Mechanism of Salt-Sensitive Hypertension: Focus on Adrenal and Sympathetic Nervous Systems

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ABSTRACT

A central role for the kidney among the systems contributing to BP regulation and the development of hypertension has been proposed. Both the aldosterone/mineralocorticoid receptor pathway and the renal sympathetic nervous system have important roles in the regulation of renal excretory function and BP control, but the mechanisms underlying these processes have remained unclear. However, recent studies revealed the activation of two pathways in salt-sensitive hypertension. Notably, Rac1, a member of the Rho-family of small GTP binding proteins, was identified as a novel ligand-independent modulator of mineralocorticoid receptor activity. Furthermore, these studies point to crucial roles for the Rac1–mineralocorticoid receptor–NCC/ENaC and the renal β-adrenergic stimulant–glucocorticoid receptor–WNK4-NCC pathways in certain rodent models of salt-sensitive hypertension. The nuclear mineralocorticoid and glucocorticoid receptors may contribute to impaired renal excretory function and the resulting salt-sensitive hypertension by increasing sodium reabsorption at different tubular segments. This review provides an in-depth discussion of the evidence supporting these conclusions and considers the significance with regard to treating salt-sensitive hypertension and salt-induced cardiorenal injury.


The most obvious connection between sodium intake and health is manifested by the relationship between sodium intake and BP. Different individuals have different susceptibilities to the BP-raising effects of salt. The BP sensitivity to salt is defined as the interindividual difference in the BP response to changes in dietary sodium chloride intake. A study by Guyton revealed an interaction between genetically determined alterations in the kidney and excess dietary sodium intake. Renal excretory function is impaired in patients with salt-sensitive hypertension and results in an elevated BP. There are reports of several allelic variants of candidate genes for hypertension; however, the susceptibility genes that cause essential hypertension remain unidentified. A study by Ji et al. provided some important evidence for the nature of inherited functional defects in renal sodium handling that cause a salt-induced increase in BP, most often associated with major alterations in the rate of renal tubular sodium chloride reabsorption. There are several factors that modulate renal function for urinary sodium excretion: the sympathetic nervous system (SNS), the renin-angiotensin system (RAS), and aldosterone and insulin. Activation of RAS increases tubular sodium reabsorption and leads to BP elevation. Apart from the classic actions of the circulating RAS, an independently functioning RAS within the kidney is thought to play a key role in regulating renal sodium excretory functions and BP. In an elegant study using a kidney cross-transplantation technique, Crowley et al. found that AT1 receptors in the kidney are primarily responsible for mediating angiotensin II (AII)–dependent hypertension. Moreover, AII–dependent hypertension is attenuated in mice lacking AT1 receptors in the proximal tubule, a finding associated with the inhibition of volume retention. Thus, the salt-induced increase in local RAS formation and the subsequent activation of AT1 receptors in the proximal tubule may contribute to salt-sensitive hypertension through increased tubular sodium absorption. Some investigators reported that fractional proximal sodium clearance was increased in most salt-sensitive hypertensive patients. Other investigators suggested that abnormalities in sodium handling at other segments of the renal tubules contribute to increased tubular sodium reabsorption as well as the consequent salt-sensitive hypertension. The adrenal hormones, aldosterone and cortisol, act by stimulating their respective receptors, the mineralocorticoid receptor (MR) and the glucocorticoid...
hypertension. In this review, I discuss the involvement of the adrenal and renal sympathetic nervous mechanisms in salt-sensitive hypertension and the roles of the MR and GR in the abnormal regulation of renal sodium handling in rodent models of salt-sensitive hypertension.

