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Genetic dissection of sodium and potassium transport along the aldosterone- sensitive distal nephron: importance in the control of blood pressure and hypertension

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Key words

Hypertension; aldosterone; angiotensin; diuretics; transgenic mouse models.

Abstract

In this review, we discuss genetic evidence supporting Guyton's hypothesis stating that blood pressure control is critically depending on fluid handling by the kidney. The review is focused on the genetic dissection of sodium and potassium transport in the distal nephron and the collecting duct that are the most important sites for the control of salt and potassium balance by aldosterone and angiotensine II. Thanks to the study of Mendelian forms of hypertension and their corresponding transgenic mouse models, three main classes of diuretic receptors (furosemide, thiazide, amiloride) and the main components of the aldosterone- and angiotensin-dependent signaling pathways were molecularly identified over the past 20 years. This will allow to design rational strategies for the treatment of hypertension and for the development of the next generation of diuretics.

Introduction

Hypertension is the most common disease in the human population, affecting over 1 billion individuals worldwide, and is one of the major treatable risk factors in cardiovascular diseases including stroke, myocardial infarction, heart and kidney failure. Despite the importance of hypertension as a cause of cardiovascular-renal disease, its pathogenesis is largely unknown. Four lines of evidence have verified Guyton's 30-year old hypothesis stating that blood pressure (BP) is critically dependent on salt handling by the kidney: i) physiological evidence: the pressure-natriuresis relationship established by Guyton; ii) pharmacological evidence: the introduction of diuretics (thiazide) in the early 60 as first line and, for the first time, effective treatment of essential hypertension; iii) genetic evidence in animal experiments (rodents): kidney cross transplantation between hypertensive strains and their corresponding "wild type" strains indicated that the hypertensive phenotype always "follows the kidney" from the hypertensive strain; iv) genetic evidence in humans: mutations with Mendelian transmission in 18 genes have now been identified as genes causing a salt- loosing (hypotensive) or a salt -

retaining (hypertensive) phenotype. All genes map to the renin-angiotensin-aldosterone system (RAAS). Molecular and genetic approaches have begun to define the key players and molecular pathways determining variations in BP. The major sodium (NKCC2, NCC and ENaC) and potassium (ROMK) transporters are expressed in the distal nephron and are the targets for all of the clinically useful diuretics - furosemide/bumetanide inhibit NKCC2, thiazides inhibit NCC and amiloride blocks the ENaC. Importantly, molecular/genetic approaches have also begun to define the signalling pathways regulating and integrating these cation transporter activities and their roles in determining blood pressure. Aldosterone and Angiotensin II signaling pathways are expressed in the distal nephron and some of their components have been found to be mutated in some Mendelian form of human hypertension (mineralocorticoid receptor MR, the 11β–HSD2 that protects MR from illicit occupation by cortisol, WNK1, WNK4, Cullin3, KHEL3). Several mouse transgenic animals (classical transgenic, knock-in and knockout) have been generated to validate and study the role of these key players in both renal salt handling and blood pressure regulation. A number of relevant and extensive reviews have been published covering different aspects of the genetics of hypertension in human and animal models [1-6]. In the present review we will discuss recent advances that have been made in this field focusing on the control of sodium and potassium balance along the renal distal nephron and the collecting duct that are tightly regulated by aldosterone and angiotensin. We will focus on experimental evidence obtained by genetic dissection of sodium and potassium transport along these nephron segments using knock out or knock in transgenic mouse models that mimick human salt retaining or salt loosing syndromes with an hyper- or hypotensive phenotype respectively. These human diseases and their corresponding animal models have defined the most significant steps that are limiting in the action of aldosterone and angiotensin. These limiting steps represent the best drug targets for the development of novel therapies specifically for the next generation of diuretics that remain a class of drugs of major clinical importance for the treatment of hypertension.

Distal nephron and collecting duct: the Aldosterone- Sensitive Distal Nephron

The nephron is the functional unit of the kidney (**Figure 1)**. In human, 180 liters of fluid is filtered per day through the glomerulus (G). Around 60% will be reabsorbed in the proximal tubule (PCT and PST) (2), 30% in the Henle's loop (TL and TAL) (4), 9% in the Distal Convoluted Tubule (DCT), the Connecting Tubule (CNT) and the Collecting Duct (CD) leaving 1.8 liter/day of final urine. Over 90% of renal ATP is used for fluid reabsorption driven by the Na,K-ATPase. Sodium and potassium balance are achieved through the final regulation of sodium and potassium transport in the distal nephron and the collecting duct under hormonal

control (aldosterone, vasopressin, angiotensin, insulin, etc). DCT cells, CNT cells and CD principal cells are the main cells involved in hormonal regulation. Most of the fine regulation of sodium and potassium transport takes place in the Thick Ascending Limb (TAL) (or Distal Straight Tubule), the Distal Convoluted Tubule (DCT), the Connecting Tubule (CNT) that branch 10 to 12 nephrons onto one collecting duct (CD) (**Figure 2**). Aldosterone is a mineralocorticoid hormone that plays a critical role in achieving sodium and potassium balance by controlling sodium reabsorption and potassium secretion in the kidney. Glucocorticoid receptor (GR) is ubiquitously expressed in the glomerulus, the entire nephron and in the collecting duct whereas the mineralocorticoid receptor (MR) is expressed in specific segments of the nephron: the Thick Ascending Limb (TAL), the Distal Convoluted Tubule (DCT1 and DCT2), the Connecting Tubule (CNT) and in the Collecting Duct (CD). Ackerman *et al.* [7] using highly specific antibodies against MR and GR showed that MR is coexpressed with GR in TAL, DCT, CNT and CD. Mineralocorticoid specificity is insured by the coexpression of 11β-HSD2 that metabolize cortisol or corticosterone in an inactive metabolite preventing the illicit occupation of MR by glucocorticoids. The expression of 11β-HSD2 is strong in DCT2, CNT and CD but decreases sharply in DCT1 and TAL. Based on the criteria of MR/11β-HSD2 coexpression, the Aldosterone Sensitive Distal Nephron (ASDN) (**Figure 2**) has been defined [8, 9] as comprising: the distal part of DCT (DCT2), the CNT and the CD, anatomically and developmentally distinct from the nephron but expressing similar regulation by aldosterone. The role of aldosterone in TAL is not yet well defined but it is interesting to note that the Angiotensin II receptor (AGTR1) expression fully overlaps that of MR suggesting that some of the synergistic action of the two hormones depend on the coexpression of MR and AGTR1 in TAL and downstream to the end of CD. Here, we will focus primarily on the role of NKCC2, NCC, ROMK, BK and ENaC on sodium and potassium transport and their regulatory proteins (MR/GR, 11β-HSD2, Sgk-1, Nedd 4-2, WNK1, WNK4, CUL3, KLHL3).

Transporters

Salt loosing syndromes

Bartter syndrome

As reviewed by Hebert [10] and Jentsch [11] Bartter syndrome represents a group of autosomal recessive disorders that are characterized by markedly reduced or absent salt transport by the thick ascending limb of Henle. Consequently, individuals with Bartter syndrome exhibit renal salt wasting and lowered blood pressure, hypokalemic metabolic alkalosis and hypercalciuria with a variable risk of renal stones. Five genes have been associated with the disease (**Table 1**). These include i) SLC12A2, the sodium-potassium-chloride co-transporter

(NKCC2) (Type I Bartter) ; ii) KCNJ1, the potassium ion channel (ROMK) (Type II Bartter); iii) CLCNKB, the basolateral chloride ion channel (ClCKb)(Type III Bartter) ; iv) BSND barttin (Type IV Bartter). Barttin is a β subunit that is required for the trafficking of CLCNKB channel to the plasma membrane in both the thick ascending limb and the marginal cells in the scala media of the inner ear that secrete potassium ion-rich endolymph. Loss-of-function mutations in BSND thus cause Bartter syndrome with sensorineural deafness ; v) CASR a extracellular calcium ion-sensing receptor. Gain-of-function mutations in this receptor can result in a Bartter phenotype (Type V Bartter) because activation of this G protein-coupled receptor inhibits salt transport in the thick ascending limb. As shown in Table 1, transgenic mouse models mimic Bartter syndrome well Type I [12], Type II [13], Type IV [14] and to some extent Type III [15].

