

## REVIEW

# Mineralocorticoid receptor as a therapeutic target in chronic kidney disease and hypertension

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The kidney has a central role in long-term control of blood pressure, and decreased kidney function is a common but difficult-to-treat cause of hypertension. Conversely, elevated blood pressure contributes to the progression of chronic kidney disease. Steroid hormone aldosterone and its receptor mineralocorticoid receptor (MR) contribute to hypertension by increasing renal salt reabsorption and promote kidney dysfunction through direct effects on renal parenchymal cells. Accumulating data indicate that various mechanisms affect aldosterone-MR signaling. Using a genetically engineered mouse model, we identified crosstalk between small GTPase Rac1 and MR. This crosstalk pathway promotes glomerular podocyte injury, and is also involved in the pathogenesis of hypertension. Notably, salt loading increases renal Rac1 activity in several models of salt-sensitive hypertension, which, in the presence of aldosterone, synergistically activates MR signaling, causing hypertension and kidney injury. There is also a mechanism regulating MR in a cell-selective manner. In the principal cells of the collecting duct, aldosterone directly binds and activate MR. In neighboring intercalated cells, however, binding of aldosterone to MR is regulated by phosphorylation at the ligand-binding domain. This mechanism serves as a switch to turn on electrolyte flux pathways in intercalated cells, allowing aldosterone to exert distinct effects in different physiological contexts. Given the potential benefit of MR blockade in hypertensive kidney disease, the delineation of these pathways may lead to the identification of alternative therapeutic targets. In this review, we discuss the roles of MR in mediating kidney disease and hypertension, with a focus on the crosstalk among related signaling pathways.

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The kidney fluid system is a predominant regulator of blood pressure, and reduced kidney function constitutes a major cause of hypertension.<sup>1–3</sup> High blood pressure, in turn, contributes to the progression of kidney dysfunction,<sup>4,5</sup> causing a vicious cycle in hypertensive kidney disease. Mineralocorticoid receptor (MR; encoded by *NR3C2*) belongs to the nuclear receptor superfamily and is abundantly present in the so-called aldosterone-sensitive distal nephron.<sup>6,7</sup> In response to the steroid hormone aldosterone, MR regulates the transcription of target genes, serving as the master regulator of Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>−</sup> flux mechanisms in this segment.<sup>8,9</sup> Among the various genes that cause Mendelian forms of blood pressure variation in humans, mutations in aldosterone synthase, MR and epithelial Na<sup>+</sup> channel (ENaC) can cause both hypertension and hypotension,<sup>3</sup> demonstrating the central role of the aldosterone-MR system in fluid volume homeostasis.

Besides its role in regulating blood pressure, MR has also been shown to be involved in the pathogenesis of end-organ damage, including heart failure and chronic kidney disease (CKD) progression.<sup>10–16</sup> Especially, experimental and clinical studies have

demonstrated that MR blockade effectively reduces proteinuria.<sup>14,16–19</sup> It is now accepted that MR is much more widely distributed than previously thought,<sup>20–24</sup> and aberrant MR signaling in nonclassical targets is likely to be involved in target organ damage associated with hypertension. Given the wide distribution of MR in various tissues and organs,<sup>20–24</sup> it is plausible that the function of MR is locally modulated in a context-dependent manner. Accordingly, we identified previously unrecognized mechanisms that regulate MR function independently of circulating ligand levels.<sup>18,22,25</sup> In this short review, we discuss the roles of MR in mediating kidney disease and hypertension, with a focus on the crosstalk among various related signaling pathways.

