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Regulation of blood pressure and renal function by NCC and ENaC: lessons from genetically-engineered mice

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Abstract

The activity of the thiazide-sensitive Na^+/Cl^- cotransporter (NCC) and of the amiloride-sensitive epithelial Na^+ channel (ENaC) is pivotal for blood pressure regulation. NCC is responsible for Na^+ reabsorption in the distal convoluted tubule (DCT) of the nephron, while ENaC reabsorbs the filtered Na^+ in the late DCT and in the cortical collecting ducts (CCD) providing the final renal adjustment to Na^+ balance. Here, we aim to highlight the recent advances which were made using transgenic mouse models towards the understanding of the regulation of NCC and ENaC function relevant to control of sodium balance and blood pressure. We thus like to pave the way for common mechanisms regulating these two sodium transporting proteins and their potential implications in the structure of the nephron segments in the control of Na^+ and Cl^- reabsorption.

Introduction

High blood pressure, or hypertension, can persist for years without any symptoms while causing damage to blood vessels and thus increasing the risk of heart attack and stroke which are the leading causes of morbidity and mortality worldwide [1,2]. The precise etiology of hypertension is still unknown mainly due to the intricate and multifactorial nature of the disease that is the outcome of interactions between genetic, physiological and environmental factors. The regulation of sodium absorption in the kidney is critically important for the maintenance of sodium balance and the long-term regulation of arterial blood pressure. The better understanding of sodium transporting proteins along the nephron is therefore crucial for improvement of drug selectivity. The Na^+-Cl^- co-transporter (NCC) and the epithelial Na^+ channel (ENaC) play key roles in blood pressure (BP) regulation. The focus of this review is to highlight recent animal models modulating the activity of these proteins in the maintenance of kidney homeostasis and in diseased state.

NCC expression and regulation

Thiazide diuretics, the pharmacological inhibitors of NCC, are still one of the most efficient drugs in the treatment of hypertension [3]. NCC reabsorbs 5-10% of filtered sodium of the urine and can induce hypertension or hypotension depending on its activity. NCC is coded by the SLC12A3 gene [4-6] and is expressed in the kidney at the apical plasma membrane of epithelial cells in the distal convoluted tubule (DCT) and co-localizes with ENaC in the DCT2 [7]. Increased NCC phosphorylation correlates with its increased activity [8] as assessed through specific phospho-antibodies detecting threonine and serine residues in human or mouse [9,10]. Low salt diet [11], but also aldosterone, angiotensin-II, vasopressin and insulin increase the activity of NCC *in vivo*, whereas potassium load [12] and high salt diet [11] reduce NCC phosphorylation. Furthermore, ubiquitylation inhibits NCC by reducing the half-life of the co-transporter or the proteins that in turn modulate its function [13]. For more details, we like to refer to recent reviews that focus on the complex regulatory network modulating the activity of this co-transporter [14-17]. The degree of NCC activity plays a crucial role in blood pressure disorders such as pseudohypoaldosteronism type II (PHAII, also known as Gordon syndrome and familial hyperkalemic hypertension, FHHt) featuring hypertension, hyperkalaemia and metabolic acidosis [18] that can be cured by thiazide diuretics. Up to date, four mutated genes have been found in patients affected by PHAII, two coding for kinases (WNK1 and 4 [19]) and two coding for ubiquitinylases (KLHL3 and CUL3 [18]). On the other hand,

inactivating mutations in the SLC12A3 gene coding NCC cause the Gitelman's syndrome, an autosomal recessive salt-losing disorder characterized by hypokalemic metabolic alkalosis, hypomagnesemia, hypocalciuria and normal to low BP (see for review; [20,21]). In addition, some rare NCC mutations had been found that significantly reduced its basal activity resulting in a lower BP and decreased risk of hypertension [22]. This review focuses on transgenic mouse models where modulation in the NCC activity results in BP changes (Table 1).

NCC mouse models

The SLC12A3 (NCC) gene was first inactivated in the mouse in 1998 and this knock-out (KO) recapitulates some features of the Gitelman's syndrome such as hypocalciuria and hypomagnesemia. The knockout animals present with morphological changes in the DCT, but are able to compensate perturbations of sodium and fluid volume homeostasis [23]. In addition, NCC-KO mice are more sensitive to reduced dietary potassium [24] and are resistant to tacrolimus-induced hypertension [25]. In contrast, the overexpression of NCC in mice does not allow reproducing the features of PHAII probably because the phosphorylation of NCC is not affected [26]. Finally, NccT58M/T58M mice carrying the corresponding human (T60M) mutation causing Gitelman's-like syndrome do not properly respond to thiazide diuretics and exhibit lower levels of phosphorylated NCC that are not affected by a low-salt diet [27]. Moreover, homozygous *NCC*$^{Ser707X/Ser707X}$ knock-in mice carrying the mutation in the NCC gene corresponding to human p.Ser710X (c.2135C4A) found in Chinese Gitelman's syndrome patients, fully recapitulate the phenotype of human Gitelman's syndrome and the expression of epithelial Na^+ channel ENaC and the late DCT volume are significantly [28]. In summary, NCC mouse models clearly show the implication of this transporter in Na^+ handling thereby closely mimicking the human disorder.

