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

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Nishimoto M, Fujita T. Renal mechanisms of salt-sensitive hypertension: contribution of two steroid receptor-associated pathways. Am J Physiol Renal Physiol 308: F377–F387, 2015. First published December 17, 2014; doi:10.1152/ajprenal.00477.2013.—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 have 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 β-adrenergic stimulant-glucocorticoid receptor (GR)-with-no-lysine kinase (WNK)4-Na⁺-Cl⁻ cotransporter pathway, which is active in distal convoluted tubule (DCT)1, and the Ras-related C3 botulinum toxin substrate (Rac)1-mineralocorticoid receptor (MR) pathway, which is active in DCT2, connecting tubules, and collecting ducts. β-Adrenergic stimulation due to increased renal sympathetic activity in obesity- and salt-induced hypertension suppresses histone deacetylase 8 activity via cAMP/PKA signaling, increasing the accessibility of GRs to the negative GR response element in the WNK4 promoter. This results in the suppression of WNK4 transcription followed by the activation of Na⁺-Cl⁻ cotransporters in the DCT and elevated Na⁺ retention and blood pressure upon salt loading. Rac1 activates MRs, 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 MRs in DCT2, connecting tubules, and collecting ducts. Thus, GRs and MRs are independently involved in two pathways responsible for renal Na⁺ 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.

salt-sensitive hypertension; mineralocorticoid receptor; glucocorticoid receptor; Rac-related C3 botulinum toxin substrate 1; sympathetic nervous system

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 Na⁺ plays a major role in Na⁺ retention and salt-sensitive hypertension (42). Several genes have been found to cause rare Mendelian forms of human hypertension (134), with these findings providing insights into the basic mechanisms of human hypertension and highlighting the key role of altered Na⁺ 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 Na⁺-handling system in a distinct region of the nephron: one in distal convoluted tubules (DCTs) and the other in connecting tubules (CNTs) and collecting ducts (CDs).

Animal Models of Salt-Sensitive Hypertension

Several animal models have been used to study salt-sensitive hypertension, with most, including ANG II infusion, aldosterone infusion, and DOCA-salt models as well as many genetically engineered mouse models, exhibiting salt sensitivity. These models use known mechanisms of salt sensitivity, making them suitable to study specific pathways of salt-sensitive hypertension. In contrast, Dahl salt-sensitive and Dahl salt-resistant rats were generated by selecting Sprague-Dawley 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 hyper-
tension. Sabra rats (5) were similarly inbred by selecting DOCA-salt-sensitive animals. Although spontaneously hypertensive rats (SHRs) 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 Na⁺ handling in salt-sensitive hypertension. Under normal physiological conditions, 99.5% of Na⁺ excreted in the glomerulus is reabsorbed through nephrons. About 70% is reabsorbed by proximal tubules, largely by Na⁺/H⁺ exchange; 20–30% by the thick ascending limb of Henle, by the Na⁺-K⁺-2Cl⁻ co-transporter (NKCC); and 5–7% by DCTs, by the Na⁺-Cl⁻ co-transporter (NCC). The last fraction is reabsorbed via epithelial Na⁺ channels (ENaC) in the CNT/CD.

Na⁺/H⁺ exchanger (NHE)3 in proximal tubules (62, 68) and NKCC2 in the thick ascending limb (3, 10, 66) are associated with salt-sensitive hypertension in animal models. Salt-induced NHE3 suppression is more impaired in Dahl salt-sensitive rats than in Dahl salt-resistant rats (68). NKCC2 activity in thick ascending limb is also upregulated in salt-sensitive rats (3, 10, 66). Single-nucleotide polymorphisms involving NKCC2 activation in thick ascending limb are associated with salt-sensitive hypertension in humans (123).

The Cl⁻ transport system associated with Na⁺ handling has also attracted attention. Pendrin, a Na⁺/H⁺-dependent Cl⁻/HCO₃⁻ exchanger, is expressed on the apical membrane of intercalated cells in the DCT/CNT/CD and is a target of the aldosterone/mineralocorticoid receptor (MR) pathway (111, 128). Pendrin, together with the Na⁺/H⁺-dependent Cl⁻/HCO₃⁻ exchanger (SLC4A8) and ENaC, absorbs Na⁺ and Cl⁻ electro-neutrally in a thiazide-sensitive but NCC-independent manner (65). β-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 (BP) 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 With No Lysine Kinases

NCC is distributed in the DCT region and is a primary target of thiazide diuretics. Mutations in with-no-lysine (WNK)1 and WNK4 were identified as possibly responsible for PHAII, also known as familial hyperkalemic hypertension or Gordon syndrome (134). Since these 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 BP and metabolic disorder, thus implicating these mutations in NCC regulation (40).