## POTENTIATION OF MR SIGNALING BY THE SMALL GTPASE RAC1 AND GLOMERULAR EPITHELIAL CELL DAMAGE

The Rho family of small G proteins, which includes RhoA, Rac1 and Cdc42, controls cell shape, polarity and migration through actin reorganization.<sup>26</sup> When activated, Rho family GTPases dissociate from the negative regulators, Rho GDP dissociation inhibitors (RhoGDIs), translocate to the cell membrane, and are converted to the active,

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GTP-bound form through interaction with guanine nucleotide-exchange factors. They are then converted to the inactive GDP-bound form by GTPase-activating proteins and bound to RhoGDIs. Interestingly, mice lacking RhoGDI- $\alpha$  (*Arhgdia*<sup>-/-</sup>) exhibit heavy proteinuria and progressive kidney failure,<sup>27</sup> highlighting the critical role of this protein in kidney function. Using this mice model, we analyzed the mechanisms of the kidney injury in detail, and unexpectedly found that the activity of Rac1 but not that of RhoA was elevated,<sup>18</sup> and pharmacological manipulation of Rac1 activity in the kidney revealed its pathological importance in glomerular podocyte injury and proteinuria.

In addition to its well-described role in actin cytoskeletal reorganization, Rac1 is also capable of regulating the nuclear accumulation and transcriptional activity of nuclear transcription factors.<sup>28,29</sup> In an effort to clarify the signaling pathways that cause kidney dysfunction in the *Arhgdia*<sup>-/-</sup> model, we found that Rac1 facilitates MR signaling independently of the ligand aldosterone levels.<sup>18</sup> Indeed, *Arhgdia*<sup>-/-</sup> mice showed MR accumulation in the nuclear fraction and increased expression of MR target genes in the absence of hyperaldosteronism. Furthermore, these changes, in addition to heavy proteinuria and kidney dysfunction, were almost completely reversed by the selective MR antagonist eplerenone, demonstrating the central role of aberrant MR signaling. Thus, in this model, Rac1 activity in the kidney is increased by deletion of RhoGDI $\alpha$ , which in turn induces MR signaling by facilitating receptor function, thereby causing kidney dysfunction.

Interestingly, recent studies have identified and characterized a type of congenital nephrotic syndrome caused by mutations in RhoGDI $\alpha$  in humans.<sup>30,31</sup> Consistent with our data, the study showed that mutations in *ARHGDI* (R120X, G173V) result in the activation of Rac1 but not that of RhoA. In addition, the nephrotic phenotype recapitulated in RhoGDI $\alpha$ -deficient zebrafish is ameliorated by Rac1 inhibitors and MR antagonists.<sup>30</sup>

### ROLE OF THE RAC1-MR PATHWAY IN THE PATHOPHYSIOLOGY OF CKD ASSOCIATED WITH HYPERTENSION

The pressor response to high salt intake (termed 'salt sensitivity') varies among individuals;<sup>32</sup> however, the related mechanisms have not been completely elucidated. Dahl salt-sensitive (Dahl-S) and salt-resistant (Dahl-R) strains have long been used to evaluate the factors influencing salt sensitivity. In these models, previous studies have reported that plasma aldosterone levels are rather low in Dahl-S rats compared with those in Dahl-R rats.<sup>33</sup> Moreover, salt loading

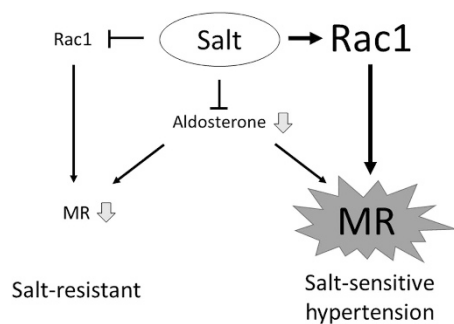
decreases aldosterone secretion similarly in Dahl-S and Dahl-R rats,<sup>22</sup> excluding the possibility of autonomic aldosterone secretion in these models. Interestingly, however, previous data indicate that serum- and glucocorticoid-induced kinase 1 (SGK1) and ENaC, the main downstream targets of aldosterone-MR signaling, are paradoxically elevated in salt-loaded Dahl-S rats,<sup>34</sup> despite suppression of aldosterone. Consistent with this, we observed increased SGK1 protein and nuclear MR contents in salt-loaded Dahl-S rats. Furthermore, we also found that the selective MR antagonist eplerenone ameliorated high blood pressure and glomerular injury in this model.<sup>22</sup> In contrast, salt-loaded Dahl-R rats showed decreased nuclear MR and SGK1 along with reduced plasma aldosterone, indicating that MR signaling is appropriately reduced.

