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Activation of Aldosterone/Mineralocorticoid Receptor in Chronic Kidney Disease and Metabolic Syndrome

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Beyond regulation of electrolyte, volume, and blood pressure (BP) homeostasis, aldosterone has recently emerged as a deleterious hormone in the cardiovascular (CV) and renal systems. This issue of *Nephrology Rounds* examines research findings from our laboratory indicating that the glomerular podocyte is a novel target for aldosterone in the kidney. In fact, activation of the mineralocorticoid receptor (MR) in the podocyte causes a disruption of the glomerular filtration barrier, proteinuria, and glomerulosclerosis. Spontaneously hypertensive rat (SHR)/NDmcr-cp, a metabolic syndrome (MetS) rat, is susceptible to renal and cardiac injuries, especially when the animal is fed a high-salt diet. Inappropriate activation of the aldosterone/MR system underlies the target organ damage. Furthermore, the small guanosine 5'-triphosphate (GTP)ase, Rac1, is identified as a novel activator of MR. Research suggests that this ligand-independent MR activation contributes to the progression of chronic kidney disease (CKD). This issue summarizes recent advances in aldosterone research and introduces results on the role of aldosterone-dependent and aldosterone-independent MR activation in CKD and the MetS.

Introduction

Since its isolation in 1953, aldosterone (formerly called "electrocortin") has been recognized as a hormone that regulates electrolyte, volume, and BP homeostasis. Aldosterone acts on its receptor, MR, in the distal nephron, thereby controlling sodium reabsorption and potassium excretion. This classic mode of action has been substantiated by many studies of genetic disorders as well as in knockout mice.

Recently, aldosterone emerged as a deleterious hormone in the CV and renal systems.¹ Historically, in the 1940s, before the isolation of aldosterone and MR, it was already known that MR activation causes target-organ damage. Hans Selye,² an advocate of "stress theory" (nonspecific adaptation response to stressors by glucocorticoids), reported that administering desoxycorticosterone acetate (DOCA), the first synthetic steroid, to rats induces inflammatory and fibrotic changes in the heart and kidney. Uninephrectomy and salt loading were performed as conditioning factors. Selye considered that these phenotypes are due to mineralocorticoid actions of DOCA. Furthermore, he postulated that glucocorticoids have anti-inflammatory actions in adaptation to stress, whereas mineralocorticoids have pro-inflammatory effects.

Half a century later, aldosterone, the physiological mineralocorticoid in the body, was shown to cause myocardial fibrosis.³ Based on these experimental findings, together with aldosterone breakthrough phenomenon (discussed below), Pitt et al^{4,5} conducted 2 milestone randomized clinical trials (RCTs): the Randomized ALdosterone Evaluation Study (RALES) and the Eplerenone Post-acute myocardial infarction Heart failure Efficacy and SURvival Study (EPHESUS). These investigations found that the addition of a low-dose MR antagonist, spironolactone or eplerenone, dramatically improved the outcomes of heart failure patients already prescribed standard medications, including angiotensin converting enzyme (ACE) inhibitors and angiotensin II (Ang II) type 1 receptor blockers (ARBs). The organ-protective efficacy of MR antagonists was further confirmed by subsequent clinical studies.^{6,7}

Aldosterone and proteinuria

Cumulative evidence suggests that aldosterone is a potent inducer of proteinuria. Large-scale RCTs demonstrated the efficacy of blocking the renin-angiotensin-aldosterone system (RAAS) with ACE inhibitors or ARBs to reduce proteinuria and retard the progression of CKD. Conventionally, Ang II was regarded as the primary factor responsible for injurious actions of the RAAS. Recent evidence, however, indicates aldosterone is an additional pathogenic factor. According to a study by Greene et al,⁸ Ang II blockade by losartan and enalapril attenuated proteinuria in remnant rat kidney, a model with enhanced RAAS activity. However, these drugs not only inhibited the action of Ang II, but also blocked aldosterone synthesis, and a reversal of plasma aldosterone levels by exogenous aldosterone infusion recapitulated the nephropathy. Conversely, adrenalectomized rats



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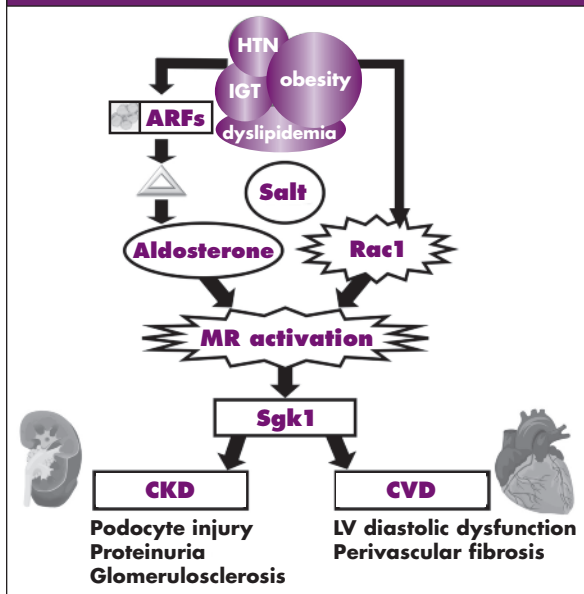
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Figure 1: A schematic representation of the proposed pathophysiological mechanisms of target-organ disease in the metabolic syndrome



Mineralocorticoid (MR) activation plays a pivotal role in the progression of chronic kidney disease (CKD) and cardiovascular disease (CVD). MR can be activated by both ligand-dependent pathway (via aldosterone-releasing factors [ARFs]), and ligand-independent pathway (via the guanosine triphosphate Rac1).