NCC regulation by WNKs

The WNK1 gene encodes for L-WNK1, a ubiquitous kinase, and KS-WNK1 (kidney-specific WNK-1) expressed in the DCT. L-WNK1 inactivation causes cardiovascular defects resulting in embryonic lethality [29]. Mice that lack KS-WNK1 show an increased activity of NCC, Na^+ retention and elevated BP in response to high Na^+ challenge [30]. In contrast, mice overexpressing KS-WNK1 in the kidney exhibit reduced expression of total and phosphorylated NCC, renal Na^+ wasting and lower BP [30]. Interestingly, mice carrying a heterozygous deletion in the first intron of the WNK1 gene (WNK1+/FHHt) that leads to increased L-WNK1 expression present hypertension, hyperkalemia, hyperchloremic metabolic acidosis, and are sensitive to thiazide diuretics,

recapitulating all features of PHAII [31]. Thus, KS-WNK1 is a negative regulator of NCC in the control of Na^+ homeostasis and blood pressure, and the level of L-WNK1 rather than the ratio of L-WNK1 versus KS-WNK1, seems to be involved in the WNK1-related PHAII [30,31]. When *WNK1*$^{+/FHHt;WNK4-/-}$ mice were produced, the increased NCC activity, hypertension, and hyperkalemia of *WNK1*$^{+/FHHt}$ mice was maintained in the absence of WNK4 [32]. Single WNK4-KO mice exhibit normal BP and reduced NCC expression and phosphorylation and the sodium balance is maintained in these animals [33]. In addition, WNK4 hypomorphic (WNK4Hypo) mice in which the exon 7 of the Wnk4 gene was deleted do not present with hypokalemia and metabolic alkalosis, but exhibit salt-sensitive hypotension and decreased NCC phosphorylation [34]. In contrast, overexpression of wild-type WNK4 causes hypotension with hypocalciuria without changes in the other electrolytes, and NCC is also reduced [35]. However, if the mutant WNK4 carrying the Q562E mutation as found in PHAII patients is overexpressed (*WNK4*^{Q562E} Tg) or *WNK4*$^{D561A/+}$ knock-in mice carrying such mutation are produced, the phenotype is reversed and these mice develop hypertension, hyperkalemia and increased NCC activity [10,35,36]. Indeed, NCC deficiency corrects abnormalities in *WNK4*^{Q562E} Tg mice indicating that the phenotype of *WNK4*^{Q562E} Tg is completely dependent on NCC [35]. Moreover, when *WNK4*$^{D561A/+}$ mice were crossed with *NCC*$^{T58M/T58M}$ mice, the salt-wasting phenotype persisted [27]. Interestingly, when WNK4 is absent or decreased and accompanied by decreased NCC function, the activity of ENaC is augmented, suggesting a compensatory mechanisms in the control of sodium reabsorption [33,34]. In summary, WNK1 and WNK4 are essential regulators of NCC function and seem to modulate NCC in an independent manner.

NCC regulation by other kinases

Further kinases are involved in the regulation of NCC activity, such as SGK1, SPAK, and ORS1. Dietary NaCl restriction increases renal NCC phosphorylation in wild-type mice, but this response is attenuated in mice lacking the serum and glucocorticoid-inducible kinase 1 (SGK1). In contrast, high K^+ diet induces NCC suppression, and this response is increased in the Sgk1-KO[37]. SGK1 is ubiquitously expressed, and inducible and kidney-specific deletion of the Sgk1 gene causes an impaired Na^+ retention under a low-salt diet, despite higher plasma aldosterone levels. This phenotype is also characterized by a decrease in the BP, phosphorylation of NCC, and to a lesser extent that of ENaC [38]. Thus, SGK1 is involved in the regulation of NCC activity under dietary NaCl. Mice deficient for the sterile 20/SPS1-related proline/alanine-rich kinase, SPAK, exhibit hypotension and also present with hypokalemia, hypomagnesemia, and hypocalciuria recapitulating the

Gitelman's syndrome. Indeed, the NCC phosphorylation was strongly decreased in the kidney of SPAK-KO mice that also did not respond to thiazide [26,28,39]. In addition, SPAK knock-in animals carrying a homozygous mutation (T243A) that prevents phosphorylation of SPAK by WNK 1 and 4 [40], also exhibit reduced BP and phosphorylation of NCC pointing to a WNK-SPAK signaling pathway *in vivo* [41]. Finally, angiotensin-II appears to have an effect on NCC phosphorylation through the WNK4-SPAK-dependent signaling pathway [42]. The kinase oxidative stress-responsive kinase-1 (OSR1) has also been demonstrated *in vitro* to be a substrate for the WNK1 and 4 [43]. Complete and kidney tubule-specific OSR1-KO mice demonstrated that the Na^+-K^+-Cl^- cotransporter NKCC2, but not NCC, is the principal target of OSR1, and that the reduced phosphorylation of NKCC2 in the kidney tubule-specific OSR1-KO mice may cause hypotension and a Bartter-like syndrome in those animals [44]. Although *Wnk4*$^{D561A/+;KSP\text{-}Osr1\text{-}/\text{-}}$ double mutant mice continue to display a PHAII phenotype, the *Wnk4* $^{D561A/+;Spak\text{-}/\text{-}}$ double mutant animals are normotensive, indicating that SPAK and OSR1 are important in the control of the BP, and the SPAK-NCC pathway may play a major role in the development of PHAII [45]. Finally, NCC phosphorylation is almost completely suppressed in the *Wnk4*$^{D561A/+;SpakT243A/T243A;Osr1T185A/+}$ triple knock-in mice and the high BP, hyperkalemia and metabolic acidosis present in the *Wnk4*$^{D561A/+}$ mice are corrected in the triple knock-in [46]. In conclusion, WNKs, SGK1, SPAK and OSR1 are all regulators of NCC function, and the SPAK/OSR1 pathway most likely mediates the WNKs effects. KCNJ10 thereby determines the expression of the apical NCC in the early distal convoluted tubule (DCT1) in response to a variety of physiological stimuli [47].