The 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 Xenopus laevis oocytes did not directly affect NCC activity; however, when coexpressed with WNK4, WNK1 increased NCC activity by suppressing WNK4 (139, 140). Subsequent studies have suggested more complicated modes of regulation. 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 the DCT/CNT. 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 downregulation of ENaC activity, whereas KS-WNK1 transgenic mice exhibit reduced NCC activity and BP (43, 69). These lines of evidence suggest 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 using 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 have shown that L-WNK1 can directly regulate NCC by phosphorylating STE20/SPS1-related proline/alanine-rich kinase (SPAK), which phosphorylates NCC and stimulates its activity (80, 99). Consistent with this result, WNK phosphorylation-resistant SPAK knockin mice exhibited salt wasting and low BP due to a 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 WNK4 gene responsible for PHAII were found to be missense mutations in an acidic region highly conserved among WNKs. Wild-type WNK4 suppressed NCC activity in X. laevis oocytes, Cos-7 and human embryonic kidney (HEK)-293 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 the wild-type 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 Sprague-Dawley 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-K⁺ diet, which promotes salt excretion and confers resistance to high salt-induced hypertension, increased WNK4 while reducing NCC levels in the 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 adaptations in response to changes in dietary salt intake. However, a recent study (34) has revealed that the regulation of NCC in several pathological conditions is more complex. Mutant WNK4 transgenic mice showed NCC activation and BP 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 (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 knockin 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 switch of WNK4 modes. WNK4 and WNK1 phosphorylate SPAK/OSR1 in the presence of ANG II, leading to NCC phosphorylation (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 showing that insulin induced changes in the expression of WNK4 itself (113). In 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 Na+ handling.

We identified a novel pathway in the DCT in which renal sympathoexcitation suppresses WNK4 and NCC activity, leading to increased Na+ reabsorption at distal nephrons and the development of salt-sensitive hypertension (81).

Renal Sympathoexcitation 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 below. We found that the renal norepinephrine (NE) turnover rate was elevated in salt-sensitive hypertensive rats 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 have shown 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 Na+ 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

Sympathoexcitation in the proximal tubule increases Na+ reabsorption, both by renin production, resulting from β1-adrenergic stimulation, and direct tubular action, resulting from α1-adrenergic 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 the kidneys (81). These effects were reversed by treatment with a blocker of nonselective β-adrenergic receptors, but not by an α1-adrenergic receptor blocker. NCC activation by renal sympathetic excitation in DOCA-salt rat was inhibited with a β2-adrenergic antagonist but not with a β1-adrenergic antagonist. Administration of isoproterenol to both β1-adrenergic receptor knockout and wild-type mice on a high-salt diet increased BP, but this effect was not observed in β2-adrenergic receptor knockout mice. In vitro, the glucocorticoid receptor (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 GRs on WNK4 expression has been recently confirmed (119). Consistent with these findings, distal nephron-specific GR knockout mice infused with isoproterenol showed no increase in BP after salt loading. This β-adrenergic effect is 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 GRs, increasing GR-nGRE binding and reducing WNK4 transcription (Fig. 1). Reductions in WNK4 levels result in NCC activation, leading to Na+ retention and salt-sensitive hypertension. Renal denervation reversed the reduction in renal WNK4 expression in DOCA-salt rats, which was associated with normalization of BP. We therefore hypothesized that the improvement in renal Na+ excretion induced by renal denervation is the primary mechanism underlying the antihypertensive effects of renal nerve ablation, as documented in recent clinical studies. As described above, WNK4 can enter the stimulatory state in response to ANG II, aldosterone, and potentially other factors. We found that neither an ANG II type 1 receptor blocker nor an MR antagonist could reverse isoproterenol-induced salt-sensitive hypertension and downregula-
Mechanism of Renal Sympathetic Nerve Excitation

Central sympathetic nerve excitation drives renal sympathetic nerve activity. Several factors, including obesity, the RAS, and salt loading, activate the SNS, as described below. In rodent models, sympathoexcitation by oxidative stress in the brain mediates BP elevations, suggesting that brain ROS are involved in both salt- and obesity-induced hypertension (30, 82). Compared with Dahl salt-resistant rats, Dahl salt-sensitive rats on a high-salt diet displayed a greater depression of renal sympathetic activity and reductions in BP 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 BP, with antioxidants in the brain successfully reversing the elevations in BP, renal sympathetic nerve activity, and renal damage (29). Thus, salt loading itself activates central and renal sympathetic nerve activation in pathological conditions.

