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Renal mechanism of salt-sensitive hypertension: the contribution of two steroid receptor-associated pathways

Mitsuhiro Nishimoto and Toshiro Fujita

Abstract

Although salt is a major environmental factor in the development of hypertension, the degree of salt sensitivity varies widely among individuals. The mechanisms responsible for this variation remain to be elucidated. Recent studies revealed the involvement of two important signaling pathways in renal tubules that play key roles in electrolyte balance and the maintenance of normal blood pressure: the $\beta_2$ adrenergic stimulant–GR–WNK4–NCC pathway, which is active in distal convoluted tubules 1 (DCT1); and the Rac1–MR pathway, which is active in distal convoluted tubules 2 (DCT2), connecting tubules (CNT), and collecting ducts (CD). $\beta_2$ stimulation due to
increased renal sympathetic activity in obesity- and salt-induced hypertension suppresses HDAC8 activity via cAMP/PKA signaling, increasing the accessibility of the glucocorticoid receptor (GR) to the negative GR response element (GRE) in the \textit{WNK4} promoter. This results in the suppression of \textit{WNK4} transcription, followed by the activation of \(\text{Na}^+\text{-Cl}^-\) cotransporter (NCC) in DCT and elevated sodium retention and blood pressure upon salt loading. Rac1 activates mineralocorticoid receptors (MR), even in the absence of ligand binding, with this activity increased in the presence of ligand. In salt-sensitive animals, Rac1 activation due to salt loading activates MR in DCT2, CNT, and CD. Thus, GR and MR are independently involved in two pathways responsible for renal sodium handling and salt-sensitive hypertension. These findings suggest novel therapeutic targets and may lead to the development of diagnostic tools to determine salt sensitivity in hypertensive patients.

Keywords: salt-sensitive hypertension, mineralocorticoid receptor, glucocorticoid
receptor, Rac1, sympathetic nervous system.
Abbreviations:

Rac1 - Ras-related C3 botulinum toxin substrate 1
MR - mineralocorticoid receptor
DCT - distal convoluted tubule
CNT - connecting tubule
CD - collecting duct
HDAC8 - histone deacetylase 8
GR - glucocorticoid receptor
nGRE - negative glucocorticoid receptor response element
WNK4 - with no lysine (K) kinase 4
NCC - Na⁺-Cl⁻ cotransporter
SNS - sympathetic nerve system
RAS - renin angiotensin system
PHAII - pseudohypoaldosteronism type II

PT - proximal tubule

TAL - thick ascending limb

NKCC - Na⁺-K⁺-2Cl⁻ cotransporter

ENaC - epithelial Na⁺ channel

NHE3 - Na⁺/H⁺ exchanger 3

Ang II - angiotensin II

KO - knockout

AT₁R - angiotensin II type1 receptor

Dahl-S/R - Dahl salt-sensitive/resistant rat

ROMK - renal outer medullary K⁺ channel

NDCBE - Na-dependent Cl⁻/HCO₃⁻ exchanger

SPAK - STE20/SPS1-related Pro/Ala-rich kinase
OSR1 - oxidative stress responsive 1

NE - norepinephrine

DOCA-salt - deoxycorticosterone acetate and salt-loading rat

ROS - reactive oxygen species

11βHSD2 - 11β-hydroxysteroid dehydrogenase type 2

Sgk1 - serum and glucocorticoid-inducible kinase1

RhoGDIα - Rho guanine nucleotide dissociation inhibitor α

SHR - spontaneously hypertensive rat

ARF - aldosterone-releasing factor
Introduction

High levels of dietary salt intake can induce hypertension, with salt restriction being one of the most effective treatments for this condition (32, 72, 79).

Hypertensive patients, however, exhibit a large spectrum of salt sensitivity (71), with the mechanisms determining salt sensitivity as yet incompletely understood.

Several factors influence salt sensitivity, including the activities of the sympathetic nervous system (SNS), the renin–angiotensin system (RAS), aldosterone, and insulin. In the Guytonian model, dysfunctional excretion of renal sodium plays a major role in sodium retention and salt-sensitive hypertension (42). Several genes have been found to cause rare Mendelian forms of human hypertension (134), with these findings providing insight into the basic mechanisms of human hypertension and highlighting the key role of altered sodium handling by the kidneys as a final common pathway in the pathogenesis of hypertension. In addition, mutations in several genes
cause secondary genetic hypertension, e.g., primary aldosteronism and pseudohypoaldosteronism type II (PHAII) (122). We identified two important pathways associated with the development of salt-sensitive hypertension in these two syndromes: the renal SNS and aldosterone pathways. Each of these pathways is associated with the sodium-handling system in a distinct region of the nephron: one in distal convoluted tubules (DCT), and the other in connecting tubules and collecting ducts (CNT/CD).

