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► To cite this version:

Toshifumi Nakamura, Sophie Girerd, Frederic Jaisser, Jonatan Barrera-Chimal. Nonepithelial mineralocorticoid receptor activation as a determinant of kidney disease. *Kidney International Supplements*, 2022, 12 (1), pp.12-18. 10.1016/j.kisu.2021.11.004 . hal-03692429

HAL Id: hal-03692429

<https://u-paris.hal.science/hal-03692429>

Submitted on 22 Jul 2024

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Non-epithelial mineralocorticoid receptor activation as a determinant of kidney disease

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Word count:

Abstract: 157/250

Main text: 2611

Figures & Tables: 1 figure, 1 table

References: 63

Running header (≤ 50 characters incl spaces): Mineralocorticoid receptor in non-epithelial cells

ABSTRACT

Chronic kidney disease is a major global health challenge and mineralocorticoid receptor signaling is thought to play a role in disease progression. The classic role of the mineralocorticoid receptor is the regulation of fluid and electrolyte homeostasis via differential gene expression, and recently its role in modulating inflammation and fibrosis has been identified. In addition to expression of the mineralocorticoid receptor in renal epithelial cells, it is also found in non-epithelial cells, such as endothelial cells, vascular smooth muscle cells, podocytes, and fibroblasts. Targeting the mineralocorticoid receptor in these cells may play a role in offering protection against inflammation and fibrosis in the kidneys and the cardiovascular system. Here, data from preclinical cell-specific mineralocorticoid receptor knockout mouse models and *in vitro* studies that help uncover the role of the MR in non-epithelial cells are presented. This review also discusses a number of potential targets that offer opportunities for the targeting of mineralocorticoid receptor signaling in non-epithelial cells.

Keywords: chronic kidney disease; mineralocorticoid receptor; non-epithelial cells

INTRODUCTION

The mineralocorticoid receptor (MR), a member of the steroid hormone receptor family, acts as a ligand-dependent transcription factor.¹ It is involved in the regulation of inflammation and fibrosis and fluid and electrolyte homeostasis via differential gene expression.^{1,2} The MR mediates the physiologic activity of the adrenal steroid hormone, aldosterone,³ and it is best known for its role in the control of electrolyte and fluid balance via the MR expressed in aldosterone-sensitive kidney epithelial cells in the distal nephron that express the 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) enzyme.^{2,4} Thus, although the MR binds aldosterone and cortisol with equal affinity, the presence of 11 β -HSD2 in epithelial cells confers specificity of the MR for aldosterone.¹⁻³ However, the MR is also expressed in non-epithelial tissues including the heart, adipocytes, podocytes, inflammatory cells, endothelial cells (ECs), and vascular smooth muscle cells (VSMCs) (Figure 1), where in some cases it is unprotected by 11 β -HSD2, leading to potential activation by cortisol.^{3,4} MR overactivation, which contributes to inflammation and fibrosis, is well documented in cardiovascular (CV) disease (CVD), as discussed by Bauersachs & Lothar (Link to accompanying review article to be included) and is recognized as a potential treatment target to slow the progression of chronic kidney disease (CKD). The aims of this narrative review are to discuss the evidence regarding the role of non-epithelial MR activation as a determinant of CKD, and to review the latest evidence for MR targets that are relevant for disease management.

MODELS OF MR DEFICIENCY IN NON-EPITHELIAL CELLS AND KIDNEY DISEASE

Increasing evidence suggests that targeting the MR in non-epithelial cells plays a key role in offering protection against inflammation and fibrosis in the kidneys and the CV system.⁴ The development of cell-specific MR knockout mouse models as well as *in vitro* studies has helped elucidate the specific role of the MR in podocytes, vascular cells (ECs and VSMCs), inflammatory cells, and fibroblasts in kidney injury associated with MR overactivation, as discussed below and presented in Table 1.³⁻⁵ Of note, sodium handling is not affected in mouse models of endothelial, smooth muscle or myeloid MR deficiency.⁶⁻⁸