HTN = hypertension; IGT = impaired glucose tolerance; LV = left-ventricular

with glucocorticoid replacement were protected from renal ablation-induced proteinuria. Indeed, Ang II excess models, such as Ang II/salt rats and double transgenic rats carrying both human renin and angiotensinogen genes, develop severe albuminuria and kidney injury that can be abrogated by aldosterone synthase inhibitor FAD286, adrenalectomy, or MR antagonists.^{9,10} The pivotal role for aldosterone was directly confirmed by creating uninephrectomized aldosterone/salt rats that were prone to proteinuria and glomerulosclerosis, despite suppressed plasma renin activity and Ang II concentrations.¹¹ Serum- and glucocorticoid-inducible kinase 1 (Sgk1), an aldosterone-effector kinase, is suggested to mediate the proteinuric effect of aldosterone because mice lacking Sgk1 were protected against DOCA/salt-induced albuminuria.¹²

Clinically, proteinuria is commonly seen in high-aldosterone conditions such as primary aldosteronism;¹³ as well, elevated plasma aldosterone concentrations are noted in patients with renal insufficiency.¹⁴ Quinkler et al¹⁵ analyzed kidney biopsy samples from proteinuric patients with diverse etiologies, and reported the correlation of renal MR and Sgk1 expressions with renal inflammation and proteinuria. The involvement of aldosterone is also suggested by “aldosterone breakthrough” phenomenon. Administration of ACE inhibitors or ARBs results in an acute decline in plasma aldosterone concentrations; however, with longer use, the initial suppression is not sustained and plasma aldosterone levels rise in a subset of patients.¹⁶ Sato et al¹⁷ found that aldosterone breakthrough was observed in 40% of type 2 diabetic patients with early nephropathy during 40 weeks of trandolapril therapy. Trandolapril reduced albuminuria in patients without aldosterone breakthrough, whereas the antialbuminuric effect of trandolapril was lost in patients with aldosterone breakthrough. Furthermore, the addition of low-dose spironolactone on top of trandolapril significantly reduced albuminuria without a change in BP for patients with aldosterone breakthrough.

More than 10 RCTs have demonstrated the antiproteinuric effects of MR antagonists. A recent systematic review and meta-analysis¹⁸ revealed that adding MR antagonists could significantly reduce proteinuria in CKD patients receiving ACE inhibitors and/or ARBs.

Podocyte injury as a major cause of proteinuria

The glomerular filtration barrier comprises 3 layers: the glomerular endothelium, the glomerular basement membrane, and the podocytes. Podocytes serve as the final filtration barrier restricting the passage of macromolecules from plasma in the process of primary urine formation.^{19,20}

Podocytes extend numerous major processes and secondary foot processes; neighboring podocytes interdigitate their foot processes, which are bridged by the “slit diaphragm.” Typically, with an injury to the podocytes, the foot processes are retracted; this is a dynamic and reversible process known as “foot process effacement.” Actin cytoskeletal organization is thought to play a role in the regulation of the “spectacular” morphological architecture and functional integrity.

In 1998, nephrin was identified by positional cloning as a causative gene for congenital nephrotic syndrome of the Finnish type. Nephrin is localized to the slit diaphragm, and is thought to constitute the major size-selective permeability barrier. Subsequently, a number of podocyte-specific molecules have been identified, including cluster of differentiation (CD)2AP, podocin, α -actinin 4, transient receptor potential channel 6 (TRPC6), and phospholipase C (PLC) ϵ 1.¹⁹ Reports on genetic mutations or gene targeting of many of these molecules indicate manifestations of proteinuria and glomerulosclerosis, establishing the pivotal roles of podocytes and their slit diaphragm as a filtration barrier.²⁰ Recent studies unveiled the role for nephrin and slit diaphragm as a signaling platform.²¹ Podocytes are actually injured in many types of proteinuric renal diseases, including nephrotic syndrome, lupus nephritis, diabetic and hypertensive nephropathy, and obesity-related glomerulopathy;^{22,23} therefore, podocytes are an important therapeutic target.