NCC regulation by ubiquitin ligases

Nedd4-2 is a component of the E3 ubiquitin ligase complex that is involved in the ubiquitylation and degradation of the ENaC channel. Ronzaud and colleagues have recently demonstrated that renal NEDD4-2 deficiency in adult mice causes salt-dependent hypertension without hyperkalemia, which is mediated by NCC. Interestingly, ENaC activity is down-regulated in the Nedd4-2-KO mice which allows at least partially to maintain a normal Na^+/K^+ balance in these animals [13]. In addition, the same authors have provided evidence for a modulation of NCC by Nedd4-2 via an aldosterone-SGK1-Nedd4-2 pathway [48]. Kelch-like 3 (KLHL3) and Cullin 3 also belong to the E3 ubiquitin ligase complex. *KLHL3*$^{R528H/+}$ knock-in mice carrying the same mutation as autosomal dominant type PHAII patients present increased levels of WNK1 and WNK4 kinases, and increased NCC phosphorylation [49]. Finally, the nephron-specific deletion of Cullin 3 in mice increases WNK kinase levels and the abundance of phosphorylated NCC co-transporter

leading to salt-sensitive hypotension in the long term [50]. In conclusion, NEDD4-2, KLHL3 and Cullin 3 are negative regulators of NCC although there is evidence that NEDD4-2 does not belong to the Cullin 3-KLHL3 pathway [49,50].

The epithelial Na^+ channel (ENaC)

The rate-limiting step of trans-epithelial Na^+ reabsorption is the apical Na^+ entry through ENaC [51]. It occurs in the aldosterone-sensitive distal nephron (ASDN) [52] that consists of the DCT2, connecting tubule (CNT) and the entire collecting duct (CD) [52]. The importance of the amiloride-sensitive ENaC was unveiled by the analyses of two human diseases, namely the Liddle's syndrome (gain-of-function mutations), a form of severe and early onset hypertension, and its mirrored disease, pseudohypoaldosteronism, a salt wasting disease (PHA1; loss-of-function mutations)(see for review [53]). In the past years, several genetically engineered ENaC mice have been generated ranging from mild to severe salt wasting and salt retaining phenotypes [53]. One of the current research topics is to identify the site of ENaC action and function along the aldosterone-sensitive distal nephron *in vivo*. Whereas inactivation of ENaC in the distal part of the nephron revealed no requirement for sodium and potassium handling [54] despite presence of an electroneutral Na^+ reabsorption [55], more proximal inactivation within the nephron unveiled the cortical connecting tubule as site of aldosterone-mediated sodium reabsorption [56] and lead to a salt-losing phenotype as observed in patients with PHA-1 (pseudohypoaldosteronism type 1), although we cannot exclude that the DCT2 may be implicated as well. Interestingly, mice with a CCD-specific ENaC inactivation are resistant to develop lithium-induced nephrogenic diabetes insipidus highly suggesting that lithium passes selectively through ENaC channels in the CCD [57]. Recent research thus focuses on the DCT2 as the nephron segment with crucial aldosterone-sensitive ENaC activity partly co-localizing with the NCC.

ENaC regulation by Renin-Angiotensin-Aldosterone System (RAAS)

CD renin is a major regulator of filtered Na^+ exit to the urinary space through ENaC. Renin might modulate BP by two potential mechanisms. The first one suggests that renin leads to increased angiotensin-II type 1 receptor (AT1R) activation through angiotensin-II that is formed locally [58]. The second pathway is angiotensin II-independent via renin or pro-renin activation (**Fig.1**). The specific role of renin in CD was studied by using CD-specific renin-KO mice and suggests that CD renin in addition to angiotensin-II modulates BP as the angiotensin-II infused hypertension was reduced in the renin-KO mice. This effect seems to be associated with changes in ENaC expression [59].

Additionally, using angiotensin-II type 1 receptor-associated protein (ATRAP) knockout mice, it was shown that ATRAP promotes AT1R internalization and suppression of pathological activation of tissue AT1R signaling. ATRAP deficiency exacerbated angiotensin-II-mediated hypertension by pathological activation of renal tubular AT1R. This directly stimulates ENaC in the distal tubules and enhances sodium retention in an aldosterone-independent manner while NCC seems not to be implicated in this mechanism [60].

Aldosterone, by acting via the mineralocorticoid receptor (MR), can also affect Na^+ transport by regulating ENaC activity through serum- and glucocorticoid-regulated kinase (SGK1), suggesting the intriguing possibility that SGK1 is an integrator of insulin- and mineralocorticoid-regulated Na^+ transport [61-63] (Fig.1). However, Nesterov and co-workers used an aldosterone synthase-knockout model and showed that ENaC function is aldosterone-sensitive in CNT/CCD while in the DCT2/CNT it is largely independent from aldosterone [64]. It was also concluded that, in this model, the kidney can adapt to a physiologic K^+ load in an aldosterone independent way after activation of renal outer medullary K^+ channel and ENaC where angiotensin-II may contribute [65].

The phosphorylation of the ubiquitin-protein ligase Nedd4-2 by Sgk1 [66-71] inhibits the interaction between Nedd4-2 and ENaC. Such inhibition would then lead to reduced ubiquitylation, internalization, and degradation of ENaC. Faresse and colleagues used an acute adult kidney-specific Sgk1-KO mouse model and presented evidence for a role of Sgk1 in the regulation of NCC, and a decrease in the expression of β,γ-subunits and a mild effect on the α-subunit of ENaC. Moreover, they observed a slight reduction of α-, but not γ-ENaC cleavage. In addition, under low-Na^+ diet Sgk1-KO mice developed higher aldosterone levels, higher natriuresis and kaliuresis, and lower BP [13,38]. The renin-angiotensin-aldosterone system in the kidney can thus efficiently compensate for sodium loss through the colonic surface epithelium [72]. These ENaC knockouts develop a colon-specific pseudohypoaldosteronism type 1 with mineralocorticoid resistance without evidence of impaired potassium balance [72]. Equally, the colon-specific knockout of the serine protease CAP1/Prss8 and the constitutive knockout of the tyrosine kinase Janus kinase 3 (JAK3) lead through intestinal Na^+ loss, increased aldosterone release and subsequent stimulation of renal tubular Na^+ reabsorption [72,73].