Aldosterone-MR-ENaC

Primary aldosteronism is a representative type of salt-sensitive hypertension, resulting from increased Na⁺ reabsorption in aldosterone-sensitive distal nephrons. Aldosterone activates MRs and ENaC, increasing Na⁺ reabsorption and salt-sensitive hypertension. The putative ligands of MRs are cortisol and aldosterone. Both ligands have equal affinity for MRs, but the plasma concentration of cortisol is hundreds to thousands of times higher, making cortisol the major ligand of MRs 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 MRs. Expression of MRs is distributed throughout the nephron, but 11βHSD2 is highly expressed only in DCT2, CNTs, and CDs. This specific distribution of 11βHSD2 is coincidental with the expression of its effector, ENaC, within distal nephrons, especially in the 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 upregulates membrane expression of preexisting ENaC by activating the transport machinery, for example, through suppression of the ubiquitin ligase Nedd4-2 via phosphorylation by serum- and glucocorticoid-induced kinase (Sgk)1 (19).

In the delayed phase, the aldosterone-activated MR binds to the promoter region of the gene encoding ENaC-α, thereby increasing de novo synthesis of ENaC. Sgk1 also plays a role during this late phase, relieving suppression of ENaC transcription by inhibiting H3K79 methyltransferase, which blocks transcription (144).

Aldosterone/MR signaling also plays an important role in 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 (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 (96) in humans found that the rhythm of urinary Na⁺ 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. In only three genes responsible for Mendelian diseases, mutations can induce both hypertension and hypotension: ALDOS, which encodes aldosterone synthase; MR, which encodes the MR; and SCNN1J, which encodes ENaC (67). These lines of evidence showed that the aldosterone/MR pathway plays a very important role in Na⁺ storage and that dysregulation of this pathway may cause impaired Na⁺ handling and BP control.

**Novel Pathway of MR Activation**

Clinical trials have shown 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 BP 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, coactivators and corepressors, other transcription factors, and cross-talk, among intracellular signaling pathways. Among the factors found to modulate the activity of nuclear receptors are steroid receptor coactivator 1 (90), Ras/MAPK (54), Smad3 (77, 138), PKA (14, 75), ubiquitin conjugating enzyme 9 (141), SUMO (108), and RhoGTPase (116). Rac-related C3 botulinum toxin substrate (Rac)1, a small RhoGTPase, may also play a role in regulating nuclear translocation of transcription factors (57, 137). We have recently reported that Rac1 activates MRs in both ligand-dependent and ligand-independent manners (110).

Transfection of the constitutively active form of Rac1 into HEK-293 cells induces nuclear translocation and transcriptional activation of MRs, even in the absence of ligand (110). Aldosterone increased this Rac1-induced MR activation. Furthermore, Rho guanine nucleotide dissociation inhibitor α (RhoGDIIα) 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 RhoGDIIα-deficient zebrafish was also improved by treatment with either eplerenone or Rac inhibitors (35). Recently, mutations in RhoGDIIα 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 Sprague-Dawley rats, salt loading decreases Rac1 activity in the kidneys and is associated with downregulation of the MR target gene Sgk1, in accordance with reduced levels of plasma aldosterone. Salt loading of Dahl salt-resistant rats also reduced active Rac1, whereas salt loading of Dahl salt-sensitive rats markedly upregulated Rac1. Consistent with these changes in Rac1 activity, nuclear MRs were downregulated by high salt loading in Dahl salt-resistant rats but up-regulated in Dahl salt-sensitive rats, despite the equal suppression of plasma aldosterone concentrations in both sets of animals. Treatment with the Rac inhibitor NSC-23766 not only decreased nuclear MRs but suppressed the increase in BP induced by salt loading as effectively as treatment with the MR blocker eplerenone. This paradoxical response of MR activation to salt loading in Dahl salt-sensitive rats is attributable solely to the abnormal activation of Rac1 in response to salt loading, which leads to increased Na⁺ reabsorption in distal nephrons and salt-sensitive hypertension. Thus, Rac1 is a determinant of both MR activation and salt sensitivity (Fig. 2).