Animal models of salt-sensitive hypertension

Several animal models have been used to study salt-sensitive hypertension, with most, including the Ang II infusion, aldosterone infusion, and DOCA-salt models, as well as many genetically-engineered mouse models, exhibiting salt sensitivity. These models utilize known mechanisms of salt sensitivity, making them suitable to
study specific pathways of salt-sensitive hypertension. By contrast, Dahl salt-sensitive (Dahl-S) and salt-resistant (Dahl-R) rats were generated by selecting SD rats by their salt sensitivity (18). Salt-sensitive hypertension in these animals was thought to result from the accumulation of genes involved in salt sensitivity, making them suitable to investigate as yet unknown pathways of salt-sensitive hypertension. Sabra rats (5) were similarly inbred by selecting DOCA-salt-sensitive animals. Although spontaneously hypertensive rats (SHR) were generated in a similar way (88) and exhibit salt sensitivity (70), they were selected to model essential hypertension, and did not necessarily accumulate genes involved in salt sensitivity. Other genetically inbred rat models of essential hypertension include Milan (7), New Zealand (112), and Lyon (23) genetically hypertensive rats.

Evidence from these animal models, as well as studies in humans, revealed the importance of renal sodium handling in salt-sensitive hypertension. Under normal
physiological conditions, 99.5% of sodium excreted in the glomerulus is reabsorbed through the nephrons. About 70% is reabsorbed by the proximal tubules (PT), largely by \( \text{Na}^+/-\text{H}^+ \) exchange; 20–30% by the thick ascending limb (TAL) of Henle via the \( \text{Na}^+/-\text{K}^+/-2\text{Cl}^- \) cotransporter (NKCC); and 5–7% by the \( \text{Na}^+/-\text{Cl}^- \) cotransporter (NCC) in DCT. The last fraction is reabsorbed via epithelial \( \text{Na}^+ \) channels (ENaC) in the CNT/CD.

\( \text{Na}^+/-\text{H}^+ \) exchanger 3 (NHE3) in PT (62, 68) and NKCC2 in the TAL (3, 10, 66) are associated with salt-sensitive hypertension in animal models. Salt-induced NHE3 suppression is more impaired in Dahl-S than in Dahl-R rats (68). NKCC2 activity in TAL is also up-regulated in salt-sensitive rats (3, 10, 66). SNPs involving NKCC2 activation in TAL are associated with salt-sensitive hypertension in humans (123).

The chloride transport system associated with sodium handling has also attracted attention. Pendrin, a sodium-independent \( \text{Cl}^-/-\text{HCO}_3^- \) exchanger, is
expressed on the apical membranes of intercalated cells in DCT/CNT/CD and is a target of the aldosterone/MR pathway (111, 128). Pendrin, together with the Na⁺-dependent Cl⁻/HCO₃⁻ exchanger NDCBE (SLC4A8) and ENaC, absorbs sodium and chloride electroneutrally in a thiazide-sensitive but NCC-independent manner (65).

Beta-intercalated cell-specific-pendrin transgenic mice exhibit salt-sensitive hypertension (50), and genetic ablation of pendrin normalizes mineralocorticoid-induced hypertension (128). Although both pendrin-ablated mice and individuals with genetic disruption of SLC26A4 have normal renal function and normal acid-base, fluid, and electrolyte balances (102), the functions of pendrin and NCC may be partially redundant. Indeed, double-knockout mice exhibit severe salt wasting and volume depletion (114). Thus pendrin is another candidate target for novel diuretic and antihypertensive agents.

Although these transporters contribute to salt-sensitive hypertension, the
main targets of antihypertensive agents at present are NCC and ENaC. NCC, the
classical target of thiazide diuretics, is a key molecule involved in blood pressure
maintenance, with regulation of this cotransporter receiving increased attention. ENaC,
mainly modulated by the aldosterone/MR system, is a major regulator of hypertension
in the salt-loading state.

NCC regulation by WNK kinases

NCC is distributed in the DCT region and is a primary target of thiazide
diuretics. Mutations in the "with-no-K" (K=lysine; WNK) kinases WNK1 and WNK4
were identified as possibly responsible for pseudohypoaldosteronism type II (PHAII),
also known as familial hyperkalemic hypertension or Gordon syndrome (134). Since
that finding, the regulation of NCC by the WNK family of kinases has attracted
increased attention. Patients with PHAII exhibit hypertension, hyperkalemia, and
hyperchloremic metabolic acidosis; in these individuals, low-dose thiazide diuretics can normalize both blood pressure and the metabolic disorder, thus implicating these mutations in NCC regulation (40).