Gitelman syndrome

As recently reviewed by Knoers and Levtchenko [16] Gitelman's syndrome (GS), also referred to as familial hypokalemia-hypomagnesemia, is characterized by hypokalemic metabolic alkalosis in combination with significant hypomagnesemia and low urinary calcium excretion. Mutations in the solute carrier family 12, member 3 gene, SLC12A3, which encodes the thiazide-sensitive NaCl cotransporter (NCC), are found in the majority of GS patients [16]. At present, more than 140 different NCC mutations throughout the whole protein have been identified. As shown in Table 1, the corresponding mouse model shows a mild Gitelman like phenotype. In a small minority of GS patients, mutations in the CLCNKB gene, encoding the chloride channel CLCNKB have been identified [16] but the corresponding mouse knock in model has not yet been engineered. This could help to understand why some mutations in CLCNKB generate a Bartter like phenotype while others generate a Gitelman like phenotype.

PHA-1

As reviewed by Bonny and Rossier [17] and Zennaro *et al.* [18] loss-of-function mutations in two key components of the aldosterone response, the mineralocorticoid receptor (MR) and the epithelial sodium channel ENaC, cause type 1 pseudohypoaldosteronism (PHA-I), a rare genetic disease of aldosterone resistance characterized by salt wasting, dehydration, failure to thrive, hyperkalemia and metabolic acidosis. The *renal form* of PHA-I is caused by mutations in MR. The phenotype tend to be milder with age [18]. The *systemic form* of PHA-1 is caused by mutations in ENaC either α or β or γ subunits. It combines a severe perinatal salt loosing syndrome, metabolic acidosis, life threatening hyperkalemia with a failure to clear fluid from lung (wet lung syndrome) [17]. As shown in Table 1, constitutive germ line inactivation of either α [19], β [20] or γ [20, 21] ENaC subunit in mice causes perinatal lethality (100% 48 h after birth) with a renal [19-21] combined with a lung phenotype [19, 21]. The data indicate that there

is no redundancy beween any of the 3 homologous subunits of ENaC confirming *in vitro* experiments in Xenopus Laevis oocytes. The early lethality precludes any observation of the relative role of each subunit in adult kidney. Nephron and/or collecting duct specific promotor allows to conditionally inactivate any gene of interest along the nephron or the collecting duct . This approach allows a true « genetic dissection » of the role of ENaC α subunit along the ASDN. Using a *HoxB7* promotor exclusively expressed in the entire collecting duct combined to Cre/lox technology, it was possible to delete conditionaly and efficiently the ENaC α subunit in this part of ASDN [22]. Surprisingly neither sodium nor potassium balance were impaired even when the mice were challenged with low salt or high potassium diet. The activity of ENaC in CD (at least in mice) is dispensable and could be compensated by other transport systems (see below). Interistingly, ENaC expressed in the CD plays a critical role in lithium-induced nephogenic diabetes insipidus (NDI) since CD specific deletion of α ENaC fully protect against lithium toxicity [23]. To investigate the relative importance of ENaC-mediated sodium absorption in the CNT, mice lacking α ENaC in the aquaporin 2-expressing CNT and CD were generated. With dietary sodium restriction, these mice experienced significant weight loss, increased urinary sodium excretion, and hyperkalemia [24]. Plasma aldosterone levels were significantly elevated under both standard and sodium-restricted diets. α ENaC expression within the CNT/CD is crucial for sodium and potassium homeostasis and causes signs and symptoms of systemic PHA-1 if missing [24]. However the phenotype was relatively mild compared to the neonatal human or mice phenotype raising the question of the importance of DCT2 the remaining nephron segment still expressing ENaC under this experimental condition. Since ENaC is co-expressed with NCC in DCT2 under the control of the aldosterone dependent-signaling pathway (Figure 1), this very short segment could play a major role in achieving sodium and potassium balance. Recently, it has been possible to induce gene inactivation along the entire nephron and the collecting duct (excluding the glomerules) using a doxycycline-inducible system under the control of the *Pax8* promotor [25]. With this technology it was shown that the induction of α ENaC deletion in adult kidney causes a severe salt loosing syndrome with life threatening hyperkalemia and death if not rescued by a high sodium low potassium diet [26] confirming the key role of DCT2 in ASDN.

Salt retaining syndromes

Liddle syndrome

Gain-of-function mutations in ENaC β or γ subunits cause pseudoaldosteronism (Liddle's syndrome), a severe form of salt-sensitive hypertension, hypokalemia, metabolic alkalosis, low plasma renin activity and suppressed aldosterone secretion with a Mendelian autosomal dominant mode of transmission [1, 27]. As shown in Table 1, Pradervand *et al* [28] generated a

transgenic mouse model expressing the human mutation identified in the princeps case, i.e. the deletion of a C terminal regionof the β subunit (R566Stop) comprising the critical consensus PPXY motif that bind to the WW domains of an E3 ubiquitylation enzyme (NEDD4L or Nedd4-2). Under normal salt diet, mice heterozygous (L/+) and homozygous (L/L) for Liddle mutation (L) develop normally during the first 3 months of life. In these mice, BP is not different from wild type despite evidence for increased sodium reabsorption in distal colon and low plasma aldosterone, suggesting chronic hypervolemia. Under high salt intake, the Liddle mice develop high BP, metabolic alkalosis, and hypokalemia accompanied by cardiac and renal hypertrophy. This animal model reproduces to a large extent a human form of salt-sensitive hypertension and establishes a causal relationship between dietary salt, a gene expressed in kidney and hypertension [28]. A subsequent study [29] investigated the renal sodium transport *in vivo*, *ex vivo* (intact perfused tubules), and *in vitro* (primary culture of cortical collecting ducts [CCD]) bringing three independent lines of evidence for the constitutive hyperactivity of ENaC in CCD from mice harboring the Liddle mutation. In another study [30] whole cell currents through epithelial Na channels (ENaCs) were measured by patch clamp of *ex vivo* cortical collecting duct (CCD) isolated from mice homozygous for the Liddle mutation (L/L) and from wild-type (WT) littermates. Mineralocorticoid regulation of ENaC is maintained in a mouse model of Liddle's syndrome and the highest currents were recorded in L/L CCD from animal kept under low salt diet. The increase in whole cell current was attributed to a difference in the density of conducting channels, in agreement with the proposed molecular and pathogenic mechanism for the Liddle mutation [31, 32].

Potassium channels : ROMK and BK

As discussed above, the critical role of ROMK is underscored by mutations causing the antenatal Bartter Type II, one of the most severe salt loosing syndrome. Holtzclaw *et al.* [33] have recently reviewed the physiological importance of potassium large conductance calcium-activated channels (Maxi K or BK), and their potential secretory roles in flow-induced K secretion and in the control of blood pressure. BK channels are also fundamental to the control of smooth muscle tone and neuronal excitability. BK channels can be formed by two subunits: the pore-forming α subunit, which is the product of the KCNMA gene family , and the modulatory β subunit product of the KCNMB gene family. Intracellular calcium regulates the physical association between the α and β subunits. In the kidney the most important BK channels are the αβ1 heteromer expressed in the apical membrane of CNT whereas αβ4 heteromers are predominantly expressed in intercalated cells (Figure 1). Constitutive germ line inactivation of BK α subunit results in extreme aldosteronism, hypertension, and an absence of flow-induced potassium secretion. Inactivation of the BK β1 subunit results in decreased

handling of a potassium load, increased plasma potassium, mild aldosteronism and hypertension that is exacerbated by a high potassium diet [34, 35]. Inactivation of BK β4 subunit leads to insufficient potassium handling, high plasma potassium, fluid retention, but with milder hypertension [36]. As discussed by Holtzclaw *et al*. [33] BK β1 hypertension may be a 'three-hit' hypertension, involving a potassium secretory defect, elevated production of aldosterone, and increased vascular tone. To distinguish between these three factors (adrenal, vessels, kidney), inducible nephron specific deletion of *α1* or β1 or β4 genes will be highly informative.

We have considered so far the transporters (NKCC2, ROMK, BK, NCC and ENaC) whose role in sodium and potassium balance is well established. In most ASDN model little attention is paid to the role of intercalated cells classically involved in pH homeostasis and acid-base regulation. As reviewed by Eladari and Hubner [37] and Wall and Pech [38, 39], recent data, however indicate, that a more integrative view of the role of intercalated and principal cells along the CD and the CNT should be proposed.