In our previous work, we evaluated the contribution of Rac1 GTPase to the mechanism of this paradoxical MR signaling in salt-loaded Dahl-S rats.<sup>22</sup> Interestingly, high salt loading increased Rac1 activity in the kidneys of Dahl-S rats. In contrast, renal Rac1 was reduced by high salt in Dahl-R rats. Pharmacological manipulation of Rac1 by Nsc23766 in Dahl-S rats abrogated hypertension and kidney injury along with reduction in nuclear MR and SGK1 contents, demonstrating the pathological roles of Rac1 overactivity. Thus, these data indicate that salt loading increases Rac1 activity, which in turn causes salt-dependent hypertension via facilitating MR signaling even when circulating aldosterone is reduced (Figure 1).

Notably, adrenalectomy in Dahl-S rats prevented Rac1 activation, which was restored by exogenous aldosterone supplementation. These data indicate that the Rac1-MR and aldosterone-MR axes are interdependent. They also exclude the possibility that MR is activated by ligands other than aldosterone in this model. Thus, high salt loading suppresses aldosterone but activates MR signaling via Rac1 in the context of salt-sensitive hypertension, contributing to blood pressure elevation and kidney disease progression. Our data indicate that, besides hyperaldosteronism, dysregulation of MR significantly contributes to the pathogenesis of hypertension. Similarly, MR blockade can confer organ protection in kidney diseases associated with pathological MR activity, highlighting the potential usefulness of MR antagonists.

The first-line treatment for proteinuric CKD is angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs).<sup>35</sup> Large-scale interventional studies have established that inhibitors of the renin-angiotensin system reduce proteinuria and block CKD progression.<sup>36-39</sup> Importantly, however, the renoprotective effects of ACEIs and ARBs are less clear in patients with high salt intake than in those with low to normal salt intake.<sup>40</sup> Given our experimental data, high salt status can be associated with enhanced MR signaling via Rac1, which may not be effectively abrogated by ACEIs or ARBs.

The EVALUATE (Eplerenone Combination vs. Conventional Agents to Lower Blood Pressure on Urinary Anti-albuminuric Treatment Effect) study investigated the effects of long-term, low-dose eplerenone (50 mg per day) added to ACEIs, ARBs or both, on the urinary albumin-to-creatinine ratio in hypertensive patients with nondiabetic CKD (with an estimated glomerular filtration rate of at least 50 ml min<sup>-1</sup> 1.73 m<sup>-2</sup>).<sup>14</sup> In this randomized, placebo-controlled trial, the authors showed that eplerenone significantly reduced albuminuria and blood pressure in patients with nondiabetic CKD who were already on ACEIs or ARBs. Although serum potassium concentrations were modestly higher in the eplerenone group than the placebo group, none of the participants had hyperkalemia as defined by serum K<sup>+</sup> levels higher than 5.5 mmol l<sup>-1</sup>, indicating that low-dose eplerenone was tolerable in this population. Most interestingly,



**Figure 1** Rac1 response to high salt loading and salt sensitivity. In salt-resistant phenotype, salt loading decreases plasma aldosterone and also renal Rac1 activity. This results in reduced mineralocorticoid receptor (MR) signaling and natriuresis. In contrast, a subgroup of salt-sensitive hypertension shows increased Rac1 activity by salt loading. The aberrant Rac1 signaling causes MR overactivity in the presence of aldosterone.

in *post hoc* analyses stratified by urinary sodium excretion, the renoprotective effects of eplerenone were prominent in those with high urinary sodium excretion (160 mmol per day or greater). In contrast, there were no significant differences in the percent change in urinary albumin-to-creatinine ratio from baseline between the eplerenone group and placebo group in participants with low urinary sodium excretion (< 160 mmol per day). Furthermore, there was no significant correlation between the percent decrease in urinary albumin-to-creatinine ratio and baseline aldosterone levels. These data indicate the strong association between salt intake and MR signaling in humans, and are consistent with the experimental data showing that salt loading enhances MR signaling without increasing circulating aldosterone. Also, this study clearly demonstrates that MR antagonists have different property to ACEIs or ARBs, and that their use on top of ACEI or ARB may be beneficial at least in a subgroup of CKD patients as far as serum  $K^+$  levels are appropriately controlled.