The \textit{WNK1} mutation responsible for PHAII is an intronic deletion, resulting in elevated expression of wild-type WNK1. Therefore, WNK1 was thought to enhance NCC activity. Indeed, WNK1 in \textit{Xenopus laevis} oocytes did not directly affect NCC activity; however, when co-expressed with WNK4, WNK1 increased NCC activity by suppressing WNK4 (139, 140). Subsequent studies suggested more complicated modes of regulation. \textit{WNK1} was found to express two isoforms as a result of alternative splicing: L-WNK1, a ubiquitously expressed kinase, and KS-WNK1, a shorter kinase-deficient form, expressed specifically in DCT/CNT. \textit{In vitro}, KS-WNK1 inhibits L-WNK1 function and suppresses NCC surface expression (140). Furthermore, KS-WNK1-deficient mice exhibit salt-sensitive hypertension in response to NCC
activation, despite the compensatory down-regulation of ENaC activity, whereas KS-WNK1 transgenic mice exhibit reduced NCC activity and blood pressure (43, 69). These lines of evidence suggested that KS-WNK1 may contribute to control of NCC activity; however, KS-WNK1 ablation alone causes a much less severe phenotype than the phenotypes resulting from WNK4 mutations in mice and humans. An elegant method utilizing the Cre-loxP system showed that mice harboring the PHAII-associated deletion of the first intron of WNK1 expressed higher levels of L-WNK in a DCT/CNT-specific manner and recapitulated the complete PHAII-like phenotype without a change in KS-WNK1 expression (129).

Notwithstanding the results of the aforementioned in vitro studies, several investigators show that L-WNK1 can directly regulate NCC by phosphorylating SPAK (STE20/SPS1-related proline/alanine-rich kinase), which phosphorylates NCC and stimulates its activity (80, 99). Consistent with this result, WNK
phosphorylation-resistant SPAK knock-in mice exhibited salt wasting and low blood pressure due to reduction of NCC activity (95). This pathway concomitantly regulates NKCC2; however, because loop diuretics are not clinically effective against PHAII, the role of NKCC2 activation remains unclear.

Mutations in the \textit{WNK4} gene responsible for PHAII were found to be missense mutations in an acidic region highly conserved among the WNKs. Wild-type WNK4 suppressed NCC activity in \textit{X. laevis} oocytes and Cos-7 and HEK293 cells (8, 37, 135, 139). In addition, transgenic mice expressing the disease-causing mutant developed hypertension associated with increased NCC expression in the DCT, whereas overexpression of a wild-type \textit{WNK4} transgene lowered BP (64, 134). The inhibitory mechanism appears to involve WNK4-mediated degradation of surface-expressed NCC via a lysosomal pathway (117, 145). Furthermore, in normal SD rats, renal expression of WNK4 was reduced by low salt intake and increased by
high salt intake, whereas NCC expression had the opposite response (63, 91). In addition, a high-potassium diet, which promotes salt excretion and confers resistance to high salt-induced hypertension, increased WNK4 while reducing NCC levels in DCT (127). These modulations by electrolyte intake were independent of both Ang II and aldosterone. Therefore, WNK4 was initially thought to suppress NCC activity, and WNK4 modulation of NCC was thought to contribute to renal adaptation in response to changes in dietary salt intake. However, recent studies revealed that the regulation of NCC in several pathological conditions is more complex (34). Mutant WNK4 transgenic mice showed NCC activation and blood pressure elevation even when they had two alleles of wild-type WNK4, suggesting that mutant WNK4 stimulates NCC, overwhelming the suppressive effect of wild-type WNK4. Indeed, WNK4 acts upstream of SPAK and oxidative stress response kinase-1 (OSR1), as do other members of the WNK family, such as WNK1. Furthermore, WNK4 was found to switch
modes, from inhibitory to stimulatory (130), and genetic ablation of WNK4 or knock-in
of WNK4-resistant SPAK significantly reduced NCC expression in the kidneys,
indicating that the WNK4–SPAK pathway is essential for NCC expression itself (12,
95).

Ang II is a key hormonal regulator involved in the switching of WNK4 modes.

WNK4 and WNK1 phosphorylate SPAK/OSR1 in the presence of Ang II, leading to
NCC phosphorylation (i.e., activation) in vitro and in vivo (12, 104, 130). Furthermore,
aldosterone was also found to switch the functional mode of WNK4 and to directly
activate NCC, even in the absence of Ang II (126); although this finding could not be
confirmed (103). Insulin also activates NCC, a pathway also thought to involve WNK4,
but it is unclear whether insulin signaling alters WNK4 status as does Ang II. Acute
insulin administration resulted in the phosphorylation of SPAK/OSR1, followed by
NCC activation, with WNK4 being essential to this pathway both in vitro and in vivo;
however, there is no evidence that insulin induced changes in expression of WNK4 itself (113). By contrast, insulin administration significantly reduced WNK4 expression (60, 115), suggesting that the effect of insulin on NCC resulted from alleviation of the suppressive effect of WNK4. These apparent discrepancies may be resolved by the hypothesis that WNK4 acts via two distinct pathways, both of which converge on NCC activity and expression. WNK4 molecules that suppress surface expression of NCC were recently shown to occupy a different subcellular compartment than WNK4 molecules that interact with SPAK (16). Thus WNK4 may intrinsically exert both inhibitory and stimulatory effects on NCC, and external hormonal factors may determine the balance of these opposing effects to fine-tune NCC activity and sodium handling.