To date, a variety of factors have been identified that promote or ameliorate podocyte injury.²⁰ Podocytes, whose foot processes overlay the glomerular capillary tufts, are sensitive to mechanical stress; therefore, glomerular hypertension or hyperfiltration may contribute to podocyte impairment. Alternatively, podocytes express a number of vasoactive substances and their receptors, as well as nuclear receptors, and podocyte function is reported to be modulated by their ligands (eg, Ang II, glucocorticoids, vitamin D).^{20,24,25}

Aldosterone evokes podocyte injury – role of oxidative stress

The intimate relationship between aldosterone and proteinuria prompted an investigation of the effects of aldosterone on podocytes. The first step was an analysis of proteinuria and podocyte injury in uninephrectomized, high salt-fed rats infused with aldosterone (0.75 μ g/hr via an osmotic minipump).²⁶ This is an established chronic mineralocorticoid excess model in which plasma aldosterone levels rise to comparative levels seen in human congestive heart failure. After 4 weeks, aldosterone-infused rats developed hypertension and massive proteinuria. Glomerular expressions of slit diaphragm-associated molecules, nephrin and podocin, were markedly decreased, whereas expression of a damaged podocyte marker, desmin, was upregulated in aldosterone-infused rats. Electron microscopic analysis revealed podocyte foot-process effacement. Podocytes were already impaired at

2 weeks of aldosterone infusion, when proteinuria was modestly increased. Proteinuria and podocyte damage were completely reversed by eplerenone. These findings suggest that podocyte injury underlies the pathogenesis of proteinuria caused by aldosterone administration.

To determine how aldosterone evokes podocyte injury, the contribution of BP elevation *per se* was first tested. Although hydralazine normalized BP, it failed to improve proteinuria and podocyte damage, suggesting the presence of BP-independent, aldosterone-mediated mechanisms. Next, the role of oxidative stress was examined because reactive oxygen species (ROS) have been proposed as important mediators of aldosterone-induced target-organ injury.²⁷ Aldosterone-infused rats exhibited enhanced oxidative stress markers such as increased urinary excretion of thiobarbituric acid-reactive substances, elevated renal nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity determined by a lucigenin chemiluminescence assay, and stimulation of membrane translocation of NADPH oxidase cytosolic components p67phox and Rac1. Indeed, an antioxidant, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL), significantly reduced oxidative stress markers and corrected podocyte damage and proteinuria. Oxidative stress markers were also suppressed by eplerenone. These results suggest that aldosterone causes podocyte injury and proteinuria, possibly through the induction of oxidant stress.

Aldosterone may modulate podocyte function directly by acting on MR within podocytes, or indirectly by affecting glomerular hemodynamics; therefore, it is important to examine whether aldosterone exerts direct cellular effects using cultured podocytes. MR transcripts and proteins were actually detected in *in vitro* cultured podocytes as well as *in vivo* glomerular podocytes. Exposure of cultured podocytes to aldosterone resulted in nuclear translocation of MR, activation of NADPH oxidase, and ROS increments. In addition, aldosterone upregulated the expression of Sgk1, in cultured podocytes as well as in the kidney of aldosterone-infused rats, which could be prevented by eplerenone. Taken together, these results suggest that at least some of the proteinuric effects of aldosterone are attributable to direct nonhemodynamic actions on podocytes.

Aldosterone and the MetS

Aldosterone excess has been implicated in obesity-related disorders. In 1981, Tuck et al²⁸ suggested the involvement of aldosterone in the pathogenesis of obesity-associated hypertension. Goodfriend et al²⁹ demonstrated a correlation between plasma aldosterone concentrations and the amount of visceral fat. Accordingly, nontraditional adrenal stimuli for aldosterone production have been reported, including oxidized products of linoleic acid,³⁰ and as-yet unidentified potent mineralocorticoid-releasing factors secreted by adipocytes.³¹

Recent clinical evidence supports the relationship between aldosterone and the MetS. Two cross-sectional clinical studies in patients of African descent demonstrated that plasma aldosterone concentration is independently associated with the MetS.^{32,33} Plasma renin activity was somewhat suppressed in the MetS. The C allele of -344C/T variant in the promoter of the aldosterone synthase (*CYP11B2*) gene, which is associated with hyperaldosteronemia, was shown to increase susceptibility to the MetS in European men.³⁴ Furthermore, prospective studies of Framingham Offspring Study participants indicated that subjects with higher circulating aldosterone levels have an increased risk of developing the MetS.³⁵

MR is also expressed in adipocytes, and it mediates the mineralocorticoid and glucocorticoid effects on adipogenesis and white adipose tissue functions.³⁶ Aldosterone may contribute to adipokine abnormalities in the MetS. One report indicates that an MR antagonist corrects the obesity-related adipokine changes in *db/db* mice.³⁷

The MetS as a modifiable risk factor for CKD

CKD, defined as glomerular filtration rate (GFR) <60 mL/minute and/or the presence of proteinuria, is becoming an urgent public-health problem worldwide, since it commonly affects 10%-20% of the adult population in many countries. CKD patients, if untreated, will progress to end-stage renal disease (ESRD) requiring renal replacement therapy. More importantly, even mild kidney dysfunction increases the risk of premature CV death; therefore, to prevent such adverse outcomes, campaigns for early detection and treatment of CKD are promoted.