### CELL-SELECTIVE REGULATION OF MR BY PHOSPHORYLATION

In the principal cells of the connecting tubules and collecting duct, aldosterone directly binds to MR and regulates the expression and activity of the amiloride-sensitive ENaC.<sup>41</sup> This process is mediated by the interplay of the Ser/Thr kinase SGK1 and ubiquitin ligase neuronal precursor cell expressed developmentally downregulated 4-2.<sup>41</sup> Aldosterone regulates ENaC also via proteolytic cleavage<sup>42</sup> and epigenetic modification.<sup>43</sup> Activation of ENaC by aldosterone drives electrogenic  $Na^+$  reabsorption, which promotes lumen-negative potential and stimulates  $Cl^-$  reabsorption, or  $K^+$  and  $H^+$  secretion. Consistent with this, in patients with autosomal recessive pseudohypoaldosteronism type I, mutations in any of the three different ENaC subunits cause salt wasting, hyperkalemia and metabolic acidosis.<sup>44,45</sup>  $K^+$  secretion in this segment is mediated by renal outer medullary  $K^+$  channels, and apical vacuolar  $H^+$ -ATPase is responsible for  $H^+$  secretion.

With regard to the route of  $Cl^-$  reabsorption in this segment, accumulating data indicate the critical role of pendrin (encoded by *SLC26A4*), a  $Cl^-/HCO_3^-$  exchanger selectively present in  $\beta$ -intercalated cells.<sup>46–48</sup> In mice lacking pendrin, Wall *et al.*<sup>48</sup> found that  $Cl^-$  flux in the cortical collecting duct is no longer evident, and

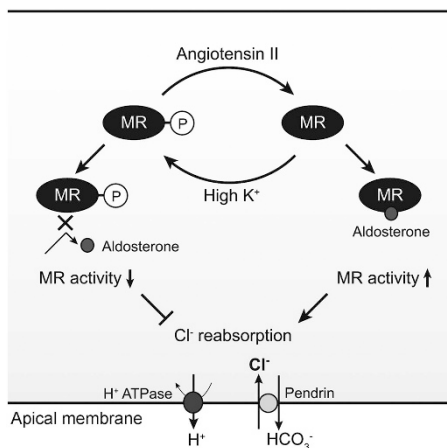
these mice show the salt-loss phenotype. Conversely, overexpression of pendrin produces salt-dependent hypertension.<sup>49</sup> The role of pendrin in the complex network of the kidney fluid system was established in the study by Soleimani *et al.*,<sup>50</sup> who reported severe volume depletion and hypotension in mice lacking pendrin and the  $Na^+-Cl^-$  co-transporter. These data demonstrate that pendrin and  $Na^+-Cl^-$  co-transporter are the main  $Cl^-$  reabsorption pathways in the distal nephron and that pendrin contributes to fluid and electrolyte homeostasis.

Previous studies have identified the factors influencing pendrin expression and/or activity, including acid/base change, angiotensin II,  $K^+$ ,  $Cl^-$  and mineralocorticoids.<sup>51–54</sup> Indeed, evidence indicates that intercalated cells express MR.<sup>7</sup> In  $\beta$ -intercalated cells, the synthetic mineralocorticoid deoxycorticosterone pivalate is shown to increase pendrin expression in the apical membrane.<sup>52</sup> However, the effects of mineralocorticoids may not be evident depending on the experimental conditions,<sup>55</sup> suggesting that MR is regulated via complex mechanisms in these cells.