We identified a novel pathway in DCT, in which renal sympatho-excitation suppresses WNK4 and NCC activity, leading to increased sodium reabsorption at the
Renal sympatho-excitation in salt-sensitive hypertension

A growing body of evidence indicates that excitation of the SNS underlies salt-sensitive hypertension (9, 31, 32, 36). Salt loading increases sympathetic activity in the kidney as well as the brain, and those excitations seem to be linked, as described in detail in the following section. We found that the renal norepinephrine (NE) turnover rate was elevated in salt-sensitive hypertensive, but not in normotensive, rats (33). Similarly, in obese hypertensive patients, the NE spillover rate was found to be specifically increased in the kidneys, but not in the liver or intestines (26), suggesting that renal sympathetic activity is selectively elevated in these individuals.

Recent clinical studies show that renal sympathetic denervation therapy is remarkably effective in the treatment of drug-resistant hypertension (61, 120),
although the underlying molecular mechanism remains to be determined. Stresses
due to the delivery of short-period continuous air jets to the heads of DOCA-salt rats
decreased urinary sodium excretion by increasing renal sympathetic activity to a
greater extent than observed in control normotensive rats, a response inhibited by
renal denervation (106). This observation suggests that natriuresis induced by renal
denervation may contribute to the antihypertensive effects of this therapy in
salt-sensitive hypertension (28, 53).

Renal sympathetic nerve activation and WNK4 suppression in salt-sensitive
hypertension

Sympatho-excitation in PT increases sodium reabsorption, both by renin
production, resulting from $\beta_1$ stimulation, and direct tubular action, resulting from $\alpha_1$
stimulation (20). In addition, using a mouse model, we observed that continuous
infusion of NE induced salt-sensitive hypertension, accompanied by reduced WNK4 expression and elevated NCC expression in their kidneys (81). These effects were reversed by treatment with a blocker of non-selective β-adrenergic receptors, but not by an α₁ blocker. NCC activation by renal sympathetic excitation in DOCA-salt rat was inhibited with a β₂-antagonist, but not with a β₁-antagonist. Administration of isoproterenol to both β₁-KO and wild-type mice on a high-salt diet increased blood pressure, but this effect was not observed in β₂-KO mice. In vitro, GR is essential for β-adrenergic regulation of WNK4/NCC expression, and we identified one negative GR-responsive element (nGRE) in the WNK4 promoter; the negative effect of GR on WNK4 expression was recently confirmed (119). Consistent with these findings, distal nephron-specific GR-knockout mice infused with isoproterenol showed no increase in BP following salt loading. This β-adrenergic effect resulted from epigenetic histone modulation of the WNK4 promoter, through suppression of histone deacetylase8
(HDAC8) activity via phosphorylation by PKA. Thus β₂-adrenergic stimulation due to increased renal sympathetic activity in salt-sensitive hypertension activates cAMP/PKA, suppresses HDAC8 activity, and increases histone acetylation. Histone acetylation makes the WNK4 promoter region more accessible to GR, increasing GR-nGRE binding and reducing WNK4 transcription (Figure 1). Reductions in WNK4 levels result in NCC activation, leading to sodium retention and salt-sensitive hypertension. Renal denervation reversed the reduction in renal WNK4 expression in DOCA-salt rats, which was associated with normalization of blood pressure. We therefore hypothesized that the improvement in renal sodium excretion induced by renal denervation is the primary mechanism underlying the antihypertensive effects of renal nerve ablation, documented in recent clinical studies. As described in the previous section, WNK4 can enter the stimulatory state in response to Ang II, aldosterone, and potentially other factors. We found that neither an AT₁R blocker nor
an MR antagonist could reverse isoproterenol-induced salt-sensitive hypertension and
down-regulation of WNK4, although it remains unclear whether the SPAK/OSR1
pathway was activated under those experimental conditions. A recent study showed
that NCC activation induced by NE-infusion requires OSR1, but not SPAK (121).

Because of its association with hypertension, WNK4 has also been the focus
of recent vascular research. WNK4 expressed in vessels suppresses TRPC3, a
member of the receptor-operated calcium channel family that modulates vascular tone
(92). The PHAII-associated mutant form of WNK4 could not suppress TRPC3
expression, with expression of this variant form resulting in enhanced vasoconstriction
in response to α-adrenergic stimulation (92). It is not known whether vascular WNK4 is
also controlled by the HDAC8/GR pathway, or whether this mechanism is associated
with salt-sensitive hypertension; both are possible.
Central sympathetic nerve excitation drives the renal sympathetic nerve activity. Several factors including obesity, RAS, and salt loading activate SNS, as described below. In rodent models, sympatho-excitation by oxidative stress in the brain mediates blood pressure elevation, suggesting that brain reactive oxygen species (ROS) are involved in both salt- and obesity-induced hypertension (30, 82).