The MetS, a constellation of comorbidities that include visceral obesity, hypertension, glucose intolerance, and dyslipidemia, is also increasing at an alarming rate, and is a major public-health concern. The MetS is known as a highly predisposing condition for CV disease (CVD). Recent epidemiological studies demonstrate that the MetS is an important modifiable risk factor for proteinuria and CKD. For instance, a cross-sectional study³⁸ reveals that MetS subjects have a higher incidence of microalbuminuria (12.3% versus 4.7%) and CKD (6.0% versus 1.2%). A prospective longitudinal study, based on data in community-living, nondiabetic adults with normal kidney function, revealed that over 9 years, MetS patients increased their risk of developing CKD by approximately 50%, and the risk increased with more syndrome traits.³⁹ Nevertheless, the mechanisms linking the MetS to CKD have not been clearly elucidated; it is not solely attributable to the additive effects of individual components (eg, HTN and diabetes mellitus),³⁹ which suggests some unifying underlying mechanism.

Podocyte injury and proteinuria in MetS rats: contribution of aldosterone/MR activation

Given the relationships between aldosterone and CKD, the MetS and CKD, and aldosterone and the MetS, a working hypothesis was postulated that aldosterone/MR activation plays a critical role in the progression of CKD in the MetS. The SHR/NDmcr-cp (SHR/cp, obese SHR) is a rat model of the MetS that is a derivative of the SHR with the introduction of a leptin-receptor gene mutation. The rat manifests a clustering of obesity, hypertension, hyperinsulinemia, and hypertriglyceridemia.

Obese SHR/cps developed marked proteinuria in an age-dependent manner, while urinary protein excretion remained low in nonobese SHRs despite similar BP levels.⁴⁰ Proteinuria in SHR/cps was accompanied by podocyte injury, as indicated by attenuation of nephrin, induction of desmin, and foot process effacement. Notably, serum aldosterone levels were higher in obese SHR/cps than in nonobese SHRs, and there was a positive correlation between circulating aldosterone concentrations and proteinuria. Expression of Sgk1 was upregulated in the whole kidney, as well as in the glomerular fraction of SHR/cps, supporting the causative role of aldosterone/MR activation. Indeed, eplerenone effectively reduced proteinuria and podocyte damage in SHR/cps. These data suggest that aldosterone-provoked podocyte injury plays a pivotal role in the pathogenesis of proteinuria in SHR/cps.

In exploring the role of oxidative stress, with similarities to aldosterone-infused rats, oxidative stress markers were upregulated in SHR/cps; furthermore, eplerenone reversed the increased oxidative stress, which suggests the participation of endogenous aldosterone. Treatment with the antioxidant, TEMPOL, also significantly alleviated proteinuria and podocyte injury in SHR/cps. These data suggest that oxidative stress is an important mediator of aldosterone-induced podocyte injury in this model.

Mechanisms of aldosterone excess in SHR/cps

In investigating the mechanisms for the high-aldosterone state in SHR/cps, the expression of CYP11B2 was enhanced in the adrenal glands of SHR/cps compared with nonobese SHRs, but was below the detection level in the kidney. This suggests that aldosterone production in the adrenals is responsible for high-circulating aldosterone, but the excess in this model was not attributable to conventional aldosterone secretagogues (eg, Ang II or hyperkalemia). As noted, Ehrhart-Bornstein et al³¹ reported that adipocytes from obese subjects secrete potent aldosterone-releasing factors (ARFs); although these ARFs are unidentified, a comparison of ARF activity between SHR/cps and SHRs was carried out according to the methods of Ehrhart-Bornstein et al. Interestingly, aldosterone production in H295R adrenocortical cells was markedly increased by fat cell-conditioned medium from SHR/cp, but not medium from nonobese SHRs. In parallel, fat cell-conditioned medium from SHR/cp, but not from SHRs stimulated the expression of CYP11B2 and steroidogenic acute regulatory protein, key factors in aldosterone synthesis. The activity was not stimulated by known adipokines, including angiotensinogen.

ARF-mediated hyperaldosteronemia is not inhibited by ACE inhibitors or ARBs; thus, eplerenone should have benefits over Ang II blockade in situations where such factors are overproduced. Together, these data indicate that adipocytes produce ARFs in SHR/cps, which may contribute to elevated circulating aldosterone, podocyte injury, and proteinuria.

Salt accelerates renal and cardiac damage in SHR/cps via MR activation

Clinical studies reveal increased salt sensitivity in target-organ injury of obese subjects; however, the mechanisms have not been clearly elucidated. Augmentation of the deleterious effects of aldosterone is found under high salt intake, prompting an examination of the effects of salt loading in SHR/cps.⁴¹

High salt feeding for 4 weeks markedly enhanced proteinuria and podocyte injury in SHR/cps; in addition, eplerenone perfectly inhibited the salt-induced exacerbation, suggesting the involvement of the aldosterone/MR cascade. Although salt loading suppressed circulating renin and aldosterone, it paradoxically activated renal MR signaling, as revealed by increased MR in the nuclear fraction, induction of Sgk1, and upregulation of putative mediators of aldosterone-evoked organ damage, such as plasminogen activator inhibitor (PAI)-1 and macrophage chemoattractant protein (MCP)-1, in the kidney of salt-loaded SHR/cps. Eplerenone completely inhibited these MR-dependent cascades.

