone–angiotensin–renin system [22], the lack of β- and γ-ENaC along the nephron instead rather revealed an implication in K^+ handling. Those mice only exhibited a transient sodium loss, but a persistent severe hyperkalemia [23,24]. Consequently, a standard diet failed to rescue the lethal phenotype (Fig. 3). A high Na^+, but low K^+ diet only partially rescued the β-, but completely rescued the αENaC knockout mice. The rescue reflected the activation of the sodium-chloride symporter NCC that is suppressed at baseline elevated plasma K^+ concentrations. The induced βENaC knockout mice developed a transient Na^+ losing and thus, the lethal phenotype could be completely prevented by a standard Na^+ and low K^+ diet [23]. The γENaC knockout mice further presented severe hyperkalemia under high Na^+ and low K^+ diet. Unlike mice lacking α- or β-ENaC, plasma K^+ concentrations did not normalize and NCC was not increased in induced γENaC knockouts [24]. However, when K^+ was eliminated by addition of a K^+ chelator as kayexalate at the time of γENaC deletion, plasma K^+ concentrations and NCC activity remained normal and weight loss was inhibited [22]. Contrary to the induced αENaC knockout, mice with induced absence of β- and γ-ENaC instead developed a transient Na^+ loss with persistent hyperkalemia. As a result, the plasma K^+ concentration became limiting in regulating NCC activity regardless of Na^+ balance.

The genetic dissection of the ENaC-mediated transport along the nephron allowed us to better define and narrow down its site of action (Fig. 2). Inactivation of αENaC in the collecting duct revealed no requirement for sodium and potassium handling on standard and low Na^+ diet [25]. This also determined the entry site for lithium, since these mice were protected from lithium-induced nephrogenic diabetes insipidus [26] and confirmed that thiazolidinedione-induced fluid retention is independent of collecting duct αENaC activity [27]. These data furthermore suggested the cortical connecting tubule as site of aldosterone-mediated sodium reabsorption. Indeed, gradual deletion of αENaC expression in the connecting tubule (CNT) combined with dietary Na^+ restriction was sufficient to induce a severe salt-losing syndrome with significant weight loss, increased urinary sodium excretion and hyperkalemia in addition to elevated plasma aldosterone levels [28]. Finally, reducing αENaC expression in the kidney connecting tubule induced clear pseudohypoaldosteronism type 1 symptoms during K^+ loading and compensatory mechanisms like higher plasma aldosterone levels, lower renal NCC activity and lower GFR occurred which pointed to a crucial role of this segment for whole body sodium homeostasis [29]. Apart from the tubular transepithelial transport, Sassi *et al.* [30▪] recently provided evidence that the γENaC subunit additionally interacts with claudin-8 to modulate paracellular sodium permeability in renal collecting duct.

In summary, renal tubular-specific deletion of any of the ENaC subunits revealed a specific role in renal Na^+ and K^+ handling and enabled to unlink the absorption of Na^+ and K^+ without affected systemic ENaC function. Overall, the genetic dissection of ENaC function allowed to narrow down its site of action. We can however not exclude a requirement of ENaC-mediated Na^+ absorption in more distal tubules under (more) challenging conditions, for example when the upstream segments are overloaded affecting Na^+ and/or K^+ transport or under specific diet conditions [31]. In a recent study, Yang and coworkers studied ENaC and ROMK activities along the distal nephron on various K^+ diets. ENaC-dependent K^+ secretion shifted from the early CNT under basal to more distal parts of the CNT under increased K^+ intake [32▪▪].