Compared with Dahl-R rats, Dahl-S rats on a high-salt diet displayed greater depression of renal sympathetic nerve activity and reduction in blood pressure in response to intracerebroventricular administration of antioxidants (30). Diet-induced obese rats reacted similarly (82). Salt loading of young uninephrectomized rats increased renal sympathetic activity and blood pressure, with antioxidants in the brain successfully reversing the elevations in blood pressure, renal sympathetic nerve activity and renal damage (29). Thus salt loading itself activates central and renal
Primary aldosteronism is a representative type of salt-sensitive hypertension, resulting from increased sodium reabsorption in aldosterone-sensitive distal nephrons. Aldosterone activates mineralocorticoid receptors (MR) and epithelial sodium channels (ENaC), increasing sodium reabsorption and salt-sensitive hypertension. The putative ligands of MR are cortisol and aldosterone. Both ligands have equal affinity for MR, but the plasma concentration of cortisol is hundreds to thousands of times higher, making cortisol the major ligand of MR in some tissues. However, aldosterone is the physiological ligand in tissues in which 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) is expressed, because this enzyme converts cortisol to cortisone, which cannot bind to MR. Expression of MR is distributed...
throughout the nephron, but 11βHSD2 is highly expressed only in DCT2, CNT, and CD.

This specific distribution of 11βHSD2 is coincidental with the expression of its effector, ENaC, within distal nephrons, especially in CNT/CD (13).

As previously described, ENaC is the major regulator of salt reabsorption in salt-sensitive hypertension. Aldosterone/MR signaling activates ENaC in two phases. In the acute phase, aldosterone/MR up-regulates membrane expression of pre-existing ENaC by activating the transport machinery; for example, through suppression of the ubiquitin ligase Nedd4-2 via phosphorylation by serum- and glucocorticoid-induced kinase-1 (Sgk1) (19). In the delayed phase, aldosterone-activated MR binds to the promoter region of the gene encoding ENaCα, thereby increasing de novo synthesis of ENaC. Sgk1 also plays a role during this late phase, relieving suppression of ENaC transcription by inhibiting the H3K79 methyltransferase, which blocks transcription (144).
Aldosterone/MR signaling also plays an important role in the DCT2. In addition to activating ENaC, the aldosterone/MR pathway also activates NCC by phosphorylation via the SPAK pathway, regardless of whether upstream WNK4 is stimulatory or inhibitory and its interaction with WNK4 remains unclear (58, 118, 126), as well as by ubiquitination through the Sgk1/Nedd4-2 pathway (4, 101).

A long-term study in humans found that the rhythm of urinary sodium excretion directly correlated with weekly rhythms of serum and urinary aldosterone concentrations, whereas the relationships of the former with daily dietary salt intake and cortisol concentrations appeared to be indirect (96). In only three genes responsible for Mendelian diseases, mutations can induce both hypertension and hypotension: ALDOS, which encodes aldosterone synthase; MR, which encodes mineralocorticoid receptor; and SCNN1, which encodes ENaC (67). These lines of evidence showed that the aldosterone/MR pathway plays a very important role in
sodium storage, and that dysregulation of this pathway may cause impaired sodium
handling and blood pressure control.

**Novel pathway of MR activation**

Clinical trials show that MR inhibition ameliorates vascular and kidney injury in individuals with hypertension (133) and prevents cardiac death in heart-failure patients (93, 94). On average, obese patients have statistically significantly higher plasma aldosterone concentrations, which may contribute to the salt-sensitive hypertension frequently observed in these individuals (6, 25); however, plasma aldosterone levels are not elevated in every patient. Nevertheless, treatment with an MR blocker decreased both blood pressure and proteinuria in patients with metabolic syndrome, including those with normal aldosterone levels (105). This finding suggests that aberrant MR activation can occur in subjects with normal aldosterone levels.
Indeed, in salt-sensitive animal models, MR blockers can successfully prevent cardiac (86) and renal (84) injury in the absence of high plasma aldosterone levels. In humans, a ligand-binding domain-deficient MR variant activates MR-dependent genes in the absence of ligands in vitro (143). Together, these findings suggest that MR activation can occur even in the absence of ligands, possibly through alternative pathways.