ENaC regulation by insulin

Insulin increases the activity of ENaC in CD or DCT cell systems [74] and binds to the basolateral insulin receptor (IR) (**Fig.1**). Li and colleagues [75]

found that IR-KO mice exhibit altered plasma and electrolyte homeostasis under high- and low-sodium diet. Furthermore, they presented a significantly blunted natriuretic response to the amiloride-analog benzamil. Interestingly, BP was significantly lower in knockout versus wild-type mice under basal conditions. In a follow-up study, Pavlov and co-workers using single-channel analysis in freshly isolated split-open tubules showed *ex vivo* that the IR-KO mice have significantly lower ENaC activity compared to their wild-type when animals were fed either normal- or sodium-deficient diets [76]. Overall, these two studies support a physiological role *in vivo* for insulin through its classic receptor in the modulation of BP and electrolyte homeostasis through ENaC.

ENaC regulation by vasopressin

Vasopressin targets two seven-transmembrane domain G-protein coupled receptor types, V1 and V2. Activation of V2 receptors in the distal nephron by vasopressin stimulates free water reabsorption by promoting cAMP-dependent trafficking of aquaporin 2 water channels to the luminal membrane of principal cells. In addition, vasopressin via V2 receptors also modulates discretionary sodium reabsorption across principal cells mediated by ENaC. This facilitates free water reabsorption by supporting the axial corticomedullary hyperosmotic gradient [77-80]. Recently the adenylyl cyclase VI was identified to implicated in vasopressin-stimulated ENaC activity [81].

Discussion and perspectives

Most molecular players seem to be identified in the Gitelman's and Gordon syndromes that finally result in decreased or increased NCC activity, respectively. As an outcome, NCC-dependent NaCl reabsorption is either significantly reduced (Gitelman's syndrome) or increased (Gordon syndrome). Grimm and coworkers described that SPAK is important to maintain the structural integrity of the distal convoluted tubule and if absent results in "structural remodeling" or dystrophy [39] (Fig.2). Such DCT1-specific dystrophic responses have been first described in thiazide-diuretic rats [82] and later on in parvalbumin-deficient [83] and NCC-deficient mice [84,85] as wll as in mice overexpressing wild-type WNK4 [35]. This is accompanied by a reduction in NCC protein abundance most likely due to the reduction in cell mass [39] (Fig. 2). Not surprisingly, this is accompanied by presence of all apical ENaC subunits [84] and increased ENaC activity [86]. No change in ENaC protein expression is found in the WNK4 wild-type transgenic mice [35]. In the Gordon syndrome, a hypertrophic response has been reported with an increase in the cellular mass and lumen surface area of DCT1 [35] (Fig.2). Consequently, total NCC surface expression is increased, although ENaC

abundance as evidenced by ENaC immunostaining is not changed [35]. Crossbreeding with NCC-KO mice completely corrected the hypertrophic DCT1 response [34]. Although these studies do not establish a direct implication of the above mentioned players in this response, they seem to be part of signaling pathways that maintain the integrity of DCT1 that controls NCC-, but therefore also affects ENaC-dependent sodium absorption. Additionally, SPAK-deficiency seems to negatively regulate salt reabsorption through increased NKCC2 phosphorylation/activity in TAL segment [39]. Thus, such crosstalk amongst transport proteins in different nephron segments and structural remodeling should be considered and analyzed in further gene targeting studies. Van Huysse and coworkers proposed the implication of increase ENaC expression in hypertension, although a constitutive knockout model has been used, that may not allow to exclude the implication of other organs lacking Nedd4-2 in the observed phenotype [87]. This has to be further evaluated by appropriate genetically-engineered conditional animal models. On the other side, the block of ENaC-mediated sodium absorption in other organs like e.g., in colon could be of therapeutical interest in chronic kidney disease (CKD). Here, patients exhibit decreased ability of the kidneys to excrete sodium and the diuretics have only limited success. The pharmacological inhibition of colonic ENaC may lead to increased intestinal excretion of sodium to finally better maintain sodium homeostasis.

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Figure legends

Figure1

Proposed crosstalk between NCC and ENaC activity in a mouse DCT2 cell by A), aldosterone, B) angiotensin II, C) insulin, and D) vasopressin.

Figure2

Dystrophic and hypertrophic responses in DCT1, DCT2, and CNT segments in models of A) Gitelman's syndrome (salt wasting), and B) Gordon syndrome

(salt retention phenotype) implicating the WNKs-SPAK-NCC pathway. Arrows indicate either dystrophy (reduction) or hypertrophy (increase of cellular mass). Not confirmed trophic responses are indicated by question marks.

Table 1

Mouse models unveiling the regulation of NCC in the control of blood pressure.

Table 2

Mouse models unveiling the regulation of ENaC in the control of blood pressure.

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Highlighted References

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

* of special interest

** of outstanding interest

****[13]Renal tubular NEDD4-2 deficiency causes NCC-mediated salt-dependent hypertension.**

Kidney-specific Nedd4-2 knockout mice exhibit a new form of mild, salt-sensitive PHAII-like syndrome with NCC upregulation. This unveils its role in mediating NCC ubiquitylation and endocytosis. Modulating Nedd4-2 activity may present a novel treatment of Gitelman syndrome.