Chloride transporters : SLC26A4(pendrin) and SLC4A8

As discussed above, the critical role of the chloride channel CLCNKB and its associated subunit (BDSN/ barttin) are underscored by mutations causing Bartter Type 3 and Type 4. We will now discuss the role of two exchangers expressed in intercalated cells that play a role in blood pressure control. The protein encoded by SLC4A8 is a membrane protein that functions to transport sodium and bicarbonate ions across the cell membrane. Leviel *et al.* [40] demonstrated that besides the classic electrogenic amiloride-sensitive, ENaC dependent-sodium transport, much of the sodium absorption in the CCD was actually amiloride- insensitive but thiazide- sensitive. The authors demonstrated the presence of an electroneutral, amiloride-resistant, thiazide-sensitive, transepithelial NaCl absorption in mouse CCDs, which persists even with genetic disruption of ENaC [40]. The data suggested that the parallel action of the sodium-driven chloride/bicarbonate exchanger (NDCBE/SLC4A8) and the sodium-independent chloride/bicarbonate exchanger (pendrin/SLC26A4) accounted for the electroneutral thiazide-sensitive sodium transport. Further evidence for the importance of this novel electroneutral transport was obtained by genetic ablation of SLC4A8 that fully abolished thiazide-sensitive NaCl transport in the CCD. SLC26A4 (solute carrier family 26, member 4) or pendrin is a membrane protein that functions as chloride/ bicabonate exchanger and mutations in this gene are associated with Pendred syndrome, the most common form of syndromic deafness, an autosomal-recessive disease. The role of SLC26A4/pendrin in the regulation of extracellular fluid volume and blood pressure has recently been reviewed [39, 41]. Royaux *et al.* reported that *Slc26a4 (-/-)* mice were severely impaired in their renal bicarbonate secretion [42]. Next it was shown that a renal phenotype could become unmasked by challenging *Slc26a4 (-/-)* mice

with the mineralocorticoid DOCP. Mice devoid of pendrin develop metabolic acidosis and isolated CCD tubule and were unable to secrete bicarbonate. Interestingly these mice were « resistant » to DOCP and did not develop salt retention and hypertension was prevented [43] . Conversely, under salt restriction, Wall *et al.* reported that Slc26a4 is upregulated and is critical in the maintenance of acid-base balance and in the renal conservation of Cl- and water [44].

Aldosterone and Angiotensin signalling pathways

NKCC2 regulation

As recently reviewed by Ares *et al.* [51], NKCC2 dependent-sodium chloride reabsorption in TAL is subject to exquisite control by hormones like vasopressin, parathyroid, glucagon, and adrenergic agonists that stimulate NaCl reabsorption. Atrial natriuretic peptides or autacoids like nitric oxide and prostaglandins (PGE2) inhibit NaCl reabsorption, promoting salt excretion. At least, three molecular mechanisms : membrane trafficking, phosphorylation, and protein-protein interactions have recently been described as mechanisms that modulate NKCC2 activity in TALs and heterologous expression systems [51].

According to NCBI, SORL1 (SORLA) encodes a mosaic protein that belongs to at least two families: the vacuolar protein sorting 10 (VPS10) domain-containing receptor family, and the low density lipoprotein receptor (LDLR) family. In this context, a recent study [52] (**Table 2**) shows that the mouse *Sorl1* (sorting protein-related receptor with A-type repeats) plays an important role in functional activation of NKCC2. *Sorl1-/-* mice are unable to interact with SPAK (Ste-20-related proline-alanine-rich kinase) that normally allows the proper trafficking of Spak to the apical membrane of TAL cell and the NKCC2 phosphorylation. The phenotype of the *Sorl-/-* mice is a salt losing syndrome mimicking a Bartter syndrome Type 1 indicating that this protein is one of the limiting factor in this signaling pathway [52]. Until now, no human SORL1 mutations have been described.

As reviewed by Breyer and Breyer [53], prostaglandin PGE(2) is a major renal cyclooxygenase metabolite of arachidonate and interacts with four G protein-coupled E-prostanoid receptors designated EP(1), EP(2), EP(3), and EP(4). Through these receptors, PGE(2) modulates renal hemodynamics and salt and water excretion. The authors propose that the capacity of PGE(2) to *bidirectionally* modulate vascular tone and epithelial transport via constrictor EP(1) and EP(3) receptors versus dilator EP(2) and EP(4) receptors allows PGE(2) to serve as a buffer, preventing excessive responses to physiological perturbations. Along this line of thought [54, 55], it has been shown that gene inactivation of the EP(2) receptor lead to a salt sensitive hypertensive phenotype. EP2-/- mice develop normally and have slightly elevated baseline systolic blood pressure. In EP2-/- mice, the characteristic hypotensive effect of intravenous PGE2 infusion was absent; PGE2 infusion instead produced hypertension. When fed a high salt

diet, the EP2-/- mice developed profound systolic hypertension, whereas wild-type mice showed no change in systolic blood pressure [54]. In another study however, a reduced blood pressure phenotype was observed [55]. The reason for this discrepancy remains unclear.

ASDN and RAAS

The role of the Renin Angiotensin Aldosterone System (RAAS) has been extensively reviewed and the role of each of its component studied by establishment of transgenic mouse models for renin, angiotensinogen, ACE, angiotensin II receptor, aldosterone synthase [3-5, 56]. In the context of the present review, a few important references will be outlined :

Renin

As reviewed by Corvol *et al*. [3] there are important differences between human and mouse RAAS. For instance human have one renin gene (REN) wheras some mouse strains (i.e C56BL6) have one copy of renin gene (*Ren1^c)* and others (i.e SV129) have two (*Ren1^d* and *Ren2).* Deletion of *Ren2* does not modify BP [57] whereas deletion of *Ren 1^d* decrease BP in heterozygote and homozygote females but not in males [58]. In mice, inactivation of any of the components of the renin-angiotensin system (i.e. renin, angiotensin-converting enzyme, angiotensinogen and AT1 receptor) is dispensable for survival at birth. Animals can survive although they are more sensitive to salt depletion than the wild type mice. By contrast, Renal Tubular Dysgenesis (RTD) is a human disease consisting of severe abnormalities of renal tubular development and resulting in profound anuria and perinatal death. Familial RTD is an autosomal recessive disease due to genetic defects in *any* of the constituents of the renin system [3]. Complete gene inactivation of the renin system in RTD leads to neonatal anuria and death. Proximal tubules are almost absent; renal artery hyperplasia is found in all cases of RTD. An intense stimulation of renin gene expression is noted in the kidney of patients with mutations affecting angiotensinogen, angiotensin-converting enzyme and AT1 receptor. The more severe phenotype in humans compared to mice devoid of a functional renin system may be attributable to the difference in nephrogenesis between mice and humans. In mice, nephrogenesis is completed 2 weeks after birth, whereas in humans it is completed before birth, at 38 weeks of gestation [3].

Angiotensinogen

As reviewed by Takahashi [59] and Smithies [5], potentially causative variations associated with quantitative differences in the expression of the angiotensinogen gene (AGT) have been identified. Experiments to directly test causation are possible in mice is possible, establishing that blood pressures are indeed altered by genetic changes in AGT expression (gene disruption « zero copy », heterozygous mutants « one copy », wild type « two copies », gene titration by gene duplication « 3 to 4 copies ». Thus, angiotensinogen-deficient mice generated by

homologous recombination in mouse embryonic stem cells do not produce angiotensinogen in the liver, resulting in the complete loss of plasma immunoreactive angiotensin I. The systolic blood pressure of the homozygous mutant mice is drastically reduced by >30% [60]. Gene titration experiment show that blood pressure rises linearly as the number of copies of the *Agt* gene increases [61].

Angiotensin Converting Enzyme (ACE)

Angiotensin-converting enzyme (ACE) is a dipeptidyl carboxy-peptidase that generates the vasoconstricting peptide angiotensin II and inactivates the vasodilating peptide bradykinin. The gene encoding ACE is composed of two homologous regions and codes for both a somatic and testis isoenzyme. The role of the *Ace* gene in blood pressure control and reproduction was investigated using mice generated to carry an insertional mutation that was designed to inactivate both forms of *Ace* [62]. When the *Ace* gene was disrupted, the BP of heterozygote was normal in males and lower in females despite a equal reduction of 50% of plasma enzyme activity in both gender. When the *Ace* gene was duplicated with a corresponding linear increase in ACE activity, there was no change in BP [62].