SELECTED RECENT REGULATORS OF EPITHELIAL SODIUM CHANNEL FUNCTION AND THEIR EFFECT ON SODIUM RETENTION

A variety of extracellular and intracellular factors regulate ENaC in kidney, and hormones, ions, phospholipids, proteases, kinases as well as

posttranslational modifications and mechanical forces can differentially activate the channel (see also recent reviews [3,33]. The implication of a classical ENaC regulator, namely the mineralocorticoid receptor (MR) was recently evaluated using kidney-specific MR knockout mice. Wu *et al.* [34▪] demonstrated that ENaC activity in the DCT2/CNT is maintained by a mechanism independent of the MR, whereas Nesterov *et al.* [35▪] proposed that MR is critically important for generally maintaining aldosterone-dependent and -independent ENaC activation, and rather controlled by the presence or absence of the 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2) (Table 1, Fig. 4). The deletion of Nedd4–2 (neural precursor cell developmentally downregulated protein 4–2) caused salt-sensitive hypertension [36], and Zhang *et al.* [37] suggested that Nedd4–2 is additionally required for high salt (HS)-induced inhibition of ENaC expression and activity. The glucose-regulated protein 170 (GP170) was previously shown to regulate biosynthesis and thus, levels of γENaC. The inducible nephron-specific knockout caused hypovolemia, hyperaldosteronism and dysregulation of ion homeostasis associated with lower γENaC expression, albeit other ion channels/transporters were equally affected [38]. The mechanistic target of rapamycin (mTOR) regulated ENaC via the phosphorylation of serum and glucocorticoid regulated kinase 1 (SGK1) [39,40]. The targeted deletion of mTOR in cortical duct principal cells of mice showed modest salt wasting with reduced ENaC, hereby activity affecting channel turnover and enhancing channel degradation [41]. A recent study showed that K^+ itself acts through the type 2 mTOR complex (mTORC2) to activate SGK1 which stimulates ENaC to enhance K^+ excretion [42].

Activation of ENaC by proteolytic cleavage independently coevolved with the vertebrate terrestrial migration and coincidence with the appearance of aldosterone [43]. The channel activating protease CAP1/prostasin was proposed to function as a scaffold protein for ENaC and/or other serine proteases [44], but in a recent study, Na^+ retention in nephrotic syndrome was reported to be independent of this protease [45]. Activation of the cAMP pathway promoted ENaC trafficking to the apical membrane [46] and increased ENaC open probability [47]. Exchange proteins directly activated by cAMP (Epacs) are abundantly expressed in the renal tubules and constitutive knockout mice for Epac1 and Epac2 provided evidence that single or combined deletion impaired stimulation of ENaC and compromised renal sodium conservation in response to dietary sodium deficiency [48▪]. Mammalian Ste20-like protein kinase 3 (MST3/STK24) is mainly expressed in the thick ascending tubule, and at lower levels in the distal convoluted tubules. Hypomorphic mice for this protein kinase exhibited higher ENaC activity causing hypernatremia and hypertension [49]. In response to K^+ loading, these maintained Na^+/K^+ homeostasis by regulation of WNK4 expression thereby enhancing Na^+ retention through increased NKCC2 and ENaC activity [50]. Ank-3 has been previously shown to modulate ENaC activity in a cultured murine principal cell model [51]. El-Aziz and coworkers now proposed that casein kinase II-phosphorylated βENaC promotes Ank-3-mediated trafficking to the membrane. Principal cell-specific Ank-3 knockout mice showed decreased ENaC activity and increased sodium excretion supporting evidence that Ank-3 binding increased channel activity [52]. The ankyrin repeat domain 36 (ANKRD36) mediates a variety of protein-protein interactions and was found to be significantly lower expressed in hypertension [53▪▪]. Ankrd36 knockout mice showed higher blood pressure and Na^+ absorption on high salt diet associated with increased tubular ENaC expression by altering expression of ENaC genes [53▪▪].

High salt diet induced salt-sensitive hypertension in male but not in female db/db mice by impairing ENaC downregulation which might explain the sexual dimorphism associated with the development of diabetic kidney disease [54]. Furthermore, Dizin *et al.* [55▪▪] revealed that γENaC expression and activity were physiologically controlled by the Hif pathway and, nephron tubular specific Hif1α knockout mice displayed increased γENaC abundance under high sodium intake. This might present a negative feedback on active sodium absorption to adjust metabolic demand and oxygen supply.