****[32]WNK-SPAK-NCC cascade revisited : WNK1-related familial hyperkalemic hypertension results from an increased expression.**

Using transgenic models, the authors proposed a new model for the regulation of NCC by WNK kinases. They provide evidence that WNK1 stimulates NCC in a WNK4-independent manner.

***[31]WNK1-related familial hyperkalemic hypertesnion results from an increased expresseion of L-WNK1.**

The WNK1-FHHt mouse model fully recapitulates the disease and thus nicely underlines the role of WNK1 in ion homeostasis and blood pressure.

***[27]Phosphorylation regulated NCC stability and transporter activity in vivo.**

Knockin mice carrying a defectively phosphorylated NCC exhibit typical features of Gitelman's syndrome and a blunted response to thiazide. The in vivo study demonstrates that blocking NCC phosphorylation may present a promising antihypertensive drug target.

****[49]Impaired degradation of WNK1 and WNK4 kinases causes PHAII in mutant.**

Using KLHL3 knockin mice, the authors demonstrate that both WNK1 and WNK4 are physiologically regulated by KLHL3-Cullin3-mediated ubiquitination in vivo.

***[50]Hyperkalemic hypertension associated cullin 3 promotes WNK signaling.**

Using inducible renal tubule-specific Cul3 knockout mice, the authors unveil the essential role of CUL3 in kidney tubules.

***[60]Deletion of the angiotensin II type 1 receptor-associated protein enhances renal sodium.**

Systemic ATRAP deficiency exacerbated angiotensin II-induced hypertension by promoting sodium retention in an aldosterone-independent manner.

***[64]Aldosterone-dependent and –independent regulation of the epithelial.**

This is the first ex vivo patch-clamp approach in the DCT2/CNT that clearly demonstrates an aldosterone-independent function of ENaC.

***[65]Mechanisms of Renal Control of Potassium Homeostasis in Complete Aldosterone Deficiency.**

This study nicely illustrates the redundancy and complexity of renal mechanism to upregulate renal K+ excretion. Loss of aldosterone in aldosterone-synthase knockout mice is compensated by angiotensin-II.

****[76]Regulation of ENaC in mice lacking renal insulin receptors in the collecting duct.**

This study provides new in vitro and in vivo information about the role of the insulin receptor for ENaC function in the CCD.

***[81]Adenylyl cyclase VI mediates vasopressin-stimulated ENaC activity.**

Using knockout mice, these data revealed adenylyl cyclase VI as a key mediator of vasopressin-stimulated ENaC activity in the kidney.

***[72]Colon-specific deletion of epithelial sodium channel causes sodium loss.**

This paper nicely illustrates the role of colonic ENaC in salt homeostasis and the compensation by the kidney. The block of colonic ENaC may therefore help to maintain sodium and potassium homeostasis in CKD patients.

Phenotype of transgenic mouse models modulating NCC activity and blood pressure 11 Nov 2014