MR activation in podocytes

Podocytes are one of the most important cell types for overall glomerular health, and podocyte injury is considered a critical event in progression of kidney disease in patients with diabetes.⁹ MR activation in podocytes may occur by aldosterone-dependent and independent mechanisms. In 2008, Shibata *et al.* reported that Rac1 mediates the activation of the MR, given that the presence of a constitutively active form of Rac1 induced MR nuclear translocation and activation in podocytes.¹⁰ Furthermore, mice in which Rac1 activity was elevated developed podocyte lesions and severe albuminuria, which were prevented by MR antagonism with eplerenone.^{10,11} Therefore, MR overactivation in podocytes appears to induce podocyte injury. Autophagy is a process essential for podocyte maintenance and homeostasis and is disrupted in CKD in T2D.¹² Spironolactone treatment of diabetic rats increased the autophagy of podocytes *in vivo*¹³ and restored autophagy in podocytes under mechanical stress.¹⁴ Moreover, aldosterone mediates podocyte injury through NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome activation.¹⁵ Furthermore,

aldosterone addition to podocytes *in vitro*, induces the downregulation of nephrin, podocin, podoplanin, and podocalyxin while activating the Wnt/ β -catenin pathway.¹⁶

Podocyte-specific MR deletion had no effect in ameliorating antiglomerular basement membrane glomerulonephritis⁸ and it is not yet clear to what extent podocyte MRs contribute to other types of renal injury, such as CKD or CKD in T2D. The lack of effect of podocyte MR deletion in glomerulonephritis does not exclude that MR inhibition in podocytes might have beneficial effects on other forms of kidney disease such as CKD in T2D in which the pathological mechanisms leading to podocyte injury and effacement differ.¹⁷

MR knockout in the endothelium

Endothelial dysfunction is reported in patients with CKD and leads to an increased risk of CVD. It has been suggested that abnormalities in endothelial function may be a linking factor between CKD and CVD,^{18,19} which may be mediated by the endothelial MR.²⁰ In endothelial cells, aldosterone increases ICAM-1, VCAM-1, E-selectin and MCP-1 expression.^{21,22} Moreover, aldosterone promotes oxidative injury through increasing the expression of the NADPH oxidase subunits Nox2, p47(phox) p22(phox) and by stimulating Rac1 activation.^{23,24} The endothelial function is also affected by aldosterone through a reduction in eNOS phosphorylation.^{22,25}

Studies have reported that the endothelial MR has no role in ischemic acute kidney injury (AKI),²⁶ cyclosporine A (CsA) nephrotoxicity,²⁷ or renal injury induced by chronic deoxycorticosterone acetate administration.²⁸ In agreement with these observations, it was reported that endothelial MR knockout does not modify the contractile properties of the renal artery or afferent arterioles and the kidney function

and morphology remain similar between phenotypes.⁶ Similarly, MR deletion in ECs did not protect the kidney against angiotensin II-induced contraction in renal arteries, albuminuria, or kidney injury.⁶ However, endothelial MR knockout in females prevented Western diet-induced kidney artery stiffening, proteinuria, oxidative stress, reduced endothelial nitric oxide (NO) synthase (eNOS) activation, and inflammatory M1 macrophage activation.²⁹ It is possible that gender-specific effects of the endothelial MR could account for the differential effects observed. However, side-by-side studies comparing male vs. female phenotypes will confirm these gender specific changes.

In the glomerular endothelium, it was reported that aldosterone disrupts the endothelial glycocalyx through increasing glomerular matrix metalloproteinase-2 activity and reducing syndecan 4 expression, thus causing albuminuria³⁰; therefore, it is thought that aldosterone-MR targeting in glomerular ECs could account for the antiproteinuric effects of MR antagonists (MRAs) in several kidney disease conditions.

MR knockout in vascular smooth muscle cells

The MR plays a role in the regulation of vascular tone.^{26,31} Thus, the MR expressed in VSMCs makes an important contribution in situations in which kidney injury is mediated by, or is associated with, vasoconstriction. For example, in a study with MR deletion in VSMCs, the findings showed blunted kidney dysfunction and tubular injury after an ischemic AKI episode.²⁶ The protection was mediated by a reduction in Rac-1-induced reactive oxygen species production in VSMCs, which prevented a sulfenic acid modification on the endothelin-B receptor that would have led to impaired endothelin-B receptor signaling and eNOS inactivation.²⁶ This is of particular interest because MR antagonists prevent sulfenic acid modification of the endothelin-B receptor occurring in ischemic AKI, and because the protective effect of