Overall, this illustrates that a complex network of signaling pathways is implicated.

EXTRARENAL EXPRESSION OF EPITHELIAL SODIUM CHANNEL AND BLOOD PRESSURE REGULATION

Beyond its classical role in promoting Na^+ retention, it was proposed that ENaC influences BP in epithelia but also in nonepithelial tissues like, for example, vascular endothelium or smooth muscles, interstitium/extracellular matrix of the skin or immune cells although the mechanisms are not yet well understood [56]. Vascular tone was proposed to be mediated by ENaC in vascular smooth muscle cells, and its subunits found to be expressed in mesenteric, cerebral and renal arteries [57]. Furthermore, a possible causative contribution of central nervous system ENaC in the etiology of salt-induced hypertension was proposed [58]. Expression of exogenous

Table 1. Selection of recent publications of positive and negative regulators of ENaC expression and activity

Mouse/Human gene	Genetic modification	Na^+ retention	ENaC protein expression			ENaC activity			Reference
			SD	LNa	HNa	SD	LNa	HNa	
Nr3c2/NR3C2	Inducible nephr. specific KO	n.d.	n.d.	n.d.	n.d.	ex vivo: ↓	n.d.	n.d.	[34▪,35▪]
Nedd4-2/ NEDD4L	Inducible nephr. specific KO	n.d.	↑βENaC ↑γENaC full	n.d.	↑βENaC ↑γENaC full	ex vivo: ↑	n.d.	ex vivo: ↑	[37]
Hyou1/HYOU1	Inducible nephr. specific KO	↓ SD	↓γENaC Immuno.	n.d.	n.d.	n.d.	n.d.	n.d.	[38]
Mtor/MTOR	CD-PC KO	↓LNa	↓αENaC full ↓βENaC ↓γENaC full + cl. Kidney cortex	↓αENaC full ↓βENaC ↓γENaC full + cl. Kidney cortex	n.d.	in vivo: ↓	n.d.	n.d.	[41,42]
Rapgef3/RAPGE3 Rapgef4/ RAPGEF4	Complete KO	↓LNa (Rapgef3$^{-/-}$ or Rapgef4$^{-/-}$ and Rapgef3$^{-/-}$; Rapgef4$^{-/-}$)	No difference	↓αENaC cl. ↓γENaC full (Rapgef3 $^{-/-}$ or Rapgef4 $^{-/-}$)	n.d.	in vivo: ↓ ex vivo: ↓ (Rapgef3$^{-/-}$ or Rapgef4$^{-/-}$ and Rapgef3$^{-/-}$; Rapgef4$^{-/-}$)	in vivo: ↓ ex vivo: ↓ (Rapgef3$^{-/-}$ or Rapgef4$^{-/-}$ and Rapgef3$^{-/-}$; Rapgef4$^{-/-}$)	n.d.	[48▪]
Ank3/ANK3	CD-PC KO	↓ SD	n.d	n.d.	n.d.	ex vivo: ↓	n.d.	n.d.	[52]
Stk24/STK24	Hypomorphic	↑ SD ↑ HK	↑γENaC cl.	n.d.	n.d.	in vivo: ↑	n.d.	n.d.	[50] *
Ankrd36/ ANKRD36	Complete KO	↑ HNa	↑αENaC ↑βENaC	n.d.	↑αENaC ↑βENaC ↑γENaC	n.d.	n.d.	in vivo: ↑	[53▪▪]
Lep/LEP	Complete KO	↑ HNa	n.d.	n.d.	↑αENaC full + cl. ↑βENaC ↑γENaC cl.	n.d.	n.d.	in vivo: ↑	[54]
Hif1a/HIF1A	Inducible nephr. specific KO	No difference	No difference	No difference	↑γENaC full	n.d.	n.d	n.d	[55▪▪]

Only transgenic mouse models have been considered.
CD-PC, cortical duct principal cells; ENaC, epithelial sodium channel; KO, knockout mouse; n.d., not determined; SD, standard diet, 0.32–1% Na^+; LNa, low sodium, 0.01–0.1% Na^+; HNa, high sodium, 3–8% Na^+; HK, high potassium, 2% K^+.
*↑γENaCfull + ↑in vitro under SD(49), cleaved fragment.