Angiotensin II receptor (AGTR1)

Angiotensin II receptors (AGTR) are expressed in tissue compartments involved in blood pressure control (heart,kidney, blood vessels, adrenal glands and brain) [63]. Stimulation of AGTR by Angiotensin II causes potent vasoconstriction, release of aldosterone by the adrenal gland in turn promoting sodium absorption in ASDN [63]. In the brain Angiotensin II triggers a dramatic hypertensive response while in the kidney it triggers renal vasoconstriction and antinatriuresis. A first study showed that pressor responses to infused angiotensin II were virtually absent in *Agtr1a(-/-)* mice and were qualitatively altered in *Agtr1a(+/-)* heterozygotes. This study demonstrated clearly that mouse type 1A angiotensin II receptor function is required for vascular and hemodynamic responses to angiotensin II and that altered expression of the *Agtr1a* gene has marked effects on blood pressures [64]. In order to distinguish the physiological role of AGTR in individual tissue compartment, the authors designed an elegant experimental approach i.e a kidney cross transplantation strategy [63, 65]. Although actions of the RAAS in a variety of target organs have the potential to promote high blood pressure and end-organ damage, the authors showed convincingly that angiotensin II caused hypertension primarily through effects on AGTR1 receptors in the kidney [65]. Importantly renal AGTR1 receptors were absolutely required for the development of angiotensin II-dependent hypertension and cardiac hypertrophy [65]. When AGTR1 receptors were eliminated from the kidney, the residual repertoire of systemic, extrarenal AGTR1 receptors was not sufficient to induce hypertension or cardiac hypertrophy [65]. Recently RAS actions in the epithelium of the proximal tubule were shown to have a critical and non redundant role in determining the level of

BP [66]. Abrogation of *Agtr1* angiotensin receptor signaling in the proximal tubule alone is sufficient to lower BP, despite intact vascular responses. Elimination of this pathway reduces proximal fluid reabsorption and alters expression of key sodium transporters, modifying pressure-natriuresis and providing substantial protection against hypertension [66].

ASDN and NCC

WNK4

PHAII (or Familial hyperkalemic hypertension (FHHt) or Gordon Syndrome) is a Mendelian form of arterial hypertension that is partially explained by mutations in WNK1 and WNK4 that lead to increased activity of NCC in the distal nephron [67]. PHAII is characterized by salt-, thiazide-sensitive hypertension, variable degree of hyperkalemia and metabolic acidosis. The transmission is autosomal dominant with an important genetic heterogeneity (4 genes identified). To study the pathophysiological mechanisms underlying the human phenotype caused by WNK4 mutations, WNK4 hypomorphic mice were generated by deleting exon 7 of the Wnk4 gene [68]. These mice did not show hypokalemia and metabolic alkalosis, but they did exhibit low blood pressure and increased sodium and potassium excretion under low-salt diet. Phosphorylation of OSR1/SPAK and NCC was significantly reduced in the mutant mice as compared with their wild-type littermates. Protein levels of ROMK and Maxi K (BK) were not changed, but ENaC appeared to be activated as a compensatory mechanism for the reduced NCC function [68]. Mutations in the gene encoding the kinase WNK4 cause pseudohypoaldosteronism type II (PHAII), a syndrome featuring hypertension and hyperkalemia [67]. Physiology in mice transgenic for genomic segments harboring wild-type (WT) or PHAII mutant WNK4 is changed in opposite directions [69]: PHAII mice have higher blood pressure, hyperkalemia, hypercalciuria and marked hyperplasia of the distal convoluted tubule (DCT), whereas the opposite is true in wild type mice. Genetic deficiency for the sodium chloride cotransporter of the DCT (NCC) reverses phenotypes seen in PHAII mice, demonstrating that the effects of the PHAII mutation are due to altered NCC activity [69]. To mimic more precisely the molecular pathophysiology of human PHAII in mice, Wnk4(D561A/+) knockin mice were generated [70]. The knockin PHAII mice showed increased apical expression of phosphorylated Na-Cl cotransporter (NCC) in the distal convoluted tubules. Increased phosphorylation of the kinases OSR1 and SPAK was also observed in the knockin mice. Apical localization of the ROMK and transepithelial chloride permeability in the cortical collecting ducts were not affected in the knockin mice, whereas activity of ENaC was increased. This increase, however, was not evident after hydrochlorothiazide treatment, suggesting that the regulation of ENaC was not a genetic but a secondary effect [70]. Overall these two studies nicely establish mouse models to study the molecular physiopathology of PHAII or Gordon syndrome. Small differences between

the two studies are probably due to the different methodological approach (bac transgene vs knock in).

STK39 (SPAK)

Serine Threonine kinase 39 (STK39) or Ste20-like proline/alanine-rich kinase (SPAK) encodes a serine/threonine kinase that is thought to function in the cellular stress response pathway. STK39/SPAK interacts with WNK kinases to regulate NKCC2 and NCC. SPAK-null mice were generated by targeting disruption of exons 9 and 10 of SPAK. Compared with SPAK(+/+) littermates, SPAK(+/-) mice exhibited hypotension without significant electrolyte abnormalities, and SPAK(-/-) mice not only exhibited hypotension but also recapitulated Gitelman syndrome with hypokalemia, hypomagnesemia, and hypocalciuria [71]. To define the importance of the WNK/SPAK in regulating blood pressure, knock-in mice in which SPAK cannot be activated by WNKs were generated [72]. The SPAK knock-in animals are viable, but display significantly reduced blood pressure that was salt-dependent. These animals also have markedly reduced phosphorylation of NCC and NKCC2 cotransporters at the residues phosphorylated by SPAK. This was also accompanied by a reduction in the expression of NCC and NKCC2 protein without changes in mRNA levels. On a normal sodium diet, the SPAK knock-in mice were normokalaemic, but developed mild hypokalemia when the renin-angiotensin system was activated by a low sodium diet [72].

WNK1

As reviewed by Bergaya *et al.* [73], the WNK1 gene gives rise to a ubiquitous kinase (L-WNK1) and to a shorter kinase-defective isoform, KS-WNK1 (for kidney-specific WNK1), expressed only in the distal convoluted tubule (DCT) and connecting tubule (CNT) (see Figure 1). WNK1 first intron deletion leads to overexpression of L-WNK1 in the DCT and ubiquitous ectopic expression of KS-WNK1. The increased expression of L-WNK1 in the DCT results in increased activity of the Na-Cl cotransporter (NCC) and thus hypervolemia and hypertension. The mechanisms underlying the hyperkalemia and metabolic acidosis remain unclear [73]. Hadchouel *et al.* [74] inactivated KS-WNK1 expressed only in DCT (Figure 1) and showed that this isoform is an important regulator of sodium transport. KS-WNK1(-/-) mice display an increased activity of NCC, expressed specifically in the distal convoluted tubule, where it participates in the fine tuning of sodium reabsorption [74]. The authors suggest that the activation of NCC is not sufficient by itself to induce a hyperkalemic hypertension and that the deregulation of other channels, such as ENaC, is probably required [74]. In another study Liu *et al*. [75] came to similar conclusions

KLHL3 and CULLIN

Recently two studies using exome sequencing identified mutations in Kelch-like 3 (KLHL3) [76, 77] or CULLIN3 (CUL3) [76] in PHAII patients. KLHL3 mutations are either recessive or

dominant, whereas CUL3 mutations are dominant and predominantly *de novo*. CUL3 and BTB-domain-containing kelch proteins such as KLHL3 are components of CULLIN -RING E3 ligase complexes that ubiquitylate substrates bound to kelch propeller domains. Dominant KLHL3 mutations are clustered in short segments within the kelch propeller and BTB domains implicated in substrate and cullin binding respectively [76]. Polymorphisms at KLHL3 were not associated with blood pressure [77]. KLHL3 is coexpressed with NCC and downregulates NCC expression at the cell surface [77]. Both studies establishes a role for KLHL3-CUL3 as new members of the complex signaling pathway regulating ion homeostasis in the distal nephron and indirectly blood pressure [77]. Three publications have recently identified WNK4 as a major substrate of the KLHL3-CUL3 complex [78-80]. Collectively, the data demonstrate that CUL3-RING ligases that contain KLHL3 target ubiquitylation of WNK4 regulate WNK4 levels, which in turn regulate levels of ROMK.