RAC1-INDUCED MR ACTIVATION

Plasma aldosterone concentration is normally counterbalanced by dietary salt intake through changes in the levels of circulating RAS, resulting in maintenance of normal sodium homeostasis and normal BP. In primary aldosteronism, increases in tubular sodium reabsorption through MR activation in the aldosterone-sensitive distal nephron lead to the development of salt-sensitive hypertension. Salt loading, with a continuous infusion of aldosterone, increases both BP and proteinuria in rats at plasma levels similar to those levels seen in primary aldosteronism. On a low-salt diet, however, aldosterone-induced hypertension and renal injury are abolished, suggesting that salt is indispensable for aldosterone-induced MR activation and subsequent hypertension. Salt loading not only increases BP but also, aggravates cardiorenal injury in obese hypertensive rats that have increased aldosterone levels because of aldosterone-releasing factors secreted abundantly from adipose tissues. This finding is associated with the inadequate suppression of serum aldosterone levels; therefore, the use of an MR antagonist could inhibit the injurious effects of aldosterone and/or salt. Thus, salt-induced MR activation in obese hypertensive rats results from the inappropriate secretion of aldosterone, whereas serum aldosterone in lean hypertensive rats and normal rats is adequately suppressed by high salt through the inhibition of the circulating RAS. Therefore, the lack of negative feedback regulation of aldosterone secretion by salt in obese hypertensive rats may cause salt-sensitive hypertension and cardiorenal damage through MR activation. Consistent with this finding, the MR antagonist spironolactone effectively reduces BP in dogs with chronic dietary-induced obesity. In a study of resistant hypertension management, patients with higher waist circumference showed better BP response to spironolactone; however, there was no correlation between plasma aldosterone levels and spironolactone response. In Dahl salt-sensitive (S) hypertensive rats, salt loading upregulates the renal expression of serum- and glucocorticoid-inducible kinase 1 (Sgk1), a downstream mediator of MRs, despite the appropriate suppression of serum aldosterone, suggesting that MRs are activated in an aldosterone-independent manner. However, the mechanism underlying the paradoxical response of MRs to salt loading in Dahl S rats has long been elusive.

Two factors activate MR in a ligand-independent manner: cAMP-dependent protein kinase A and reactive oxygen species. We identified a new role for Rac1, a member of the Rho–guanine triphosphate hydroxylases family, in aldosterone-independent MR activation.

In Dahl S rats, salt loading activates renal Rac1, which, in turn, leads to MR activation, sodium retention, and BP elevation, despite reduced levels of plasma aldosterone. In Dahl resistant rats and normotensive rats, Rac1 activity is normally reduced by salt loading and associated with a decrease in MR activity, a normal sodium state, and normal BP. This paradoxical response of MRs to salt loading in salt-sensitive hypertension is attributable to the abnormal response of Rac1 to salt. Treatment with the Rac1 inhibitor reduces BP and ameliorates renal injury by reversing the increase in renal Rac1 and MR activity. Thus, Rac1 is an upstream regulator of MRs and serves as a determinant of BP salt sensitivity. Serum aldosterone is suppressed by salt loading; however, adrenalectomy nullifies salt-evoked Rac1 activation, which is reversed by aldosterone supplementation. A certain level of aldosterone is then required for salt-induced Rac1 activation and the subsequent development of hypertension. On a low-salt diet, aldosterone-induced activation of Rac1 and MRs and the
resultant BP elevation are abolished in rats continuously infused with aldosterone, suggesting the necessity of salt for aldosterone-induced Rac1 activation. Thus, salt and aldosterone interdependently activate Rac1 in Dahl S rats. The mechanism underlying the dysregulation of Rac1 in Dahl S rats remains unclear.

Regarding aldosterone-independent MR activation, AII activated MRs in vitro in vascular smooth muscle cells in the absence of aldosterone. Luther et al. recently reported that aldosterone-independent MR activation contributes to AII/salt-induced hypertension and cardiac injury in aldosterone synthase knockout (KO) mice. More recently, Kawarazaki et al. found that salt-loaded AII-overproducing transgenic mice developed severe hypertension and prominent renal injury. This finding was associated with increased Rac1 activity and MR activation in the kidney. However, treatment with either an MR antagonist or an Rac inhibitor ameliorated salt-induced renal injury and reduced BP, suggesting that AII/salt-induced hypertension and renal injury are mediated by Rac1-MR activation. Given the observation that local RAS in the kidney was augmented by salt loading in Dahl S rats, despite reduction in circulating RAS, but was unchanged in Dahl resistant rats, an inappropriate increase in renal AI in a high-salt diet may contribute to the development of salt-sensitive hypertension in Dahl S rats. Taken together, renal-specific Rac1 determines the salt sensitivity of BP. However, the effects of extrarenal Rac1 could be AII-mediated. Inflammation and T-cell accumulation in the kidneys, arteries, and central nervous system are common characteristics of experimental models of salt-sensitive hypertension. T-cell signaling is induced by chemokines and other stimulants and mediated by the activation of Rho–guanine triphosphate hydroxylases, such as Rac1 and TGF-β. Tregulatory lymphocytes prevent AII- and aldosterone-induced hypertension and vascular injury, and crosstalk occurs between aldosterone and angiotensin signaling. MR activation in macrophages contributes to BP elevation and vascular damages in AII/No–nitro-L-arginine methyl ester hypertension and deoxycorticosterone acetate (DOCA) salt hypertension. Additional studies are required to investigate whether the Rac1-MR pathway in T cells and macrophages is involved in the development of salt-sensitive hypertension.