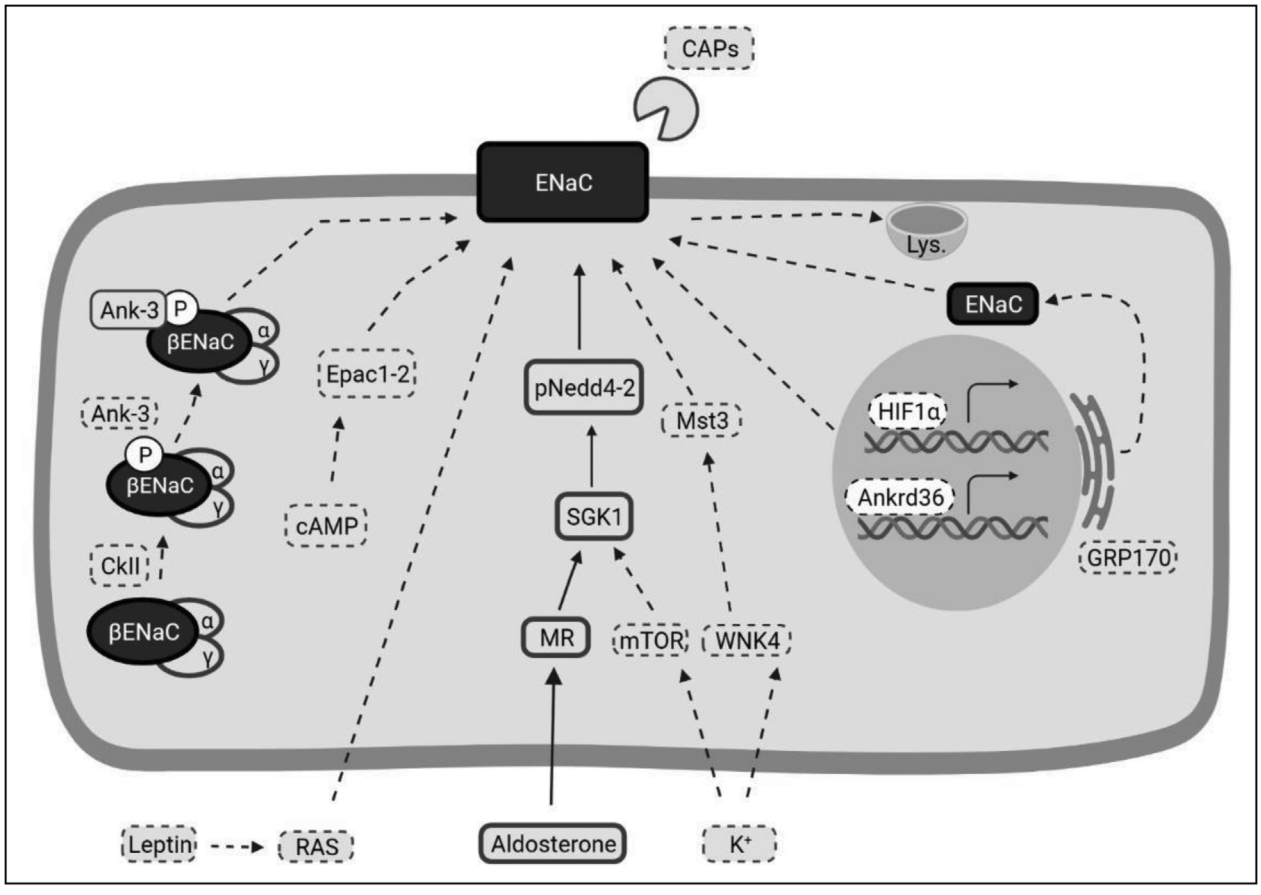

FIGURE 4. Selected recently identified regulators of ENaC function. Confirmed (full) and hypothetical (dashed line) pathways of ENaC regulation. Please note that only selected canonical and novel pathways are illustrated integrating recently identified in vivo regulators. Lys, lysosome; HIF1α, hypoxia-inducible factor 1-alpha; Ankrd36, ankyrin repeat domain 36; GRP170, glucose-regulated protein 170 *(Hyou1)*; MST3, mammalian St20-like protein kinase 3 *(Stk24)*; WNK4, WNK lysin deficient protein kinase 4; mTOR, mammalian target of rapamycin; MR, mineralocorticoid *(Nr3C2)*; SGK1, serum and glucocorticoid-regulated kinase 1; pNedd4–2, neural precursor cell expressed developmentally down-regulated 4-like; RAS, renin-angiotensin system; cAMP, cyclic adenosine monophosphate; Epac1–2-, Exchange proteins directly activated by cAMP *(Rapgef3, Rapgef4)*, Ank3, ankyrin-3; CKII, casein kinase II; CAPs, channel activating proteases; ENaC, epithelial sodium channel .

epithelial sodium channel β-subunit in mouse middle cerebral artery increased pressure-induced constriction [59▪]. Enhanced ENaC expression from a mouse model for Liddle's syndrome correlated with increased cortical stiffness reflecting mechanical rigidity [60]. Western diet contributed to renal arterial stiffening and was endothelium- and αENaC-dependent [61▪]. The absence of the endothelial αENaC subunit reduced renal ischemia reperfusion injury through improving eNOS activation and kidney perfusion [62]. Deletion of γENaC subunit in endothelial cells did not protect against renal ischemia reperfusion injury [33]. However, whether ENaC inhibits nitric oxide production thereby mediating vascular tone and myogenic response or rather helps preserving a homeostatic response still needs to be evaluated [63].

Kirabo *et al.* [64] demonstrated a novel role for ENaC in immune cells and its contribution to salt-induced inflammation and hypertension. Dendritic cells formed γ-ketoaldehydes known as immunogenic isolevuglandins (isoLGs) upon hypertensive stimuli linking vascular oxidant stress to immune activation and aortic stiffening [65]. Thereby, high salt activated CD11c(+) antigen-presenting cells via the serum glucocorticoid kinase 1 (SGK1) to mediate salt-sensitive hypertension and renal inflammation [66]. More recently, Veiras *et al.* [54] unveiled renal inflammation as a major contributor to ENaC dysregulation and salt-sensitivity in male diabetic mice.