The mechanisms of nuclear receptor activation suggest that several factors may be involved in MR activation, including ligand levels, ligand type, nuclear translocation, histone modulation, co-activators and co-repressors, other transcription factors, and crosstalk among intracellular signaling pathways. Among the factors found to modulate the activity of nuclear receptors are SRC1 (90), RAS/MAPK (54), Smad3 (77, 138), PKA (14, 75), UBC9 (141), SUMO (108) and RhoGTPase (116).

Rac1, a small RhoGTPase, may also play a role in regulating nuclear translocation of transcription factors (57, 137). We recently reported that Rac1 activates MR in both
ligand-dependent and ligand-independent manners (110).

Transfection of the constitutively active form of Rac1 into HEK293 kidney cells induces nuclear translocation and transcriptional activation of MR, even in the absence of ligand (110). Aldosterone increased this Rac1-induced MR activation.

Furthermore, RhoGDIα-knockout mice, a kidney-specific Rac1 activation model, showed MR activation in the kidneys and massive podocyte injury; this phenotype was reversed not only by a Rac1 inhibitor, but also by the MR blocker, eplerenone (110).

Periorbital edema in RhoGDIα-deficient zebrafish was also improved by treatment with either eplerenone or Rac inhibitors (35). Recently, mutations in RhoGDIα have been identified in human subjects with steroid-resistant nephrotic syndrome (35).

Although not yet reported, MR antagonists may be effective for the disease.

Rac1-induced MR activation in salt-sensitive hypertension
Several factors modulate Rac1, including cytokines (107, 125, 131, 136), mechanical stress (1, 124), and dietary salt intake (109). In normal SD rats, salt loading decreases Rac1 activity in the kidneys and is associated with down-regulation of the MR target gene, Sgk1, in accordance with reduced levels of plasma aldosterone.

Salt loading of Dahl-R rats also reduced active Rac1, whereas salt loading of Dahl-S rats markedly up-regulated Rac1. Consistent with these changes in Rac1 activity, nuclear MR was down-regulated by high-salt loading in Dahl-R rats, but up-regulated in Dahl-S rats, despite the equal suppression of plasma aldosterone concentrations in both sets of animals. Treatment with the Rac inhibitor NSC23766 not only decreased nuclear MR, but suppressed the increase in blood pressure induced by salt loading as effectively as treatment with the MR blocker eplerenone. This paradoxical response of MR activation to salt loading in Dahl-S rats is attributable solely to the abnormal activation of Rac1 in response to salt loading, which leads to increased sodium
reabsorption in the distal nephrons and salt-sensitive hypertension. Thus, Rac1 is a
determinant of both MR activation and salt sensitivity (Figure 2).

Mechanism of salt-induced Rac1 activation

Several factors, including genetic polymorphisms in Rac1 modulators, may be responsible for activation of Rac1, leading to salt-sensitive hypertension. Patients harboring mutations in endogenous Rac1 modulators have steroid-resistant nephrotic syndrome (2, 35, 41). However, no genetic polymorphisms associated with Rac1 modulators have been reported in humans with hypertension or Dahl rats with salt-sensitive hypertension. Other potential Rac1-activating factors include inflammation-related substances derived from adipocytes, such as IL-6 and TNFα, and oxidative stress, all of which activate Rac1 (38, 47, 107, 136). Local activation of RAS may also result in activation of Rac1. Salt loading increases local RAS activity in
Rac1 activation determines the induction of salt sensitivity in response to MR activation, but salt itself can activate Rac1, particularly together with aldosterone, under certain pathological conditions. Levels of the active form of Rac1 in the kidneys of Ang II-overexpressing mice were elevated following salt loading, concomitant with up-regulation of Sgk1, and both Rac inhibition and MR blockade decreased nuclear MR and Sgk1 expression and prevented podocyte injury (56). Moreover, adrenalectomy clearly suppressed salt-induced Rac1 activation in both Ang II-overexpressing mice and salt-loaded Dahl-S rats, whereas supplementation with aldosterone re-activated Rac1, suggesting that salt and aldosterone interdependently activate Rac1 and may determine the degree of salt sensitivity by modulating the Rac1/MR pathway. Consistent with this hypothesis, albuminuria and hypertension were absent in both Dahl-S and aldosterone-infused rats placed on a low-salt diet.
This phenomenon was associated with inactivation of the Rac1/MR pathway in the kidneys, suggesting that salt is required for aldosterone-induced activation of the Rac1-MR pathway in these animal models.

As for other factors modulating salt sensitivity, recent studies show that MR activation in the brain plays a key role in salt-induced sympatho-excitation (48, 49), leading us to hypothesize that an interaction between Rac1 and the aforementioned brain ROS modulates MR activity in the brain, as well as in the heart (83), resulting in activation of the central SNS (55). However, the involvement of the Rac1–MR pathway in the brain has not been observed to date.