Gene	Genetic modification	Blood pressure	NCC activity (phosphorylation)	Electrolytes	References
cullin $3^{-/-}$	inducible and kidney-specific	↓ (salt-sensitive)	↑	hypochloremic alkalosis	[50]
$Fgf23^{-/-}/VDR^{-/-}$; $\alpha Klotho^{-/-}/VDR^{-/-}$	complete and constitutive	↓	↓	↑ Na^+ excretion	[88]
protein phosphatase 1 inhibitor-$1^{-/-}$	complete and constitutive	↓	↓	normal Na^+/K^+ balance	[89]
$Ncc^{T58M/T58M}$; $Wnk4^{D561A/+}$	complete and constitutive	↓	↓	hyperkalemia and metabolic acidosis	[27]
adenylyl cyclase $6^{-/-}$	complete and constitutive	↓	prevented increase following vasopressin treatment	↑ K^+ excretion	[90]
$Spak^{-/-}$	complete and constitutive	↓	↓	↑ Na^+ excretion (Na^+-deficient diet)	[39]
$WNK3^{-/-}$	complete and constitutive	↓ (salt-sensitive)	unchanged	none	[91]
$Sgk1^{-/-}$	inducible and kidney-specific	↓	↓	↑ Na^+, K^+ excretion (Na^+-deficient diet)	[38]
$Ncc^{-/-}$; $Pendrin^{-/-}$	complete and constitutive	↓	abolished	metabolic alkalosis	[92]
$Osr1^{-/-}$ and KSP-$Osr1^{-/-}$	$Osr1^{-/-}$: complete and constitutive KSP-$Osr1^{-/-}$: kidney-specific	$Osr1^{+/-}$: ↓ KSP-$Osr1^{-/-}$: normal	$Osr1^{+/-}$: ↑ KSP-$Osr1^{-/-}$: unchanged	$Osr1^{+/-}$: normal Na^+/K^+ balance KSP-$Osr1^{-/-}$: hypokalemia	[44]
$Spak^{-/-}$	complete and constitutive	↓ (salt-sensitive)	↓	normal Na^+/K^+ balance	[26,93]
$Ncc^{S707X/S707X}$	complete and constitutive	↓	absent NCC expression	Hypokalemia, hypomagnesemia, ↑ Mg^{2+} and K^+ fractional excretion, hypocalciuria, metabolic alkalosis	[94]
$Spak^{-/-}$	complete and constitutive	↓	↓	hypokalemia, hypomagnesemia, and hypocalciuria	[28]
$Spak^{T243A/T243A}$	complete and constitutive	↓ (salt-sensitive)	↓	normal Na^+/K^+ balance	[41]
$Sgk1^{-/-}$	complete and constitutive	↓ (salt-sensitive)	↓ (Na^+-deficient diet)	normal Na^+/K^+ balance	[37,95]
$Wnk4^{Hypo}$	complete and constitutive	↓ (salt-sensitive)	↓	↑ Na^+ and K^+ excretion (low-salt diet)	[34]
Pendrin (Slc26a4) $^{-/-}$	complete and constitutive	↓	non determined	↑ Na^+ excretion	[96,97]
$Ncc^{-/-}$	complete and constitutive	↓ (salt-sensitive)	abolished	hypomagnesemia and hypocalciuria	[23]
KS-Wnk1 Tg and KS-$Wnk1^{-/-}$	KS-Wnk1 Tg: kidney-specific KS-$Wnk1^{-/-}$: complete and constitutive	KS-WNK1 Tg: ↓ KS-$WNK1^{-/-}$: ↑	KS-WNK1 Tg: ↓ KS-$WNK1^{-/-}$: ↑ (salt-sensitive)	KS-Wnk1 Tg: ↑ Na^+ excretion KS-$Wnk1^{-/-}$: ↓ Na^+ excretion	[30,98]
$Wnk4^{WT}$ Tg and $Wnk4^{Q562E}$ Tg	complete and constitutive	$Wnk4^{WT}$: ↓ $Wnk4^{Q562E}$: ↑	$Wnk4^{WT}$: ↓ (expression) $Wnk4^{Q562E}$: ↑ (expression)	$Wnk4^{WT}$: hypokalemia, hypocalciuria $Wnk4^{Q562E}$: hyperkalemia, hypercalciuria	[35]
11b-hydroxysteroid dehydrogenase type 2 $(Hsd11b2)^{-/-}$	complete and constitutive	↑	↑	transient Na^+ retention	[99,100]
Kelch-like 3 $(Klhl3)^{R528H/+}$	complete and constitutive	↑	↑ (salt-sensitive)	hyperkalemia and metabolic acidosis	[18,49]
$Wnk1^{+/FHHt}$; $Wnk4^{-/-}$	complete and constitutive	↑	↑	hyperkalemia	[32]
$Wnk1^{+/FHHt}$	complete and constitutive	↑	↑	hyperkalemia and acidosis	[31]
Pendrin Tg	intercalated cells-specific	↑ (Cl^--sensitive)	↓	↑ Cl^- absorption	[101]
$Wnk4^{D561A/+}$; KSP-$Osr1^{-/-}$ and $Wnk4^{D561A/+}$; $Spak^{-/-}$	$Wnk4^{D561A/+}$: complete and constitutive $Osr1^{-/-}$: kidney-specific $Spak^{-/-}$: complete and constitutive	$Wnk4^{D561A/+}$; KSP-$Osr1^{-/-}$: ↑ $Wnk4^{D561A/+}$; $Spak^{-/-}$: normal	$Wnk4^{D561A/+}$; KSP-$Osr1^{-/-}$: ↑ $Wnk4^{D561A/+}$; $Spak^{-/-}$: normal	$Wnk4^{D561A/+}$; KSP-$Osr1^{-/-}$: exaggerated salt excretion in response to thiazide $Wnk4^{D561A/+}$; $Spak^{-/-}$: normal responses to thiazide	[45]
$Nedd4L^{-/-}$	inducible and kidney-specific	↑ (salt-sensitive)	↑	normal Na^+/K^+ balance	[13]
$Wnk4^{Q562E}$ Tg	complete and inducible	↑	↑	hyperkalemia	[36]
β2-adrenergic receptor $(AR)^{-/-}$	complete and constitutive	suppressed hypertension following high Na^+ diet	prevented increase following high Na^+ diet	normal Na^+/K^+ balance	[102]
$Wnk4^{D561A/+}$	complete and constitutive	↑	↑	metabolic acidosis and hyperkalemia	[10]

Phenotype of transgenic mouse models modulating NCC activity and blood pressure 11 Nov 2014

angiotensin II type 1 receptor-associated protein (Agtrap)Tg	kidney-dominant	suppressed hypertension (angiotensin II infusion)	↓ (angiotensin II infusion)	↑ Na^+ excretion	[103]
angiotensin-converting enzyme (ACE)$^{-/-}$	kidney-specific	suppressed hypertension (angiotensin II infusion)	prevented increase (angiotensin II infusion)	prevented Na^+ and H_2O retention induced by angiotensin II infusion	[104]
Wnk4$^{-/-}$	complete and constitutive	normal	↓	↑ Na^+, K^+, and Cl^- excretion	[33,105]
Ncc$^{-/-}$ and Ncc Tg	Ncc Tg: Ncc promoter Ncc$^{-/-}$: complete and constitutive	Ncc$^{-/-}$: resistant to tacrolimus-induced hypertension Ncc Tg: increased tacrolimus-induced hypertension	Ncc$^{-/-}$: absent Ncc Tg: normal	Ncc$^{-/-}$: hypomagnesemia and hypocalciuria Ncc Tg: normal Na^+/K^+ balance	[23,25]
Ncc Tg	Ncc promoter	normal	normal	normal Na^+/K^+ balance	[85]
Wnk4$^{D561A/+}$; Spak$^{T243A/T243A}$; Osr1$^{T185A/+}$	complete and constitutive	normal	almost completely abolished	normal Na^+/K^+ balance	[46]
Ncc$^{-/-}$	complete and constitutive	normal	abolished	hypokalemia (low-K^+ diet)	[24]