Overall, these data further provided evidence for the contribution of inflammation to the progression of kidney disease and the development of salt sensitive hypertension.

CONCLUSION

Since its cloning about 25 years ago [67,68], we still find novel functions of ENaC in epithelial and nonepithelial tissues as in neurons and/or dendritic cells that could influence salt and water homeostasis and blood pressure. Independent of whether ENaC is renamed EnNaC or EpNaC, its subunits are encoded by the same αENaC (*Scnn1a*), βENaC (*Scnn1b*) and γENaC (*Scnn1g*) genes as found within the tubular nephron or in the vasculature. Induced deletion of each of the ENaC subunits within the tubular nephron is lethal within two to three days, whereas the knockout of ENaC regulators so far unveiled if any only mild phenotypes. This could be indicative for the need of complexity of regulators that maintain ENaC expression and activity. Not surprisingly, the analysis of respective mouse models revealed expected phenotypes as weight loss or changes in blood pressure, but also unexpected phenotypes as inflammation. Even within one organ or structure like the nephron, there is evidence for distinct regulation of the same channel being dependent or independent of aldosterone or other hormones and may highly depend on the stoichiometry of the subunits, presence of absence of regulators, and cell-specific conditions.

Further regulators of ENaC will be identified in future, thus illustrating the complexity of regulation and the pathways involved. The future challenge will be to dissect them when they work in concert or differently activated under physiological and pathophysiological conditions to modulate ENaC activity and expression.

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Conflicts of interest

There are no conflicts of interest.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

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