ASDN and ENaC

MR and GR

Mineralocorticoid receptor (MR)-deficient mice have a normal prenatal development but die within 10 days after birth from a salt-losing syndrome mimicking human PHA 1 [81]. Interistingly this phenotype could be rescued by subcutaneous infusion of saline and this treatment enables the animals to develop through this critical phase of life, after which they adapt their oral salt and water intake to match the elevated excretion rate. However, the renal salt-losing defect persists [82]. Due to the obvious experimental limitations of germline inactivation of MR, Ronzaud *et al.* [83] generated mice with MR deficiency in principal cell using the *Cre-loxP* system driven by regulatory elements of the mouse aquaporin 2 (AQP2) promotor. This strategy should inactivate MR in the late portion of CNT and CD (Figure 2). The authors demonstrated that inactivation of MR in CD and late CNT can be compensated under standard diet but no longer when sodium supply is limited. Because the mutant mice show preserved renal ENaC activity, this study provides evidence that the late distal convoluted tubule and early CNT can compensate to a large extent deficient ENaC-mediated sodium reabsorption in late CNT and CD [83]. Since AQP2 is already expressed during renal development, MR ablation took place long before the analysis performed at the adult stage leving open the possibility of non defined long term compensatory mechanisms. To investigate whether the early onset of MR ablation affected the adult renal sodium handling, Ronzaud *et al.* [84] have recently used a inducible somatic gene inactivation strategy by developing a transgene expressing the CreER(T2) fusion protein under control of the regulatory elements of the AQP2 gene (AQP2CreER(T2)). Under a low-salt diet and at adult stage, the induced ablation of MR at the adult stage recapitulates the renal sodium wasting observed in mice with constitutive early-onset MR ablation [84]. In human,

a S810L mutation in the mineralocorticoid receptor (MR) causes early-onset hypertension that is markedly exacerbated in pregnancy as described by Geller *et al*. [85]. This mutation results in constitutive MR activity and alters receptor specificity, with progesterone and other steroids lacking 21-hydroxyl groups, normally as MR antagonists, becoming potent agonists [85]. To our knowledge, no mouse knock in model have been developed to mimick this highly interesting pathophysiological situation.

Experiments in Cushing patients and healthy control subjects receiving adrenocorticotropic hormone (ACTH) indicate that transient renal sodium retention may contribute to the generation of hypertension [86]. Bailey *et al.* [86] have investigated the effect of chronic ACTH infusion on renal sodium handling in adult male C57BL/6J mice using selective antagonists to dissect mineralocorticoid and glucocorticoid receptor-mediated pathways. ACTH caused an increase in blood pressure and a reduction in fractional sodium excretion associated with enhanced activity of ENaC. ACTH excess promotes renal sodium reabsorption, contributing to the increased blood pressure; both glucocorticoid and mineralocorticoid receptor pathways are involved. These *in vivo* data are interesting and relevant to the relative occupancy of MR vs GR by aldosterone and cortisol that is controlled by 11β-HSD2. *In vitro* studies indicate that aldosterone occupies both MR and GR under physiological conditions to mediate the sodium transport response but cortisol or corticosterone may also occupy MR and GR under extreme stress [87].

HSD11B2 (11β-HSD2)

HSD11B2 (11β-HydroxySteroidDehydrogenase Type 2 = 11β HSD2) controls ligand access to the mineralocorticoid receptor, and ablation (or inhibition by drug or toxic) of the enzyme causes severe hypertension in human (Apparent Mineralocorticoid Excess =AME) [88]. Bailey *et al.* [89] generated a *Hsd11b2* null mouse on an inbred C57BL/6J genetic background, allowing survival to adulthood. Initially, impaired sodium excretion associated with increased activity of the epithelial sodium channel was observed. Later KO mice had BP approximately 20 mmHg higher on average compared with wild-type mice but were volume contracted, not volume expanded as expected. Volume contraction was not attributable to intrinsic vascular dysfunction but rather to high catecholamine levels, an important pathogenic factor since alpha1-adrenergic receptor blockade rescued the hypertensive phenotype, suggesting that vasoconstriction contributes to the sustained hypertension in this model. It was proposed that renal sodium retention remains a key event in apparent mineralocorticoid excess but that the accompanying hypertension changes from a renal to a vascular etiology over time [89]. In a subsequent study Bailey *et al.* [90] used mice heterozygote for a null mutation in *Hsd11b2* to define the mechanisms linking reduced enzyme activity to salt sensitivity of blood pressure. A high-sodium diet caused a rapid and sustained increase in blood pressure in heterozygote mice (leading to increased heart

weight) but not in wild-type littermates. Interistingly, mineralocorticoid receptor antagonism partially prevented the increase in heart weight but not the increase in blood pressure. Glucocorticoid receptor antagonism prevented the rise in blood pressure suggesting a novel interaction among 11β-HSD2, dietary salt, and circulating glucocorticoids [90].

SGK1

The serum- glucocorticoid induced-kinase 1 (SGK1) belongs to the superfamily of AGC protein kinase [91]. *Sgk1* is an early aldosterone- induced gene (see review in ref [92, 93]). No Mendelian form of human salt-losing or salt retainig syndromes have been mapped to mutations of the SGK1 gene. However, the physiological role of Sgk1 has been extensively studied in mouse models *in vitro* and *in vivo*. Wullf *et al.* [94] showed that under a standard NaCl intake, renal water and electrolyte excretion was indistinguishable between *Sgk1(-/-)* mice and wild-type mice. In contrast, dietary NaCl restriction reveals an impaired ability of *Sgk1(-/-)* mice to adequately decrease sodium excretion despite increases in plasma aldosterone levels and proximal-tubular sodium and fluid reabsorption, as well as decreases in blood pressure and glomerular filtration rate. Overall a mild PHA-1 phenotype was observed. In a second study, Huang *et al.* [95] studied the importance of *Sgk1* in renal elimination of potassium. Electrophysiological and immunohistochemical studies under high potassium diet indicated that reduced epithelial sodium channel ENaC and/or Na,K-ATPase activity in the aldosterone-sensitive distal nephron accounted for the impaired response in *Sgk1-/-* and that an enhanced apical abundance of renal outer medullary potassium channel ROMK partly compensated for the defect. The authors concluded that the acute and chronic regulation of renal potassium elimination involves Sgk1. Vallon *et al.* [96] studied the contribution of *Sgk1* to the regulation of renal function, salt intake, and blood pressure during DOCA-salt excess in *Sgk1* deficient mice. There was no difference in DOCA salt induced increased blood pressure and in creatinine clearance. A more pronounced increase of proteinuria in *Sgk1(-/-)* mice was observed. Overall the observed phenotype was mild and the authors concluded that SGK1 contributes to the stimulation of salt intake, kidney growth, proteinuria, and renal potassium excretion during mineralocorticoid excess [96]. More recently, Faresse *et al.* [97] used a somatic nephron specific inducible gene deletion strategy to study the role of renal nephron specific Sgk1 in adult kidney. Under a standard sodium diet, renal water and Na/K excretion had a tendency to be higher in doxycycline-treated Sgk1 KO mice compared with control mice. The impaired ability of Sgk1 KO mice to retain Na increased significantly with a low-salt diet despite higher plasma aldosterone levels. On a low sodium diet, the Sgk1 KO mice were also hyperkaliuric and lost body weight. This phenotype was accompanied by a decrease in systolic and diastolic blood pressure. This phenotype mimics a mild human PHA-1 phenotype indicating that Sgk1 is, to some extent, limiting in the action of aldosterone in ASDN. In another study, Fejes-Toth *et al.*

[98] also showed a mild salt-losing phenotype under salt restriction and concluded that SGK1 was essential for optimal processing of ENaC but was not required for activation of the channel by aldosterone [98].

NEDD4L (Nedd4-2)