**THE RENAL SNS AND THE WNK4-NCC PATHWAY**

Another important factor influencing the salt sensitivity of BP is the renal SNS. Salt loading increases renal SNS activity in salt-sensitive hypertensive rats, and renal sympathetic overactivity may contribute to salt-induced BP elevation through impaired excretory function. Hypertensive individuals with salt-sensitive hypertension have higher plasma norepinephrine levels on a high-salt diet than salt-resistant individuals, which suggests the persistence of an autonomic drive in salt-sensitive individuals to salt loads. Obese hypertensive patients and animals are often associated with both salt-sensitive hypertension and increased SNS activity, specifically in the kidney. Renal denervation decreases BP in not only patients with resistant hypertension but also, obese patients with resistant hypertension. The antinatriuretic effect of increased renal SNS activity is mediated by three major mechanisms: increased renin secretion, reduced renal blood flow, and increased renal tubular reabsorption. In a previous study, the antinatriuretic response to air stress through augmented central renal SNS activity in DOCA-salt rats was abolished by renal denervation without any changes in renal hemodynamics, suggesting that there may be a direct tubular effect of renal SNS. However, it remains unclear how increased SNS activity in the kidney enhances tubular sodium reabsorption and leads to the development of salt-sensitive hypertension.

With no-lysine kinase 4 (WNK), a serine-threonine kinase, is a negative regulator of the thiazide-sensitive sodium chloride cotransporter (NCC). Under normal conditions, WNK4 inhibits NCC activity and leads to a decrease in sodium reabsorption in the distal convoluted tubule (DCT) segments to maintain normal BP. Several investigators report that the expression of WNK kinases is modulated by changes in dietary sodium and therefore, influences NCC activity. The low-salt diet in Sprague Dawley rats decreases the renal expression of WNK4 and increases NCC activity in the kidney. Changes in dietary salt intake influence neurohumoral factors, such as the circulating RAS and SNS, and thus, modulate the WNK4-NCC pathway. WNK involved in NCC activation on a low-salt diet in an STE20/SPS-1-related proline/alanine-rich kinase (SPAK)–dependent manner. Aldosterone also participates in the dietary salt-induced modulation of NCC protein levels through the WNK4-extracellular signal-regulated kinase 1/2 signaling pathway. We revealed the involvement of the renal SNS in salt-induced changes in WNK4 expression and NCC activity in salt-sensitive hypertensive rats. Salt loading in DOCA-treated rats resulted in increased SNS activity and decreased WNK4 expression in the kidneys. These parameters were reversed by renal denervation, which was associated with the suppression of increased NCC activity and the resultant normalization of DOCA-salt hypertension. The continuous infusion of norepinephrine in mice downregulates WNK4 expression and upregulates NCC, leading to salt-induced BP elevation. Treatment with the β-blocker propranolol reversed these norepinephrine-induced changes. Thus, the β-adrenergic receptor (β-AR) plays a key role in the norepinephrine-induced activation of the WNK4-NCC pathway. Moreover, the β2-AR is involved in activating the WNK4-NCC pathway, because salt-induced BP elevation occurred in wild-type and β1-KO mice infused with isoproterenol but not β2-KO mice. The augmented natriuretic response to hydrochlorothiazide, an NCC blocker, in DOCA-salt rats was normalized by pretreatment with the β2-blocker ICI 11851 but not affected by the β1-blocker metoprolol, suggesting that NCC activation is induced through stimulation of the
β2-AR and plays a key role in the salt-induced elevation of BP by increased sodium reabsorption in the DCT segments. In support of this hypothesis, a micro-puncture study showed an increase in fractional sodium reabsorption at the DCT segment during isoproterenol treatment. In the nephron, the presence of β2-AR in the DCT cells suggested to us that sodium retention during increased SNS activity is mediated by β2-adrenergic signals acting on the DCT cells. Individuals with β2-AR polymorphisms show low-renin, salt-sensitive hypertension and impaired renal function in terms of urinary sodium excretion.