To obtain insights into the mechanisms regulating MR function, we have comprehensively analyzed the phosphorylation sites in MR.<sup>25</sup> Among the identified sites, phosphorylation at S843 (MR<sup>S843-P</sup>) is of particular interest because of its location in the ligand-binding domain. Previous reports have highlighted the importance of S843 in determining ligand selectivity.<sup>56</sup> Indeed, biochemical assays using phosphomimetic MR<sup>S843E</sup> revealed that phosphorylation severely impairs aldosterone binding. Consistent with this, phosphomimetic MR is exclusively cytoplasmic and shows no transcriptional activity, even in the presence of aldosterone at a physiological concentration ( $1 \text{ nmol l}^{-1}$ ). A recent study suggested that phosphorylated MR exerts a dominant negative effect,<sup>57</sup> further underscoring the biological impact of MR<sup>S843-P</sup>.

In terms of physiological significance, analysis of mouse tissues using phospho-specific antibodies revealed that MR<sup>S843-P</sup> is exclusively present in the cytoplasm of the intercalated cells and is dephosphorylated in volume depletion via angiotensin II signaling. Conversely,  $K^+$  loading increases MR<sup>S843-P</sup> in these cells. The decrease in MR<sup>S843-P</sup> in hypovolemic conditions is associated with the increase in pendrin and apical  $H^+$ -ATPase, which can be corrected by the MR antagonist spironolactone. These data indicate that volume depletion induces MR dephosphorylation, thereby activating  $Cl^-$  flux mechanisms involving intercalated cells (Figure 2).

As elegantly demonstrated by a mathematical model in the previous reports,<sup>58,59</sup> maximal  $Na-Cl$  reabsorption in the collecting duct occurs when electrolyte flux mechanisms in intercalated cells and principal cells are activated simultaneously. Under these conditions,  $K^+$  secretion is minimized as a result of electroneutral  $Na-Cl$  transport in this segment, limiting  $K^+$  secretion in the presence of high aldosterone levels. Identification of intercalated cell MR phosphorylation provides a mechanism that integrates the function of principal cells and intercalated cells, which determines the balance of  $Na^+/K^+$  exchange and  $Na-Cl$  co-transport in the collecting duct. Interestingly, we found reduced MR<sup>S843-P</sup> in a mouse model of pseudohypoaldosteronism type II, a rare Mendelian disorder featuring hypertension and hyperkalemia,<sup>25</sup> indicating that the Ser/Thr kinase WNK4 is involved in the regulation of this site. These data also suggest a possibility that the dysregulation of this site may have a role in its pathogenesis. It is likely that pseudohypoaldosteronism type II mice exhibit impaired  $K^+$  secretion partly because of the constitutive dephosphorylation of this site.



**Figure 2** Mechanism for mineralocorticoid receptor (MR) regulation in renal intercalated cells. High  $K^+$  intake increases MR phosphorylation, which prevents ligand binding and MR activation in hyperaldosteronism. Dephosphorylation of the receptor in response to angiotensin II signaling restores receptor competence, increasing  $Cl^-$  flux mechanisms involving these cells in the collecting duct. 'P' denotes phosphorylation. Adapted from ref. 25.

## FUTURE RESEARCH DIRECTIONS

Identification of a novel MR phosphorylation site raises several questions for further research. First, additional studies are required to determine the identity of the kinase that mediates MR<sup>S843-P</sup>. Given that phosphorylation at this site non-competitively inhibits ligand binding, artificial manipulation of this site may have therapeutic potential. Second, the mechanisms that control MR<sup>S843</sup> phosphorylation in a cell-selective manner are unclear. One possibility is that the responsible kinases are highly enriched in intercalated cells. Alternatively, other post-translational modifications, such as O-linked  $\beta$ -N-acetylglucosamine,<sup>60,61</sup> may have a role. Third, the role of cortisol in intercalated cells may require further investigation. Although the role of aldosterone in intercalated cells has been demonstrated, it is possible that cortisol also acts as a ligand. As suggested previously, the difference in phenotype between MR-knockout mice and aldosterone synthase-knockout mice is consistent with the role of cortisol.<sup>62</sup> Finally, the role of MR<sup>S843-P</sup> in the pathophysiology of hypertension and kidney disease needs further evaluation. Future studies are warranted to clarify these issues, which will hopefully lead to the identification of potential therapeutic targets for selective MR modulation.

## CONFLICT OF INTEREST

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