NEDD4L encodes a member of the Nedd4 family of HECT domain E3 ubiquitin ligases. HECT domain E3 ubiquitin ligases transfer ubiquitin from E2 ubiquitin-conjugating enzymes to protein substrates, thus targeting specific proteins for lysosomal degradation. The encoded protein mediates the ubiquitylation of multiple target substrates and plays a critical role in epithelial sodium transport by regulating the cell surface expression of the epithelial sodium channel, ENaC [32]. Single nucleotide polymorphisms in this gene may be associated with essential hypertension [32]. Shi *et al.* [99] generated Nedd4-2 null mice. The knockout mice had higher BP on a normal diet and a further increase in BP when on a high-salt diet. Overall, the authors concluded that in vivo Nedd4-2 is a critical regulator of ENaC activity and BP. The absence of this gene is sufficient to produce salt-sensitive amiloride sensitive hypertension mimicking Liddle syndrome[99]. This data are consistent with a genetic interaction between ENaC β or γ subunit and Nedd4-2, in turn, in agreement with a large number of in vitro expression studies demonstrating biochemical and physiological interactions between the two proteins [32]. Ronzaud *et al.* [100] generated doxycycline-inducible, nephron-specific *Nedd4L* KO mice. Under standard and high sodium diets, conditional KO mice displayed decreased plasma aldosterone but normal Na/K balance. Under a high sodium diet, KO mice exhibited hypercalciuria and increased blood pressure, which were reversed by thiazide (but not amiloride treatment). Unlike the constitutive germ line model by Shi *et al.* [99] the results demonstrate that loss of NEDD4-2 in adult renal tubules causes a new form of mild, salt-sensitive hypertension without hyperkalemia that is characterized by upregulation of NCC, elevation of $\beta\gamma$ ENaC, but not α ENaC, and a normal Na/K balance maintained by downregulation of ENaC activity and upregulation of ROMK, a phenotype approaching more that of a PHA II than a Liddle syndrome. The reasons for this difference may be due to one or a combination of the following factors i) the constitutive “chronic” deletion *Nedd4L* in all organs and tissues could trigger a number of compensatory mechanisms that could contribute to the Liddle-like phenotype; ii) the “acute” deletion (within two weeks) of Nedd4-2 exclusively in the nephron could trigger different compensatory mechanisms and unmask an unexpected but interesting PHA II (normokalemic) phenotype demonstrating that Nedd4-2 might not only control ENaC activity but also NCC; iii) despite of the fact that in the two studies the same floxed allele was used (inactivation of exon 6 to 8), differences in genetic background and/or recombination efficiency in adult versus embryo could be one of the uncontrolled factor of this kind of experiment; iv) the generation of tissue specific spliced variant at N terminal start could also be

a confounding factor. Along this line, two other *Nedd4L* knockout models have been reported. Boase *et al.* generated a total knockout of *Nedd4L* by inactivating Exon 15 and Kimura *et al.* [101] a lung-specific KO of exon 15 and downstream regions of the HECT (catalytic) domain to avoid possible splicing around the N-terminal start site. Boase *et al*. [102] observed increased ENaC activity and partial lethality due to premature lung fluid clearing. Kimura *et al.* reported the development of a cystic fibrosis-like lung disease, with airway mucus obstruction, goblet cell hyperplasia, massive inflammation, fibrosis, and death by three weeks of age. These effects of Nedd4L loss are likely caused by enhanced ENaC function as described in cystic fibrosis. The lung defects were rescued with administration of amiloride into the lungs of young knockout pups via nasal instillation [101]. This is the first demonstration that ENaC activity in the lung is controlled by E3 ubiquitylation. These lung phenotype(s) were not observed in the original model of Shi *et al.*, the major difference being the targeted allele.

USP2-45 (Ubiquitin Specific Peptidase2-45)

The deubiquitylation (DUB) enzyme USP2-45 is an aldosterone early induced gene [103, 104] and was identified in the kidney of animal stimulated by aldosterone for 30 minutes. USP2-45 is also cycling according to a circadian rhythm. Based on *in vitro* experiment [105-107], It was proposed that the effect of aldosterone on the deubiquitylation of ENaC could be synergistic with the effect of Sgk1 on Nedd4-2 whereas another study suggested that USP2-45 might down regulate the aldosterone response by interacting with the mineralocorticoid receptor [108]. Recently, Pouly et al [109] investigated the effect of Usp2 gene inactivation. USP2-45 protein has a rhythmic expression with a peak at ZT12. Usp2-KO mice did not show any differences to wild-type littermates with respect to the diurnal control of Na or K urinary excretion and plasma levels neither on standard diet, nor after acute and chronic changes to low and high Na+ diets, respectively. Moreover, they had similar aldosterone levels either at low or high Na+ diet. Blood pressure measurements using telemetry did not reveal variations as compared to control mice. The authors suggest that USP2 does not play a primary role in the control of Na+ balance or blood pressure [109]. One interpretation of these negative results is redundancy between DUBs and or interactions with other compensatory mechanisms.

TSC22D3 (TSC22 domain family, member 3 or GILZ)

As annotated, the protein encoded by this gene shares significant sequence identity with the murine TSC-22 and Drosophila shs, both of which are leucine zipper proteins, that function as transcriptional regulators. The expression of this gene is stimulated by glucocorticoids and interleukin 10, and it appears to play a key role in the anti-inflammatory and immunosuppressive effects of this steroid and chemokine.

GILZ was identified in the transcriptome of a kidney cell line stimulated by aldosterone [110]. In an *in vivo* rat model, Muller *et al.* [111] showed that the Induction of GILZ may play a permissive

role in the enhancement of the early and/or late responses; these effects may be necessary for a full response but do not by themselves promote early changes in urinary sodium and potassium excretion.

Suarez *et al.* [112] used *Cre/loxP* technology to generate mice deficient for *Tsc22d3-2*. Male knockout mice were viable but surprisingly did not show any major deficiencies in immunological processes or inflammatory responses. *Tsc22d3-2* knockout mice adapted to a sodium-deprived diet and to water deprivation conditions but developed a subtle deficiency in renal sodium and water handling but, unexpectidely,the analysis of the *Tsc22d3-2*-deficient mice demonstrated a previously uncharacterized function of glucocorticoid-induced leucine zipper protein in testis development [112].

Conclusions and Perspectives

The genetic dissection of sodium and potassium transport along the ASDN allowed to define the most important limiting factors (transporters,hormone receptors, kinases, ubiquitylase) that are involved in the aldosterone- and angiotensin- dependent signaling pathways specifically expressed in the kidney. These limiting factors are, by definition, good candidate as drug targets for the treatment of hypertension. For example, ROMK, BK, Nedd4-2, WNK1, WNK4, Cullin 3, Kelch like 3, SPAK, ClCK/barttin, pendrin, SLC4A8 and to some extent Sgk1 are potential receptors for the development of new antihypertensive drugs. Conversely genetic evidence in transgenic mouse model indicate that Usp2-45 or GILZ are not limiting and thus not good potential drug targets.

The genetic dissection has also helped to begin to understand the molecular mechanisms underlying the « aldosterone paradox » (see recent reviews in references [115, 116]. Aldosterone promotes different and somewhat opposed effects either in promoting potassium secretion (with minimun sodium retention) in case of *hyperkalaemia* or in promoting sodium reabsorption (with minimum potassium loss) in case of *hypovolemia.* WNK4 phosphorylation has been proposed as potential molecular switch [67]. It is likely, however, that other molecular switches will soon be discovered (MR phosphorylation ? E3 enzyme phophorylation ?) and thus defining two separate phoshorylation states of the signaling cascade(s) triggered by either aldosterone or angiotensin that could explain the aldosterone paradox. This would be a significant step for the successful development of the next generation diuretics that should have the following characteristics : i) block synergistically ENaC and NCC; ii) no change in efficacy in presence of high salt intake; iii) no effect on potassium balance (no hypo- or hyper- kalemia). These ideal diuretics would qualify for the name of *anti-salt pill*. As shown in the ALLHAT study [117], the present diuretics specially the thiazides were unsurpassed in long-term drug adherence, controlling elevated blood pressure and were

superior to other therapies in preventing one or more forms of cardiovascular events, having lower drug cost. Thiazides, however, have still significant side effects i.e. no control of potassium balance (hypokalemia and metabolic alkalosis), Type 2 diabetes and hyperuricemia. To minimize the side effects of an anti-salt pill, the following criteria should be met: i) pharmacodynamically, the anti salt pill should bind to its receptor with stereospecific, very high affinity (nM range), have high selectivity (no cross reactivity with related gene products), display nephron segment/tubular or cell specific expression of the receptor; ii) pharmacokinetically, the aim would be to maximally increase urinary apical drug concentrations in ASDN for instance by active secretion of the drug by the proximal tubule.

What are the future developments that could contribute to our knowledge of ASDN function? The present mouse models have obvious limitations: i) despite of the fact that RAAS seem to operate to a great extent similarly in mouse and humans, the fine regulation of blood pressure is different (heart rate of 500 beats/min!); ii) in in most mouse models, the precise mutation(s) observed in human disease were often not introduced in the mouse genome due to methodological difficulties; iii) the cardiovascular phenotyping of the mouse remains challenging; iv) some pathologies (for instance nephrotic syndrome) are difficult to reproduce in mouse but possible in rats. Before the introduction of transgenic mouse models, the best characterised animal model for renal physiology and pathophysiology was the rat. The recent development of the zinc finger technology should allow, in a near future, to obtain transgenic rat models that could be useful for drug development.

Finally, due to space limitations, important signaling pathways contributing significantly to the control of blood pressure could not be discussed here but should also be considered as a source of novel drug targets for the treatment of hypertension : circadian rthythm [118, 119], insulin [120], vasopressin [121, 122], endothelin [123], ANP [124, 125], TGFβ [126] to mention the most important.