Mechanism of salt-sensitive hypertension in obese subjects

Several reports show that obesity is associated with salt-sensitive hypertension in animals and humans (15, 100). Although the mechanisms underlying
obesity-induced, salt-sensitive hypertension remain to be elucidated, the aforementioned pathways may contribute.

Elevated levels of plasma aldosterone and urinary norepinephrine, along with impaired renal excretion of sodium into the urine, have been observed in obese SHR (118). Moreover, renal denervation attenuated the increased sodium retention and decreased blood pressure observed in obese animals (52) and patients (46).

Focusing on NCC activation, hyperinsulinemia in obese animals and humans may contribute to salt-sensitive hypertension. Although the effects of insulin on NCC remain unclear, acute insulin stimulation of mDCT cells increases NCC activity through SPAK-OSR1-induced phosphorylation, without altering WNK4 expression (60, 113). Moreover, chronic insulin treatment is associated with lower levels of WNK4 expression (60), suggesting that while reduced WNK4 expression does not induce
early NCC activation, it helps to maintain NCC in an activated state during chronic hyperinsulinemia. Furthermore, NCC activation was observed in hyperinsulinemic db/db mice (87) and obese Zucker rats with lower expression of WNK4 (60). However, these models cannot provide direct evidence for the involvement of hyperinsulinemia-induced NCC activation in the development of salt-sensitive hypertension. Indeed, continuous infusion of insulin alone failed to induce the development of hypertension in dogs or humans, even when accompanied by Ang II infusion (44). The underlying reason is not clear, but because insulin by itself relaxes vascular tone, and insulin sensitivity is altered in a tissue-dependent manner and by obesity, insulin infusion alone may not reproduce the conditions observed in physiological obesity.

Pathways other than those induced by insulin contribute to salt-induced hypertension in obesity. Leptin, a well-known adipocytokine, elevates sympathetic
activity in obese Zucker rats by activating melanocortin signaling in the central nervous system, thereby increasing blood pressure (21). Since angiotensinogen, which is cleaved to Ang II, is also secreted by adipose tissue (11), leptin and angiotensinogen may be involved in NCC activation induced by obesity through the WNK4–NCC and SPAK–OSR1 pathways, respectively; however, no data supporting this hypothesis have yet been published.

The MR activation pathway may also be involved in obesity-related salt-sensitive hypertension via excess aldosterone expression and aberrant Rac1 activation. Many obese hypertensive patients suffer from relative hyperaldosteronism (6, 25). Obese SHRs exhibit increased plasma aldosterone levels and develop massive proteinuria in an age-dependent manner. By contrast, lean SHRs exhibit relatively low and unchanged urinary protein excretion levels. In addition, renal damage in obese SHRs can be reversed by the MR blocker eplerenone (85). Similar
to the original findings (24), we found that the culture medium of adipocytes taken from
obese SHRs could stimulate aldosterone release from a cultured adrenal cell line (85).

Some candidates for this "aldosterone-releasing factor (ARF)" include TNF-α (78),
CTRP-1 (51), linoleic acid oxidation products (39), leptin (73), IL-6 (76), and an
unknown molecule whose size is greater than 50 kDa (24). Notably, these ARFs are
not subject to negative feedback by the RAS, which is modulated by salt intake. In
normal subjects, however, plasma aldosterone is counterbalanced by high salt intake
through the inhibition of the RAS, thereby decreasing MR activity. Thus in obese
subjects on a high-salt diet, inappropriate secretion of aldosterone from the adrenal
glands induces salt-sensitive hypertension through MR activation.

As described above, an MR antagonist is effective in blood pressure
reduction and cardiovascular protection in patients with metabolic syndrome, even
those with normal aldosterone levels (105). Furthermore, in a rodent model, kidney
localized Rac1/MR activation contributed to obesity-related diabetic kidney disease (142). These findings suggest that aberrant Rac1 activation followed by enhancement of MR activation with aldosterone may have occurred, but further studies are required for confirmation.

Salt-sensitive hypertension with abnormal circadian rhythms

Increasing evidence demonstrates that the circadian clock regulates renal sodium handling and blood pressure. Clinical studies show that patients with salt-sensitive hypertension exhibited impaired circadian rhythms and failed to undergo a nocturnal decline in blood pressure (45). This phenomenon is related to hypertension in obese subjects. Indeed, a recent clinical study showed that the lack of reduction in nocturnal blood pressure was twice as frequent among severely obese children than among the general child population (132). Circadian rhythms are
generated in the individual cells by the oscillation of clock genes interlocked in an auto regulatory transcription–translation feedback loop; these oscillations are integrated into the rhythm at the whole-body level via neural and hormonal pathways controlled by the suprachiasmatic nucleus (89). At the cellular level, several clock genes are involved in rhythm generation. By transcription of Per1–3, the cellular circadian clock begins a new cycle. Expression of these genes is stimulated by the binding of the Clock–Bmal complex at E-box cis-regulatory elements in their promoters. Per proteins are synthesized in the cytoplasm, translocating into the nucleus to interact with Cry proteins; this complex inhibits Per transcription by epigenetic modulation, interacting with the Clock–Bmal complex (27).