**Mechanism of Salt-Induced Rac1 Activation**

Several factors, including genetic polymorphisms in Rac1 modulators, may be responsible for the 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 TNF-α, and oxidative stress, all of which activate Rac1 (38, 47, 107, 136). Local activation of the RAS may also result in the activation of Rac1. Salt loading increases local RAS activity in the kidneys of Dahl salt-sensitive rats, despite suppressed activation of the circulating RAS (59). 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 after salt loading, concomitant with upregulation 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 salt-sensitive rats, whereas supplementation with aldosterone reactivated Rac1, suggesting that salt and aldosterone independently 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 salt-sensitive 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 (48, 49) have shown that MR activation in the brain plays...
a key role in salt-induced sympathoexcitation, 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, 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 (15, 100) have shown that obesity is associated with salt-sensitive hypertension in animals and humans. 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 NE, along with impaired renal excretion of Na⁺ into the urine, have been observed in obese SHRs (118). Moreover, renal denervation attenuated the increased Na⁺ retention and decreased BP 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 BP (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 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. In 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” include TNF-α (78), C1q and TNF-related protein 1 (51), linoleic acid oxidation products (39), leptin (73), IL-6 (76), and an unknown molecule whose size is >50 kDa (24). Notably, these aldosterone-releasing factors 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 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 BP 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 Na\(^+\) handling and BP. A clinical study (45) has shown that patients with salt-sensitive hypertension exhibited impaired circadian rhythms and failed to undergo a nocturnal decline in BP. This phenomenon is related to hypertension in obese subjects. Indeed, a recent clinical study (132) showed that the lack of reduction in nocturnal BP was twice as frequent among severely obese children than among the general child population. Circadian rhythms are generated in individual cells by the oscillation of clock genes interlocked in an autoregulatory 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 period circadian clock (*Per*) genes *Per1–Per3*, 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).

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

In contrast to Per1 heterozygous knockout mice, Cry1/2 knockout 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 BP when fed normal chow but had elevated BP when placed on a high-salt diet. Thus, clock genes may be involved in abnormalities of both the aldosterone-MR and WNK4-NCC pathways in patients with salt-sensitive hypertension.

**Roles of MRs and GRs in the Two Pathways of Renal Na\(^+\) Handling**

We identified two novel pathways in salt-sensitive hypertension: the β\(_2\)-adrenergic stimulant-GR-WNK4-NCC pathway in DCT1 and the Rac1-MR pathway in DCT2, CNTs, and CDs (Figs. 1 and 2). Notably, MRs and GRs, the two important nuclear receptors that control Na\(^+\) status and BP, are independently involved in renal Na\(^+\) transport pathways in different tubular segments, although both receptors are distributed throughout the nephrons. The enzyme 11βHSD2, 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 MRs in this organ. However, 11βHSD2 is absent from DCT1 (13). Consequently, cortisol, which is abundant in DCT1, rather than aldosterone, may be the ligand for MRs and may also serve as the ligand for GRs after β-adrenergic stimulation-induced downregulation of WNK4. This hypothesis is consistent with the results of our study, which showed that glucocorticoid-activated GRs activated in DCT1 are indispensable for β-adrenergic stimulation-induced salt-sensitive hypertension. In 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 MRs at DCT2, CNTs, and CDs, which, in turn, activates ENaC through Sgk1. Notably, our results showed that either renal denervation or treatment with an MR antagonist can effectively decrease BP in Dahl salt-sensitive rats with renal sympathetic hyperactivity and activated MRs. However, both treatments were required to decrease BP to normal levels, suggesting that the pathophysiology of salt-sensitive hypertension is heterogeneous (81, 109). Therefore, the 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.

**Conclusions**

We recently identified two novel pathways in salt-sensitive hypertension: the β\(_2\)-adrenergic stimulant-GR-WNK4-NCC pathway in DCT1 and the Rac1-MR pathway in DCT2, CNTs, and CDs. MRs and GRs, nuclear receptors that play key roles in controlling electrolyte balance and maintaining normal BP, are independently involved in these two novel pathways in distinct segments of the distal nephron. Our results offer new insights into the regulation of renal Na\(^+\) 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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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: M.N. prepared figures; M.N. drafted manuscript; M.N. and T.F. edited and revised manuscript; M.N. and T.F. approved final version of manuscript; T.F. conception and design of research.

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