The paradoxical MR activation may be partly attributable to ARFs. While the RAS-regulated aldosterone

generation is counterbalanced by salt, preliminary data suggest that aldosterone production by ARFs lacks negative feedback regulation in response to high salt intake. As a result, suppression of the circulating aldosterone levels may be less than expected and cause inappropriately high aldosterone for the amount of salt intake.

Cross-talk between the kidney and the heart has recently become a major topic; as a result, the rat model was examined to determine whether the same mechanism for the pathogenesis of cardiac injury can be extrapolated.⁴² Left-ventricular diastolic function, as revealed by cardiac catheterization and Doppler echocardiographic analysis, was impaired in salt-loaded SHR/cps, and was accompanied by increased ROS and perivascular fibrosis in the heart; again, the cardiac abnormalities fully recovered with eplerenone. These findings corroborate the hypothesis that obesity and salt, 2 cardinal features of modern society, causes MR activation, leading ultimately to CKD and CVD.

Vertebrate evolution and the aldosterone/MR system

Evolutionary perspectives on the aldosterone/MR system are interesting to consider. Phylogenetically, aldosterone first appears in land-living tetrapods, but aquatic fish lack the hormone, implying that terrestrial animals acquired the aldosterone/MR allowing the recapitulation of the seawater environment within the body to sustain life on land with little salt and water. The probability of acquiring both ligand and receptor at the same time is speculated as quite low; MR was already present during aquatic life, where it served as a receptor for other ligands and conveyed its own function. When aldosterone evolved, the hormone “exploited” the MR receptor of other ligands and compelled the assumption of a new role in electrolyte homeostasis, which eventually became the main function of MR.⁴³

When humans first appeared, the amount of available salt was limited, and salt became a precious commodity. During long periods of salt scarcity, people with stronger “salt retention” genes had a significant survival advantage. In modern, industrialized societies there is an abundance of salt, as well as energy imbalances causing the pandemic of obesity. Cultural changes have outpaced any possible genetic adaptations, with obesity and salt synergistically causing inappropriate activation of the aldosterone/MR system. Individuals with genes for enhanced aldosterone/MR activity are especially predisposed to diseases of civilization, such as salt-sensitive hypertension, CKD, and CVD, when faced with the contemporary environment.

Novel mechanism of MR activation by Rac1 and its implication in CKD

High-aldosterone models are indicated as causing MR activation and renal injury; however, some results^{27,48} demonstrate that MR can be activated even in normal or low aldosterone states. For example, Dahl salt-hypertensive rats develop podocyte injury, proteinuria, and glomerulosclerosis; despite low circulating aldosterone levels, MR signaling was enhanced in the kidney, and eplerenone dramatically retarded disease progression.²³ Clinical studies reveal that the efficacy of MR antagonists are not predicted by plasma renin-aldosterone profiles.⁷

These findings raise the possibility that molecules other than circulating aldosterone may activate MR.

As for ligands other than circulating aldosterone, MR may be activated by locally generated aldosterone; however, neither aldosterone nor CYP11B2 expression could be detected in the kidney homogenates of rat models. Alternatively, glucocorticoids may cause MR activation; clearly, MR has high affinity for both aldosterone and corticosterone/cortisol, therefore glucocorticoids may activate MR under suppressed 11 β -hydroxysteroid dehydrogenase-2 activity.

Currently, little is known about the mechanisms of ligand-independent MR activation. MR belongs to the nuclear receptor superfamily acting as a transcription factor; upon ligand binding, the ligand-receptor complex translocates into the nucleus, where it interacts with the mineralocorticoid response element in the promoter region of the target genes to activate gene transcription. From this point of view, MR activity should be modulated by multiple factors other than ligand level, such as the amount of MR, nuclear translocation, chromatin and histone modification, recruitment of coregulators, and cross-talk with other intracellular signaling molecules. Increased MR content may be one mechanism because MR transgenic mice are reported to develop cardiac and renal abnormalities.^{44,45} MR activity may be modulated by cross-talk with intracellular signaling, because such regulations are reported for other steroid receptors.⁴⁶⁻⁴⁹

The small GTPase protein, Rac1, was identified as a novel activator of MR⁵⁰ through the enhanced activity found with *in vitro* transfection assays in HEK 293 cells. In luciferase reporter assays, MR-dependent transcriptional activity in response to aldosterone was potentiated by overexpression of constitutively active (CA)-Rac1. CA-Rac1 increased the luciferase activity in the absence of aldosterone as well. Furthermore, CA-Rac1 promoted the nuclear translocation of green fluorescence protein (GFP)-tagged MR, both with and without aldosterone. Similar potentiation was also observed in cultured podocytes. These results indicate that CA-Rac1 causes MR activation.