			ENaC			
Gene/protein	**Genetic modification**	**BP (Treatment)**	**Expression**	**Activity**	**Electrolytes**	**Ref.**
AS$^{-/-}$ (Aldosterone synthase)	Constitutive Complete	↓(standard diet) n.d. (2% K+ diet) n.d. (Inhibition of AT1R)	↑(2% K+ diet) ↓ apical ENaC (Inhibition of AT1R)	n.d.	↑plasma K^+ (5% K^+) ↑plasma K^+ (Inhibition of AT1R) ↓urine K^+ (Inhibition of AT1R)	[65, 106]
Renin$^{-/-}$	Constitutive Conditional: Collecting-duct	↓ (Ang-II)	↓ (γ)	n.d.	Unchanged	[59]
JAK3$^{-/-}$ (Janus kinase 3)	Constitutive Complete	↓	n.d.	↓(colonic)	↑feces Na^+ ↓urine Na^+	[73]
ATP6V1B1$^{-/-}$ (B1 proton pump subunit)	Constitutive Complete	↓	↓ (α,γ/CCD) ↑ (α,γ/MCD)	↓(CNT/CCD) ↑ (MCD)	Hypokalemia	[107]
IR$^{-/-}$ (Insulin receptor)	Constitutive Conditional: Principal cells of the collecting duct	↓	Unchanged	↓	n.d.	[76]
AC6$^{-/-}$ (Adenylyl cyclase type VI)	Constitutive Conditional: Principal cells of the collecting duct	↓(Vasopressin)	n.d.	n.d.	Unchanged	[81]
IR$^{-/-}$ (Insulin receptor)	Constitutive Conditional: Principal cells of the collecting duct	↓	↓ (β)	↓	↑plasma K^+ (HS)	[75]
Sgk1$^{-/-}$ (Serum- and glucocorticoid-regulated kinase 1)	Inducible Conditional: Renal tubule	↓(NS and LS)	↓	n.d.	Unchanged (NS) ↑urine Na^+ and K^+ (LS)	[38]
ATRAP$^{-/-}$ (Angiotensin II type 1 receptor (AT1R)–associated protein)	Constitutive Complete	↑ (Ang-II)	↑ (α)	n.d.	↑urine Na^+	[60]
Cyp2c44$^{-/-}$ (Cytochrome P-450, family 2, subfamily c, polypeptide 44 epoxygenase)	Constitutive Complete	↑(HS)	n.d.	↑	↓urine Na^+ ↓ plasma K^+	[108]
Cyp2c44$^{-/-}$ (Cytochrome P-450, family 2, subfamily c, polypeptide 44 epoxygenase)	Constitutive Conditional: Collecting-duct	↑(5% KCl)	n.d.	↑	↓urine Na^+	[109]
PKCa$^{-/-}$ (Protein kinase Cα)	Constitutive Complete	↑(HS)	↑membrane	↑	n.d.	[110]
TRPV1-/- (Renal transient receptor potential vanilloid 1)	Constitutive Conditional: cortical collecting duct	↑(HS)	↑ (α,β)	n.d.	- urine Na^+	[111]
Tg$^{(B1\text{-}hPDS)}$ (Overexpression the	Constitutive Conditional:	↑(HS)	↓ (α,γ)	↑	↑urine Na^+ ↑urine Cl^-	[101]

chloride transporter pendrin)	Intercalated cells of the distal nephron					
Nedd4-2$^{-/-}$ (E3 ubiquitin ligase NEDD4-2)	Inducible Conditional: Renal tubule	↑(HS)	n.d.	n.d.	Unchanged	[13]
BK-β4$^{-/-}$ (Potassium large conductance calcium-activated channel, subfamily M, beta member 4)	Constitutive Complete	↑(HS)	n.d.	↑	↓urine Na^+	[112]
AMPK-1$^{-/-}$ (AMP-activated protein kinase β1-subunit)	Constitutive Complete	Unchanged (salt deficiency)	↑ (apical)	n.d.		[113]
Nedd4-2$^{-/-}$ (E3 ubiquitin ligase NEDD4-2)	Inducible Conditional: Renal tubule	Unchanged	↑ (β,γ) ↓ (α proteolysis)	n.d.	n.d.	[13]
AC6$^{-/-}$ (Adenylyl cyclase type VI)	Constitutive Conditional: Principal cells of the collecting duct	Unchanged	↓	n.d.	Unchanged	[81]
IR$^{-/-}$ (Insulin receptor)	Constitutive Conditional: Principal cells of the collecting duct	No effect (Insulin)	n.d.	n.d.	↑urine Na^+	[75]
P2Y2 receptor (ATP/UTP-activated P2Y receptor)	Total	Unchanged (standard diet) n.d. (Lithium)	↓ (α)	n.d.	↓urine Na^+ ↓urine K^+	[114]
PDS$^{-/-}$ (Chloride transporter pendrin)	Constitutive Complete	Unchanged (standard diet) n.d (Aldosterone AngII)	n.d.	n.d.	n.d.	[115]
B1R$^{-/-}$, B2R$^{-/-}$ (Bradykinin receptors)	Constitutive Complete	↑ [116] n.d. (NS)	n.d.	↑		[117] [116]
AS$^{-/-}$ (Aldosterone synthase)	Constitutive Complete	↓(standard diet) [106] n.d. (NS) n.d. (LS)	n.d.	↓ (CNT/CCD) (NS) Unchanged (DCT2/CNT) (NS) Unchanged (CNT/CCD, DCT2/CNT) (LS)	n.d.	[64] [106]
Nedd4-2$^{-/-}$ (E3 ubiquitin ligase NEDD4-2)	Constitutive Complete	↑(HS) n.d.	↑ (brain)	n.d.	n.d.	[118] [13]