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Legend to Figure 1

Model of a nephron, the functional unit of the kidney.

The nephron is composed of a filtering apparatus the Glomerulus (G) (1), the proximal tubule (Proximal Convoluted Tubule (PCT)(2) and Proximal Straight Tubule (PST)(3), the Henle's loop (Thin descending Limb (TL) (4) and Thick Ascending Limb (TAL) (5)), the Distal Convoluted Tubule (DCT)(6), the Connecting Tubule (CNT)(7). 10 to 12 nephrons branch on one Collecting Duct (CD) (8 to 11) made of the Cortical Collecting Duct (CCD), Outer medullaray Colecting Duct (OMCD) and the Inner Medullary Collecting Duct (IMCD). Adapted from Kritz and Kaissling [127]

In human, 180 liters of fluid is filtered per day through the glomerulus (G). Around 60% of the filtrate will be reabsorbed in the proximal tubule (PCT and PST) (2),30% in the Henle's loop (TL and TAL) (4), 9% in DCT, CNT and CD leaving 1.8 liter/day of final urine. Over 90% of renal ATP is used for fluid reabsorption drivent by the Na,K-ATPase. Sodium and potassium balance are achieved through the final regulation of sodium and potassium transport in the distal nephron and the collecting duct under hormonal control (aldosterone, vasopressin,angiotensin, etc). DCT cells, CNT cells and CD principal cells are the main cells involved in hormonal regulation. See for details linear model in Figure 2

Legend to Figure 2

Linear model of the distal nephron and collecting duct.

The distal nephron is composed of the Straight Distal Tubule or Thick Asending Limb (TAL), the distal convoluted tubule (DCT1 and DCT2, the connectiong tubule (CNT) and the collecting duct (CD). The Aldosterone sensitive Distal Nephron (ASDN) is composet of DCT2, CNT and CD that co express 11bHSD2 and MR. The corresponding cells (TAL cells, DCT celles, CNT celles, CCD cells are shown above tehier corresponding nephron segments (drawings adapted from Kritz an Kaissling [127]. The nephron segment/cell specificexpression of the relevant transporters and the aldo and angiotensin dependent siganling pathways are shown (see text for discussion)

Table 1 Phenotype of transgenic mouse models mimicking salt- retaining and salt- losing phenotypes along the distal nephron (TAL, DCT,CNT) and collecting duct (CD) : Transporters

Human gene	Human disease	Mouse gene	Mouse Phenotype	Ref
SLC12A2	**Bartter type I**	*Slc12a2* *Null allele*	Bartter I-like :severe extracellular volume depletion, hyperkalaemia, metabolic acidosis, hydronephrosis, and high plasma renin and aldosterone concentrations.	[12]
KCNJ1	**Bartter Type II**	*KcnJ1* *Null allele*	Bartter II -like :ROMK-deficient mice exhibit polyuria, natriuresis, and kaliuresis similar to individuals with type II antenatal Bartter syndrome	[13] [45]
CLCNKB	**Bartter Type III**	*Clcnkb* *Null allele*	The mouse phenotype is distinct from that Bartter III : overt nephrogenic diabetes insipidus with a decrease of approximately 27% in body weight.	[14] [11]
BSND	**Barrter Type IV**	*Bsnd* *R8L*	Bartter IV-like : Hypokalemia, metabolic alkalosis, and decreased NaCl reabsorption in distal tubules under a low-salt diet. aberrant intracellular localization of R8L barttin	[15]
CASR	**Bartter Type V**		No mouse model available : expected phenotype is a Bartter-like syndrome associated to autosomal dominant hypocalcemia	[46] [47]
SLC12A3	**Gitelman syndrome**	*Slc12a3* *Null allele*	Gitelman-like : subtle perturbations of sodium and fluid volume homeostasis, but renal handling of Mg2+ and Ca2+ are altered, as observed in Gitelman's syndrome.	[48]
SCNN1A	**PHA Type I** (sytemic)	*Scnn1a$^{tm1/tm1}$* *Null allele*	Systemic PHA 1 like : Severe PHA-1 phenotype with100% perinatal lethality	[19]
SCNN1B	**PHA Type I** (sytemic)	*Scnn1b* *Null allele*	PHA-1 like : Severe PHA-1 phenotype with100% perinatal lethality	[20]
SCNN1C	**PHA Type I** (sytemic)	*Scnn1c* *Null allele*	PHA-1 like : Severe PHA-1 phenotype with100% perinatal lethality	[21]
SCNN1A		*Scnn1a lox/lox*	No phenotype	[49]
SCNN1A		*Scnn1a lox/lox Hoxb7Cre*	No phenotype as far as sodium and potassium balance Full protection against lithium induced NDI	[22] [23]

SCNN1A		*Scnn1a lox/lox Aqp2Cre*	Mild PHA-1 phenotype in adult mice	[24]
SCNN1A		*Scn1a lox/loxPAX8 rtTA/LC1*	Severe renal PHA-1 phenotype with salt loosing syndrome and life threatening hyperkalaemia. 100% lethality within 10 days without treatment	[26]
SCNN1B SCNN1C	**Liddle Syndrome**	*Scnn1b*$^{Lid/Lid}$	Liddle syndrome-like : Salt sensitive hypertension, metabolic alcalosis, hypokalemia	[28-30
KCNMA1 KCNMB1 KCNMB4	No human disease reported	*Kcnma1 Kcnmb1 Kcnmb4*	Disorders observed in BKa1, BKb1 or BKb4 KO mice have shed new insights on the importance of proper renal K handling for maintaining volume balance and blood pressure	[33-35,
SLC26A4	**Pendred syndrome**	*Slc26a4*	Aldosterone and angiotensin II modulate the renal regulation of blood pressure, in part, by regulating pendrin-mediated Cl- absorption and ENaC-mediated Na+ absorption.	[38, 39, 42]
SLC4A8	no human disease	*Slc4a8*	Evidence for a novel electroneutral, amiloride-resistant, thiazide-sensitive, transepithelial NaCl absorption in mouse CCDs, which persists even with genetic disruption of ENaC	[37, 40,

Table 2 Phenotype of trangenic mouse models mimicking salt – retaining and salt- losing phenotypes along the distal nephron (TAL, DCT,CNT) an collecting duct (CD) :Aldosterone and Angiotensin dependent signaling pathways

Human gene	Human disease	Mouse gene	Mouse Phenotype	Ref
SORL1	None described	*Sorl1 (SORLA) null allele*	Barrter-like : intracellular trafficking of SPAK by the sorting receptor SORLA (i.e *Sorl1*) is critical for proper NKCC2 activation	[52]
PTGER2		*Ptger2 null allele*	PGE2, acting through the EP2 receptor, exerts potent regulatory effects on blood pressure homeostasis	[53-5
REN		*Ren 1^{c} null* *Ren 2 null*	Hypotensive phenotype in females No BP phenotype	[58] [57]
AGT		*Agt* *Null allele*	Profound hypotension in angiotensinogen-deficient mice demonstrates an indispensable role for the renin-angiotensin system in maintaining BP	[60] [61]
ACE		*Ace* *Null allele*	Heterozygous males but not females low blood pressures although both male and female heterozygotes had reduced serum ACE activity.	[62]
AGTR1		*Agtr1a* *null allele*	Angiotensin II receptor Type 1 gene ablation Hypotensive phenotype and resistance to Angiotensin II administration	[64]
		Kidney cross transplantation	Critical role of the kidney in the pathogenesis of hypertension and its cardiovascular complications. Evidence supporting Guyton's hypothesis	[113]
			Targeting *Agtr1a* receptor of the proximal tubule of the kidney decreases BP and could be a useful therapeutic strategy in hypertension.	[66]
WNK4		*Wnk4* hypomorphic	Gitelman-like Salt loosing hypotensive phenotype : wt WNK4 is proposed to be a *positive* regulator for the WNK-OSR1/SPAK-NCC cascade	[68]
STK 39 (SPAK)		*Stk 39/SPAK* *Disruption of exon 9 and 10*	Gitelman-like :Stk39/SPAK-null mice have defects of NCC in the kidneys and NKCC1 in the blood vessels, leading to hypotension through renal salt wasting and vasodilation.	[71]
		Stk39/SPAK *kinase-dead ki*	SPAK plays an important role in controlling blood pressure in mammals. SPAK inhibitors would be effective at reducing blood pressure	[72]
WNK4	PHAII	Transgenic *Wnk4 PHAII*	PHAII like : Wnk4 is proposed to be a molecular switch that regulates the balance between NaCl reabsorption and K+ secretion	[69]
WNK4	PHAII	Knock in *Wnk4 D56A/+*	PHAII like : WNK4 mutant activates NCC through activation OSR1-SPAK-NCC phosphorylation cascade.	[70]
WNK1		*KS-Wnk1 -/-*	PHAII like : mild intermediate phenotype	[74]