Subsequent investigations examined whether this "Rac1-evoked MR activation" contributes to the pathogenesis of renal injury *in vivo*, using Rho guanosine diphosphate (GDP) dissociation inhibitor (RhoGDI) α knockout (KO) mice,⁵¹ a kidney-specific Rac1 activation model. At 12 weeks of age, the KO mice exhibited massive albuminuria, podocyte damage, and glomerulosclerosis. Rac-specific inhibitor, NSC23766,⁵² substantially ameliorated the renal impairment, concomitantly with repression of Rac1 activity. Aldosterone-independent MR activation was detected in the kidney of this model, as evidenced by increased nuclear MR and enhanced Sgk1, PAI-1, and MCP-1 expression, despite normal aldosterone level. Indeed, eplerenone dramatically abrogated the renal impairment; furthermore, Rac inhibitor suppressed MR activation. These results indicate that Rac1-mediated MR activation plays a central role in the renal phenotype of RhoGDI α KO mice. Traditionally, Rac1 GTPase is known to have diverse biological functions, such as actin cytoskeletal organization, cell migration, and generation of oxidative stress as component of NADPH oxidase.⁵³ Recently, Rac1 was demonstrated as indispensable for the nuclear localization of β -catenin in canonical Wnt signaling,⁵⁴ and of signal transducer and activator of

transcription (STAT)-5 in cytokine signaling.⁵⁵ These results, in addition to the findings of nuclear MR translocation, highlight the novel roles for Rac1 in the nucleocytoplasmic shuttling of transcription factors.

This alternative pathway of MR activation actually plays a significant role in the progression of renal injury in more common CKD models, which implicates Rac1 as a novel therapeutic target for CKD. For example, Rac1 was activated in the kidneys of salt-loaded Dahl salt-sensitive rats, whereas NSC23766 significantly suppressed MR activation, proteinuria, and glomerulosclerosis. These findings suggest that the Rac1-MR pathway may contribute, at least in part, to the nonaldosterone-mediated MR activation in this model.

Rac1 is identified as a ligand-independent activator of MR; this alternative pathway of MR activation plays a significant role in the progression of renal injury in some CKD models, which implicates Rac1 as a novel therapeutic target for CKD. Preliminary data suggest that Rac1 is activated in response to various stimuli relevant to the MetS (data not shown); therefore, MR can be activated in the kidney of the MetS patient by several different pathways, both aldosterone-dependent and -independent, and mediates renal injury.

Conclusions and perspectives

Recent clinical and experimental evidence reveal the pathogenic roles of aldosterone in CKD and the MetS. Data demonstrate that SHR/cp, a MetS model, is susceptible to renal and cardiac injuries, especially when the animal is fed a high-salt diet. Inappropriate activation of the aldosterone/MR system underlies target organ diseases and MR can be activated by several different pathways, both aldosterone dependent (via ARFs) and independent (eg, via Rac1). These findings support the hypothesis that obesity and salt, 2 central features of modern society, cause MR overactivation, leading to CKD and CVD.

Future studies are necessary to identify the clinical conditions in which Rac1 is overactivated and Rac inhibitor is effective, and to determine the intrarenal localization of activated Rac1. For this purpose, clinical studies using renal biopsy specimens of CKD patients are under investigation, with the expectation that ARFs and Rac1 can be novel targets of therapy for MetS and CKD. Further, handy diagnostic tools must be established to evaluate tissue Rac1 activation state, because circulating aldosterone concentration does not necessarily reflect MR activity in the target organ.

Currently, hyperkalemia remains a concern with the use of MR antagonists, especially in patients with impaired renal function. Unfortunately, the administration of eplerenone is contraindicated in diabetic patients with albuminuria because of the reported severe hyperkalemia evoked by high doses of eplerenone. Subsequent studies suggest that lower doses of eplerenone may be safe and sufficiently beneficial. Presently, a double-blind, placebo-controlled RCT is being conducted to evaluate the antialbuminuric effect of low-dose eplerenone, the Eplerenone combination Versus conventional Agents to Lower blood pressure on Urinary Antialbuminuric Treatment Effect (EVALUATE) trial. The study subjects will be 340 Japanese hypertensive patients with albuminuria receiving ACE inhibitors or ARBs. Large-scale RCTs are also awaited to assess the efficacy and safety of MR antagonism in patients with the MetS.