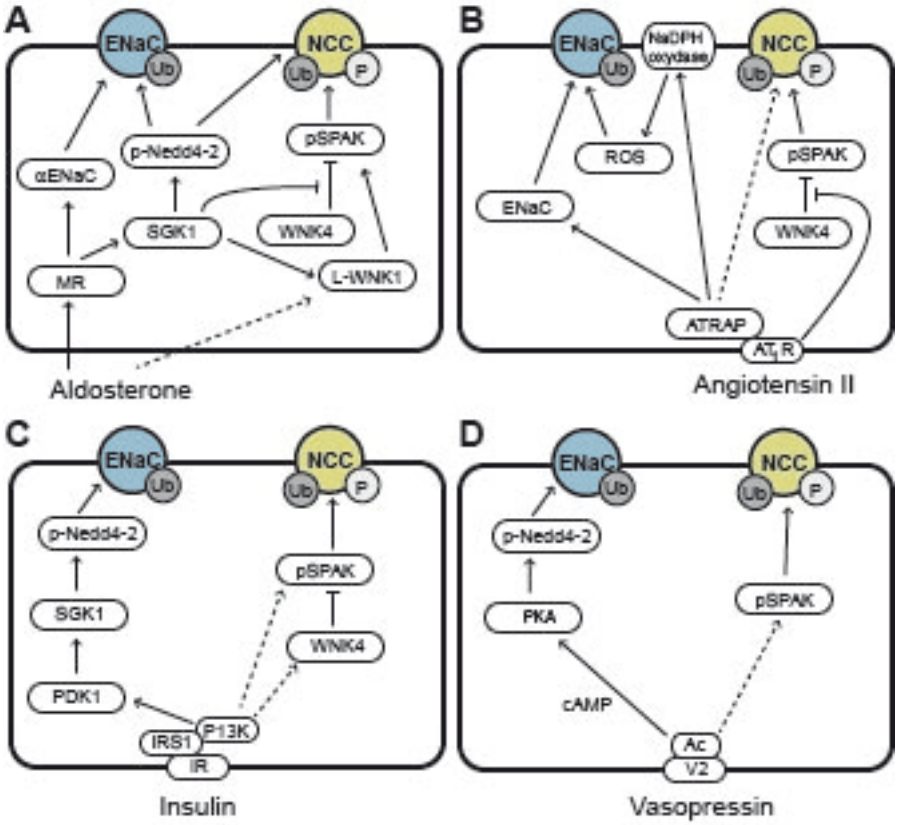

Figure 1

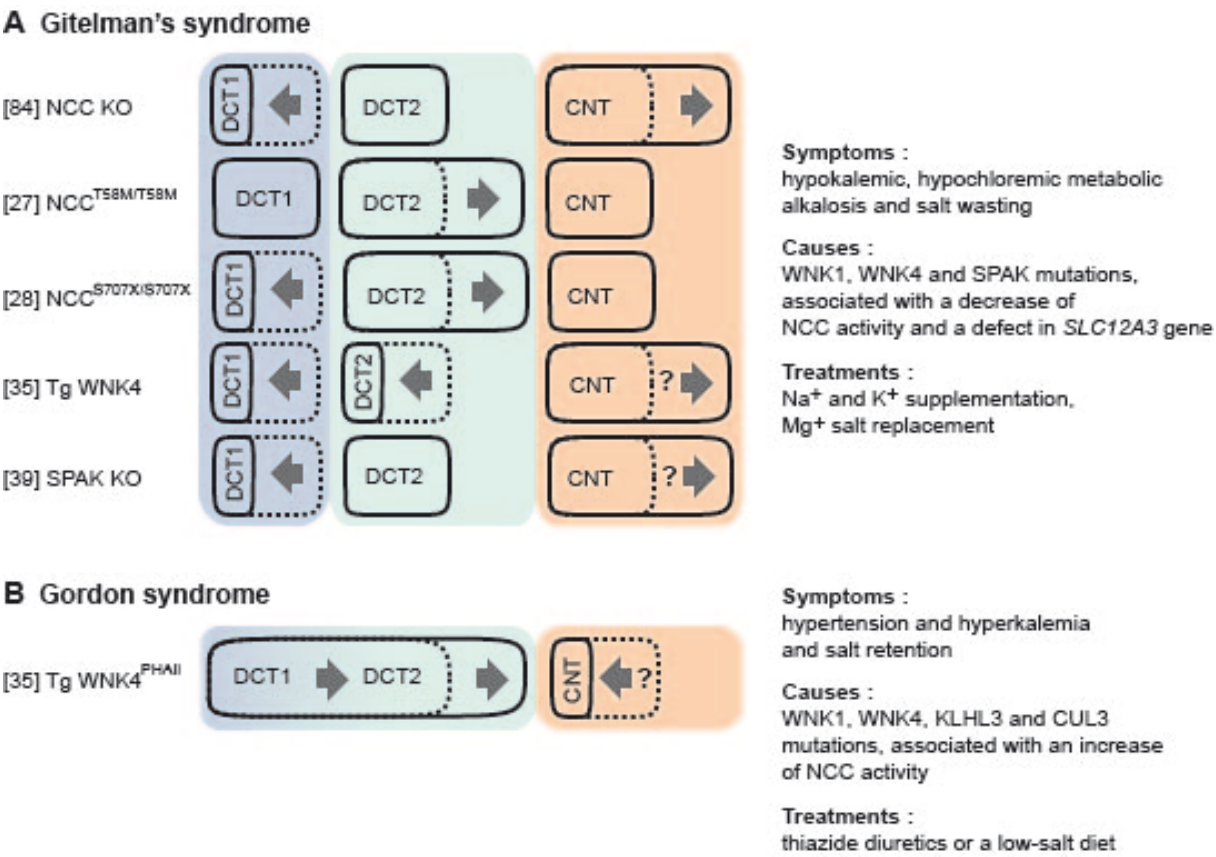

Figure 2