CUL3 KLHL3	PHAII	*Cullin 3* *Kelch-like 3*	No mouse model reported.	[76, 77]
NR3C2	PHA-1	*Nrc32 null allele*	PHA-1 like (renal) : high perinatal lethality but rescued by saline infusion	[81] [82]
NR3C2		*Nrc32*	DCT2 and early CNT can compensate to a large extent deficient ENaC-mediated sodium reabsorption in late CNT and CD.	[83]
NR3C2		Nrc32	Under a low-salt, tamoxifen induced ablation of MR at the adult stage recapitulates the renal sodium wasting observed in mice with constitutive early-onset MR ablation.	[84]
NR3C2 (S810L)	MR gain of function		No mouse model reported. Salt sensitive hypertension exarcebated during pregnancy	[85]
	Cushing		Cushing-like ACTH induced hypertension involves both MR and GR	[86]
HSD11B2	AME	*Hsdb2*	Renal sodium retention remains a key event in apparent mineralocorticoid excess (AME) but that the accompanying hypertension changes from a renal to a vascular etiology over time.	[89]
11BHSD 2		*Hsdb2*	Salt sensitive hypertension is mediated by complex interactions between MR and GR in haploid insufficient mice	[90]
SGK1		*Sgk1*	Mild PHA-like phenotype under salt restriction Slight protection against DOCP induced hypertension. Overall, Sgk1 is not absolutely required for sodium reabsorption and potassium secretion in the ASDN.	[94] [95, 98] [114]
		Sgk1	Nephron specific inducible deletion in adult animals generates a mild PHA-1 phenotype under salt restiction together with low blood pressure	[97]
NEDD4L		*Nedd4l* *Exon 7-9*	Salt-, amiloride- sensitive hypertension mimicking many features of Liddle syndrome No lung phenotype	[99]
		Nedd4l *Exon 7-9*	Nephron specific inducible deletion in adult animals generate a novel mild salt sesnitive thiazide- sensitive, amiloride-insensitive hypertension mimicking some features of PHAII	[101]
		Nedd4l *Exon 15*	Lung specific deletion generates a CFTR like phenotype	[100]
		Nedd4l *Exon 15*	Constitutive germ line KO : severe respiratory distress and perinatal lethality in Nedd4-2-deficient mice	[102]
USP2-45		*Usp2-45*	USP2 does not play a primary role in the control of sodium balance or blood pressure	[109]
TSC22D3		*Tsc22d3*	GILZ does not play a primary role in the control of sodium balance or blood pressure	[112]

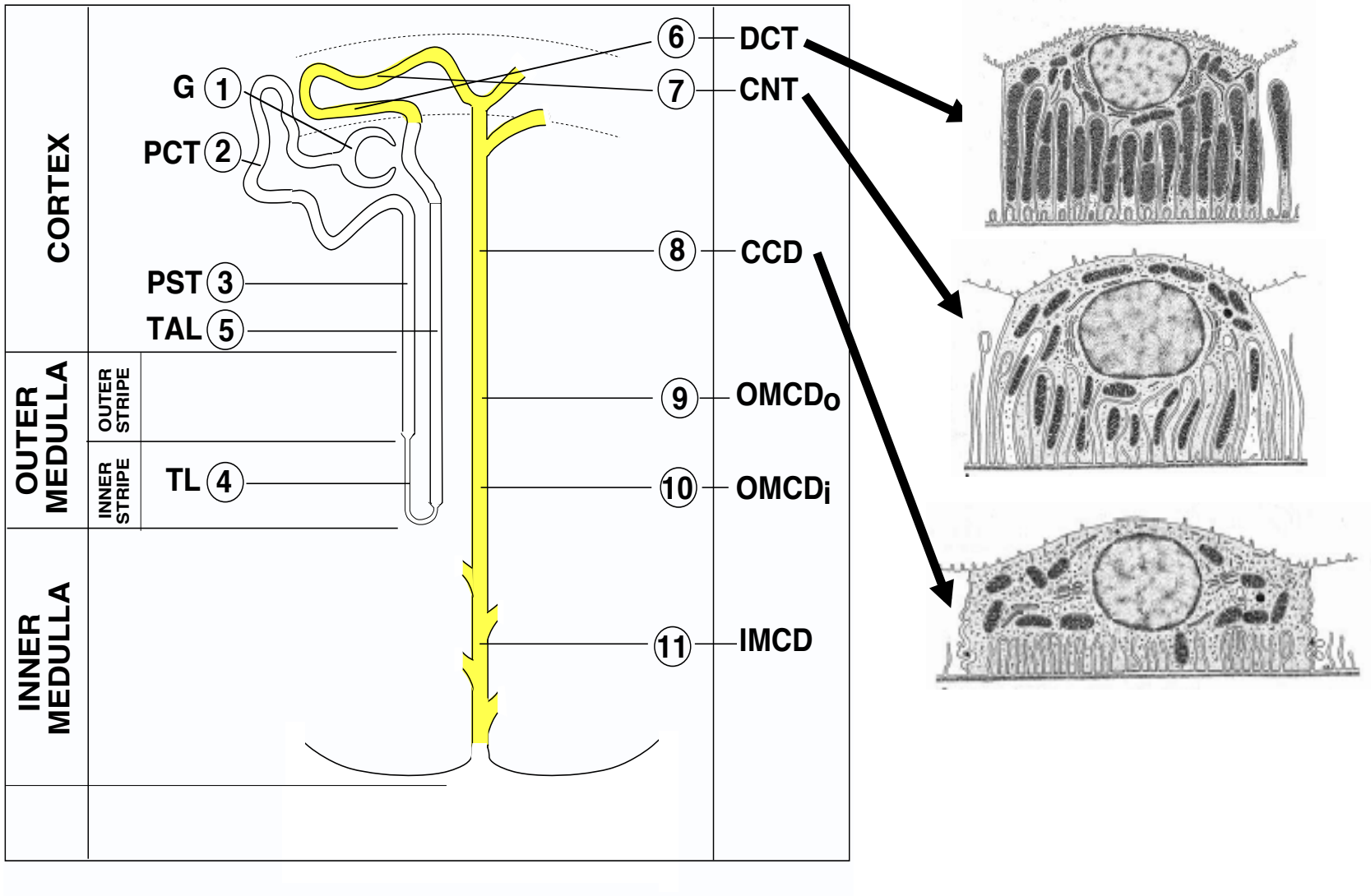
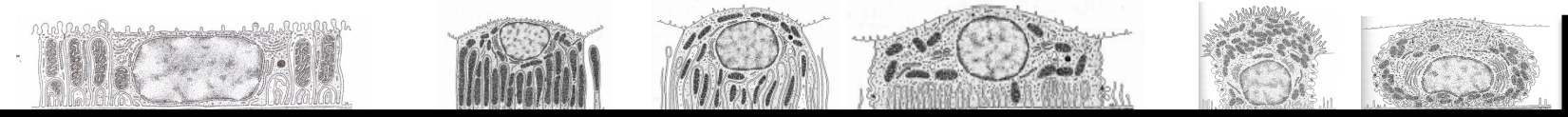

CORTEX
OUTER MEDULLA
OUTER STRIPE
INNER STRIPE
INNER MEDULLA
G 1
PCT 2
PST 3
TL 4
TAL 5
6 DCT
7 CNT
8 CCD
9 OMCDo
10 OMCDi
11 IMCD

Figure 1



Cells

cTAL cell	DCT cell		CNT cell	Principal cells	α IC	β IC
TAL	DCT1	DCT2	CNT	CCD		

Tubule/ Duct

NKCC2					Pendrin SLC26A4	
	NCC				SLC4A8	
		ENaC				
CLCNKB					CLCNKB	
BSND / Barttin					BSND / Barttin	
KCNJ1/ROMK						
BK						

Transporters

MR/GR						
AT1 R						
	11βHSD2					
SGK 1						
	Nedd4-2					
L-WNK1						
	KS-WNK1					
WNK4						
SPAK						
WNK3						
CULLIN 3						
KHEL 3						

Signaling pathways

Figure 2