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References

1. Epstein M. Aldosterone blockade: an emerging strategy for abrogating progressive renal disease. *Am J Med.* 2006;119(11):912-919.
2. Selye H, Hall C. Pathologic changes induced in various species by overdosage with desoxycorticosterone. *Arch Pathol.* 1943;36:19-31.
3. Brilla CG, Pick R, Tan LB, Janicki JS, Weber KT. Remodeling of the rat right and left ventricles in experimental hypertension. *Circ Res.* 1990;67(6):1355-1364.
4. Pitt B, Zannad F, Remme WJ, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med.* 1999;341(10):709-717.
5. Pitt B, Remme W, Zannad F, et al. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *N Engl J Med.* 2003;348(14):1309-1321.
6. Pitt B, Reichel N, Willenbrock R, et al. Effects of eplerenone, enalapril, and eplerenone/enalapril in patients with essential hypertension and left ventricular hypertrophy: the 4E-left ventricular hypertrophy study. *Circulation.* 2003;108(15):1831-1838.
7. Williams GH, Burgess E, Kolloch RE, et al. Efficacy of eplerenone versus enalapril as monotherapy in systemic hypertension. *Am J Cardiol.* 2004;3(8):990-996.
8. Greene EL, Kren S, Hostetter TH. Role of aldosterone in the remnant kidney model in the rat. *J Clin Invest.* 1996;98(4):1063-1068.
9. Lea WB, Kwak ES, Luther JM, et al. Aldosterone antagonism or synthase inhibition reduces end-organ damage induced by treatment with angiotensin and high salt. *Kidney Int.* 2009;75(9):936-944.
10. Fiebeler A, Nussberger J, Shagdarsuren E, et al. Aldosterone synthase inhibitor ameliorates angiotensin II-induced organ damage. *Circulation.* 2005;111(23):3087-3094.
11. Blasi ER, Rocha R, Rudolph AE, Blomme EA, Polly ML, McMahon EG. Aldosterone/salt induces renal inflammation and fibrosis in hypertensive rats. *Kidney Int.* 2003;63(5):1791-1800.
12. Artunc F, Amann K, Nasir O, et al. Blunted DOCA/high salt induced albuminuria and renal tubulointerstitial damage in gene-targeted mice lacking SGK1. *J Mol Med.* 2006;84(9):737-746.
13. Ribstein J, Du Cailar G, Fessler P, Mimran A. Relative glomerular hyperfiltration in primary aldosteronism. *J Am Soc Nephrol.* 2005;16(5):1320-1325.
14. Hene RJ, Boer P, Koomans HA, Mees EJ. Plasma aldosterone concentrations in chronic renal disease. *Kidney Int.* 1982;21(1):98-101.
15. Quinkler M, Zehnder D, Eardley KS, et al. Increased expression of mineralocorticoid effector mechanisms in kidney biopsies of patients with heavy proteinuria. *Circulation.* 2005;112(10):1435-1443.
16. Bomback AS, Klemmer PJ. The incidence and implications of aldosterone breakthrough. *Nat Clin Pract Nephrol.* 2007;3(9):486-492.
17. Sato A, Hayashi K, Naruse M, Saruta T. Effectiveness of aldosterone blockade in patients with diabetic nephropathy. *Hypertension.* 2003;41(1):64-68.
18. Navaneethan SD, Nigwekar SU, Sehgal AR, Strippoli GF. Aldosterone antagonists for preventing the progression of chronic kidney disease: a systematic review and meta-analysis. *Clin J Am Soc Nephrol.* 2009;4(3):542-551.
19. Tryggvason K, Patrakka J, Wartiovaara J. Hereditary proteinuria syndromes and mechanisms of proteinuria. *N Engl J Med.* 2006;354(13):1387-1401.
20. Pavenstadt H, Kriz W, Kretzler M. Cell biology of the glomerular podocyte. *Physiol Rev.* 2003;83(1):253-307.
21. Jones N, Blasutig IM, Eremina V, et al. Nck adaptor proteins link nephrin to the actin cytoskeleton of kidney podocytes. *Nature.* 2006;440(7085):818-823.
22. Pagtalunan ME, Miller PL, Jumping-Eagle S, et al. Podocyte loss and progressive glomerular injury in type II diabetes. *J Clin Invest.* 1997;99(2):342-348.
23. Nagase M, Shibata S, Yoshida S, Nagase T, Gotoda T, Fujita T. Podocyte injury underlies the glomerulopathy of Dahl salt-hypertensive rats and is reversed by aldosterone blocker. *Hypertension.* 2006;47(6):1084-1093.
24. Yamauchi K, Takano Y, Kasai A, et al. Screening and identification of substances that regulate nephrin gene expression using engineered reporter podocytes. *Kidney Int.* 2006;70(5):892-900.
25. Wada T, Pippin JW, Marshall CB, Griffin SV, Shankland SJ. Dexamethasone prevents podocyte apoptosis induced by puromycin aminonucleoside: role of p53 and Bcl-2-related family proteins. *J Am Soc Nephrol.* 2005;16(9):2615-2625.
26. Shibata S, Nagase M, Yoshida S, Kawachi H, Fujita T. Podocyte as the target for aldosterone: roles of oxidative stress and Sgk1. *Hypertension.* 2007;49(2):355-364.
27. Sun Y, Zhang J, Lu L, Chen SS, Quinn MT, Weber KT. Aldosterone-induced inflammation in the rat heart: role of oxidative stress. *Am J Pathol.* 2002;161(5):1773-1781.
28. Tuck ML, Sowers J, Dornfeld L, Kledzik G, Maxwell M. The effect of weight reduction on blood pressure, plasma renin activity, and plasma aldosterone levels in obese patients. *N Engl J Med.* 1981;304(16):930-933.