All could be a hormonal signal involved in switching WNK4 to the functional state, thereby promoting NCC activation. All relieves the inhibitory effect of WNK4 on NCC in an SPAK-dependent manner, resulting in NCC activation. Aldosterone also activates NCC through either the WNK4-SPAK-dependent or the WNK4-extracellular signal-regulated kinase 1/2 signaling pathway. Therefore, we can speculate on the involvement of all or aldosterone in NCC activation by β-AR stimulation; however, neither an MR antagonist nor an angiotensin receptor blocker affected salt-induced elevation of BP in isoproterenol-infused mice or the isoproterenol-induced downregulation of WNK4 levels. It remains unknown whether β-AR stimulation activates SPAK/oxidative stress responsive kinase-1 independently of all and aldosterone.

The GR, but not the MR, plays a key role in β-AR stimulation-induced WNK4 downregulation and salt-sensitive hypertension (Figure 2). Treatment with isoproterenol did not affect WNK4 expression in mouse DCT cells cultured with charcoal-stripped medium (to remove corticosteroids), but pretreatment with dexamethasone (a synthetic glucocorticoid) recovered the inhibitory effects of isoproterenol on WNK4. Thus, glucocorticoids and GRs are required for β-AR stimulation-induced WNK4 downregulation. Epigenetic modulation is involved in the activation of the β-AR-GR-WNK4 pathway. In mouse DCT cells, isoproterenol decreases the activity of the histone deacetylase 8. The protein kinase A-dependent inactivation of histone deacetylase 8 by isoproterenol increases the binding of the acetylated histones 3 and 4 to the promoter region of the WNK4 gene, which results in a decrease in transcriptional activity through the recruitment of GRs to the promoter region, and it includes the negative glucocorticoid response element. Given the finding that both renal WNK4 downregulation and salt-sensitive hypertension during the isoproterenol infusion were absent in distal nephron-specific GR-KO mice, GRs are indispensable for the β-AR stimulation-induced activation of the WNK4-NCC pathway in DCT cells and the resulting salt-sensitive hypertension (Figure 2).

THE ROLES OF MR AND GR IN NCC ACTIVATION

NCC plays a critical role in the control of renal sodium chloride transport and BP maintenance. We showed that NCC activation is involved in salt-sensitive hypertension in rodent models through two novel pathways: the Rac1-MR-Sgk1-NCC and β-AR-GR-WNK4-NCC pathways. An aberrant Rac1-MR pathway increases sodium reabsorption by activating NCC in the DCT2 segment in addition to activating epithelial sodium channels (ENaCs) in the DCT2 and connecting tubule and cortical collecting duct segments, whereas an aberrant β-AR-GR-WNK4 pathway activates NCCs in the DCT1 segment. Mineralocorticoid specificity is achieved by the 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which converts cortisol to cortisone, an inactive metabolite. The aldosterone-sensitive distal nephron is characterized by high expression levels of 11β-HSD2; however, 11β-HSD2 is absent from the DCT1 segment (Figure 3). As a result, aldosterone serves as a ligand for MR in the DCT2, connecting tubule, and cortical collecting duct segments, whereas cortisol, rather than aldosterone, serves as a ligand for MR in the DCT1 segment. With the presence of abundant cortisol in the DCT1 cells, the activated GR leads to β-AR competence to allow activation of the WNK4-NCC pathway in response to stress. However, MR activation in the DCT2 segment also causes NCC activation by Sgk1; Sgk1 activation
induces phosphorylation of WNK4 and releases the inhibitory effect of WNK4 on NCC, thereby leading to NCC activation. Given the evidence that salt-sensitive obese humans show both an activated Rac1-MR pathway and increased renal SNS activity, NCC in the DCT1 and DCT2 segments must be strongly activated by the aberrant β-AR-GR-WNK4 and Rac1-MR-Sgk1 pathways, respectively. Therefore, both the MR antagonist and renal denervation are required for the treatment of salt-sensitive hypertension in obese hypertensive patients, which is observed in salt-loaded Dahl S rats. Nevertheless, salt restriction is most efficacious for the treatment of obesity-associated hypertension.