The clock genes have been implicated in blood pressure regulation. Cry1/2 KO mice developed salt-sensitive hypertension, whereas Per1 hetero KO mice exhibited hypotension with impaired renal sodium conservation associated with
reduced plasma aldosterone (97). Notably, Per1 hetero KO mice expressed higher levels of WNK4, elevating NCC expression in the kidneys and increasing urinary sodium excretion and hypotension (98). A human-gene expression profiling study found that the level of Per1 was higher in the renal medulla of hypertensive than normotensive individuals (74). Clock KO mice are hypotensive (146) and Bmal1 KO mice exhibit reduced BP during the active phase (17).

In contrast to Per1 hetero KO mice, Cry1/2 KO mice exhibit high aldosterone levels resulting from increased levels of 3β-dehydrogenase, an enzyme of the aldosterone synthesis pathway that is expressed exclusively in the zona glomerulosa of the adrenal glands (22). Interestingly, these mice exhibited normal blood pressure when fed normal chow, but had elevated blood pressure when placed on a high-salt diet. Thus the clock genes may be involved in abnormalities of both the aldosterone–MR and WNK4–NCC pathways in patients with salt-sensitive
hypertension.

The roles of MR and GR in the two pathways of renal sodium handling

We identified two novel pathways in salt-sensitive hypertension: the $\beta_2$-adrenergic stimulant–GR–WNK4–NCC pathway in DCT1, and the Rac1–MR pathway in DCT2, CNT and CD (Figure 1, 2). Notably, MR and GR, the two important nuclear receptors that control sodium status and blood pressure, are independently involved in renal sodium transport pathways in different tubular segments, although both receptors are distributed throughout the nephrons. The enzyme 11$\beta$HSD2, which converts cortisol to cortisone, an inactive metabolite, is present in the kidneys; therefore, aldosterone, rather than cortisol, serves as the physiologically important ligand of MR in this organ. However, 11$\beta$HSD2 is absent from DCT1 (13). Consequently, cortisol, which is abundant in DCT1, rather than aldosterone, may be
the ligand for MR, and may also serve as the ligand for GR following β-adrenergic
stimulation-induced down-regulation of WNK4. This hypothesis is consistent with the
results of our study, which showed that glucocorticoid-activated GR activated in DCT1
is indispensable for β-adrenergic stimulation-induced salt-sensitive hypertension. By
contrast, aldosterone/Rac1-induced MR activation in DCT2 not only stimulates NCC
through Sgk1-induced release from the inhibitory effects of WNK4 (103), but also
through activation of MR at DCT2, CNT, and CD, which in turn activates ENaC through
Sgk1. Notably, our results showed that either renal denervation or treatment with an
MR antagonist can effectively decrease blood pressure in Dahl-S rats with renal
sympathetic hyperactivity and activated MR. However, both treatments were required
to decrease blood pressure to normal levels, suggesting that the pathophysiology of
salt-sensitive hypertension is heterogeneous (81, 109). Therefore, appropriate
treatment of hypertension must be designed on an individual basis. Nevertheless,
dietary salt restriction remains the most important lifestyle modification for both the prevention and treatment of hypertension, particularly salt-sensitive hypertension.

Conclusion

We recently identified two novel pathways in salt-sensitive hypertension: the $\beta_2$-adrenergic stimulant–GR–WNK4–NCC pathway in DCT1; and the Rac1–MR pathway in DCT2, CNT, and CD. MR and GR, nuclear receptors that play key roles in controlling electrolyte balance and maintaining normal blood pressure, are independently involved in these two novel pathways in distinct segments of the distal nephron. Our results offer new insight into the regulation of renal sodium handling, suggesting novel therapeutic targets for the treatment of salt-sensitive hypertension and raising the prospect of developing new diagnostic tools for determining the salt sensitivity of hypertensive patients. This, in turn, should allow treatment to be
customized to individual patients.

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Conflicts of interest

There are no conflicts of interest.
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Figure legends

Figure 1. Response to a high-salt diet of salt-resistant and salt-sensitive phenotypes in DCT, which lack 11β HSD2

In salt-sensitive hypertension, salt loading activates the β-adrenergic stimulant–GR–WNK4–NCC pathway, whereas salt suppress the SNS in salt-resistant hypertension in DCT.

Figure 2. Response to a high-salt diet of salt-resistant and salt-sensitive phenotypes in CNT/CD, which have 11β HSD2

In salt-sensitive hypertension, salt loading activates the aldosterone/Rac1–MR pathway, whereas salt suppresses Rac1 in salt-resistant hypertension, although salt suppresses the aldosterone level to some extent.