29. Goodfriend TL, Calhoun DA. Resistant hypertension, obesity, sleep apnea, and aldosterone: theory and therapy. *Hypertension.* 2004;43(3):518-524.
30. Goodfriend TL, Ball DL, Egan BM, Campbell WB, Nithipatikom K. Epoxy-keto derivative of linoleic acid stimulates aldosterone secretion. *Hypertension.* 2004;43(2):358-363.
31. Ehrhart-Bornstein M, Lamounier-Zepter V, Schraven A, et al. Human adipocytes secrete mineralocorticoid-releasing factors. *Proc Natl Acad Sci U S A.* 2003;100(24):14211-14216.
32. Bochud M, Nussberger J, Bovet P, et al. Plasma aldosterone is independently associated with the metabolic syndrome. *Hypertension.* 2006;48(2):239-245.
33. Kidambi S, Kotchen JM, Grim CE, et al. Association of adrenal steroids with hypertension and the metabolic syndrome in blacks. *Hypertension.* 2007;49(3):704-711.
34. Russo P, Lauria F, Loguercio M, et al. -344C/T Variant in the promoter of the aldosterone synthase gene (CYP11B2) is associated with metabolic syndrome in men. *Am J Hypertens.* 2007;20(2):218-222.
35. Ingelsson E, Pencina MJ, Tofler GH, et al. Multimarker approach to evaluate the incidence of the metabolic syndrome and longitudinal changes in metabolic risk factors: the Framingham Offspring Study. *Circulation.* 2007;116(9):984-992.
36. Zennaro MC, Caprio M, Feve B. Mineralocorticoid receptors in the metabolic syndrome. *Trends Endocrinol Metab.* 2009;20(9):444-451.
37. Guo C, Ricchiuti V, Lian BQ, et al. Mineralocorticoid receptor blockade reverses obesity-related changes in expression of adiponectin, peroxisome proliferator-activated receptor-gamma, and proinflammatory adipokines. *Circulation.* 2008;117(17):2253-2261.
38. Chen J, Muntner P, Hamm LL, et al. The metabolic syndrome and chronic kidney disease in U.S. adults. *Ann Intern Med.* 2004;140(3):167-174.
39. Kurella M, Lo JC, Chertow GM. Metabolic syndrome and the risk for chronic kidney disease among nondiabetic adults. *J Am Soc Nephrol.* 2005;16(7):2134-2140.
40. Nagase M, Yoshida S, Shibata S, et al. Enhanced aldosterone signaling in the early nephropathy of rats with metabolic syndrome: possible contribution of fat-derived factors. *J Am Soc Nephrol.* 2006;17(12):3438-3446.
41. Nagase M, Matsui H, Shibata S, Gotoda T, Fujita T. Salt-induced nephropathy in obese spontaneously hypertensive rats via paradoxical activation of the mineralocorticoid receptor: role of oxidative stress. *Hypertension.* 2007;50(5):877-883.
42. Matsui H, Ando K, Kawarazaki H, et al. Salt excess causes left ventricular diastolic dysfunction in rats with metabolic disorder. *Hypertension.* 2008;52(2):287-294.
43. Bridgham JT, Carroll SM, Thornton JW. Evolution of hormone-receptor complexity by molecular exploitation. *Science.* 2006;312(5770):97-101.
44. Le Menuet D, Isnard R, Bichara M, et al. Alteration of cardiac and renal functions in transgenic mice overexpressing human mineralocorticoid receptor. *J Biol Chem.* 2001;276(42):38911-38920.
45. Ouvrard-Pascaud A, Sainte-Marie Y, Benitah JP, et al. Conditional mineralocorticoid receptor expression in the heart leads to life-threatening arrhythmias. *Circulation.* 2005;111(23):3025-3033.
46. Kato S, Endoh H, Masuhiro Y, et al. Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. *Science.* 1995;270(5241):1491-1494.
47. Yanagisawa J, Yanagi Y, Masuhiro Y, et al. Convergence of transforming growth factor-beta and vitamin D signaling pathways on SMAD transcriptional coactivators. *Science.* 1999;283(5406):1317-1321.
48. Su LF, Knoblauch R, Garabedian MJ. Rho GTPases as modulators of the estrogen receptor transcriptional response. *J Biol Chem.* 2001;276(5):3231-3237.
49. Kino T, Souvatzoglou E, Charmandari E, et al. Rho family Guanine nucleotide exchange factor Brx couples extracellular signals to the glucocorticoid signaling system. *J Biol Chem.* 2006;281(14):9118-9126.
50. Shibata S, Nagase M, Yoshida S, et al. Modification of mineralocorticoid receptor function by Rac1 GTPase: implication in proteinuric kidney disease. *Nat Med.* 2008;14(12):1370-1376.
51. Togawa A, Miyoshi J, Ishizaki H, et al. Progressive impairment of kidneys and reproductive organs in mice lacking Rho GDIalpha. *Oncogene.* 1999;18(39):5373-5380.
52. Gao Y, Dickerson JB, Guo F, Zheng J, Zheng Y. Rational design and characterization of a Rac GTPase-specific small molecule inhibitor. *Proc Natl Acad Sci U S A.* 2004;101(20):7618-7623.
53. Takai Y, Sasaki T, Matozaki T. Small GTP-binding proteins. *Physiol Rev.* 2001;81(1):153-208.
54. Wu X, Tu X, Joeng KS, Hilton MJ, Williams DA, Long F. Rac1 activation controls nuclear localization of beta-catenin during canonical Wnt signaling. *Cell.* 2008;133(2):340-353.
55. Kawashima T, Bao YC, Nomura Y, et al. Rac1 and a GTPase-activating protein, MgcRacGAP, are required for nuclear translocation of STAT transcription factors. *J Cell Biol.* 2006;175(6):937-946.

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