Accumulating evidence suggests that NCC activation plays a key role in obesity-associated hypertension, cyclosporine-induced hypertension, and AII-dependent hypertension. However, NCC overexpression alone is insufficient to induce salt-sensitive hypertension and hyperkalemia, the phenotype of pseudohypoaldosteronism type II (PHAII). Kidney-specific WNK1, a dominant negative regulator of WNK1, suppresses the effects of WNK1 to stimulate NCC; however, kidney-specific WNK1-KO mice do not display PHAII-like phenotype, despite a significant increase in renal NCC abundance, which was shown in NCC transgenic mice. The study found reduced ENaC expression. The resulting reduction in ENaC activity compensates for the increased NCC expression. Therefore, NCC activation, in addition to the dysregulation of other transporters/channels, plays a significant role in the etiology of PHAII and obesity-associated salt-sensitive hypertension. In vitro, the WNK kinases regulate the activity of a broad range of sodium and potassium transport mechanisms in both the kidney and the epithelia outside the kidney. A recent report showed that vascular WNK4 suppresses transient receptor potential cation channel 3, one of the receptor-operated calcium channels that modulates vascular tone, but it remains unclear whether vascular WNK4 is also controlled by the β-AR-GR pathway. To clarify the

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**Figure 3.** Localization of MR, GR, and 11β-HSD2 in the different renal tubular segments. Arrowhead lines and T-shaped lines indicate activation and inhibition, respectively. CCD, cortical collecting duct; CNT, connecting tubule; p-WNK4, phosphorylation of WNK4.

**Figure 4.** The adrenal glands and central renal SNSs are involved in the development of salt-sensitive hypertension. MR and GR, stimulated by Rac1 and renal SNS overactivity, are involved in the activation of NCC/ENaC at DCT2 and ENaC at the CNT and cortical collecting duct (CCD) through Sgk1 and NCC activation at DCT1 through WNK4 downregulation, respectively. 11β-HSD2 is absent from the DCT1 segment (gray area). Arrowhead lines and T-shaped lines indicate activation and inhibition, respectively. G, glomerulus.
pathogenesis of salt-sensitive hypertension in obesity, additional research is required into the effects of increased renal SNS activity on the WNK kinases, NCC, and/or the activity of other transporter/channels in the kidneys and vasculature.

Salt-sensitive hypertension can be produced in animals by genetically engineered key neurohormonal regulators. We found that two novel pathways involving the adrenal and sympathetic nervous systems (Rac1-MR-Sgk1-NCC/ENaC and the renal SNS-GR-WNK4-NCC pathways) play crucial roles in certain rodent models of salt-sensitive hypertension. These pathways stimulate the nuclear receptors, MR and GR, to certain rodent models of salt-sensitive hypertension. Two novel pathways producing in animals by genetically engineered key neurohormonal regulators. We found that two novel pathways involving the adrenal and sympathetic nervous systems (Rac1-MR-Sgk1-NCC/ENaC and the renal SNS-GR-WNK4-NCC pathways) play crucial roles in certain rodent models of salt-sensitive hypertension. The two pathways provide alternative therapeutic targets for salt-sensitive hypertension and salt-mediated cardiovascular injury. However, additional studies are required to assess the therapeutic value of manipulating these particular pathways.

ACKNOWLEDGMENTS

T.F. is supported by research grants from the Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research (S).

DISCLOSURES

None.

REFERENCES


BRIEF REVIEW