MR inhibition against AKI is at least partially dependent on endothelin-B receptor activity.^{32,33} Similarly, in kidney injury associated with vasoconstriction due to CsA administration, MR deficiency in VSMCs prevented kidney dysfunction and tubular vacuolization and stabilized renal vascular resistance through diminishing the CsA-induced phosphorylation of contractile proteins.²⁷ MR activation in isolated smooth muscle cells promotes the activation of NADPH oxidase and increases the expression of genes involved in vascular calcification (alkaline phosphatase, parathyroid hormone receptor-2, and bone morphogenetic protein-2), vascular fibrosis (collagen I and III), vascular stiffness, (integrin alpha-5 subunit), and inflammation (IL-16, and cytotoxic T-lymphocyte antigen-4).^{7,34,35}

MR knockout in myeloid cells

It has been reported that renal injury can be reduced by targeting MR signaling in myeloid cells.⁸ In myeloid cells, aldosterone stimulates the expression of TNF- α , MCP-1, RANTES and IL-12.³⁶ In a mouse model of glomerulonephritis, myeloid-specific MR deletion prevented increases in cystatin C levels and reduced crescent numbers, tubular injury, fibrosis, and inflammatory cytokine levels to a similar extent as the protection observed from treatment with the selective, steroidal MRA eplerenone.⁸ Similarly, myeloid MR knockout protected against CKD progression after an ischemic AKI episode in the mouse model by favoring M2 repairing macrophage polarization via interleukin-4-enhanced signaling,³⁷ suggesting that the myeloid MR is important and is a target for MRAs to limit CKD progression. Continued infiltration of inflammatory cells and macrophages impact the repair process after ischemic AKI. Infiltration of M1-proinflammatory macrophages favor maladaptive repair and injury perpetuation, whereas the early switch from M1 to M2 macrophages facilitates an effective repair.^{38,39}

Indeed, infiltrating macrophages display increased M2 polarization markers in mice lacking the MR in the myeloid lineage and subjected to bilateral renal ischemia–reperfusion, and the population of proinflammatory Ly6C^{high} macrophages is smaller than that of control littermates³⁷. This effect was associated with the prevention of kidney fibrosis and the transition from AKI to CKD.³⁷ Thus, MR gene deletion in myeloid cells recapitulates the beneficial effects of MR antagonism with a target treatment such as the novel, nonsteroidal, selective MRA finerenone, which has been shown to protect against the progression from AKI to CKD in a mouse model.³⁷

MR activation in fibroblasts

Evidence suggests that increased MR activation may lead to fibroblast proliferation and the production of profibrotic molecules, thus inducing kidney fibrosis.⁴⁰ Aldosterone stimulates fibronectin synthesis in isolated renal fibroblasts.⁴¹ In addition, fibroblasts incubated with spironolactone or eplerenone have shown reduced extracellular matrix component production induced by platelet-derived growth factor or connective tissue growth factor,⁴² suggesting that MR activation in renal fibroblasts may also contribute to kidney remodeling during CKD through mechanisms similar to those reported in cardiac fibroblasts. In isolated renal fibroblasts, aldosterone also stimulates collagen I, III, and IV synthesis and promotes its proliferation.^{43,44} However, no studies in which the MR has been specifically deleted in fibroblasts have been done to evaluate its consequences on CKD or CKD in T2D progression.

MR TARGETS RELEVANT FOR KIDNEY DISEASE

Upregulation of the MR stimulates fibrosis and inflammation resulting in progression of CKD.⁴ Common polymorphisms also influence MR protein expression

and modify the phenotypes, for example, the common MR polymorphism c.-2G>C (rs2070951) affects blood pressure, renin, and aldosterone levels.⁴⁵ Therefore, it is plausible that polymorphisms might also lead to differential fibrotic and inflammatory phenotypes elicited by the MR. Given the current lack of an approved treatment that primarily targets inflammation and fibrosis, this is an area of ongoing interest, and research has focused on identifying potential targets that may be suitable in kidney disease. The latest evidence of potential interest regarding downstream targets of the MR is discussed here.

Endothelial ENaC sodium channel

Overactivation of the MR in ECs may contribute to kidney injury by modulating the expression of the epithelial sodium channel in the endothelium (EnNaC). Indeed, it has been shown that increased EnNaC expression induced by aldosterone stimulation is associated with stiff ECs and reduced NO generation.^{46,47} The decrease in NO levels after MR activation may be at least partially mediated by the effect of the MR on endothelial stiffness, through its influence on epithelial sodium channel expression in the endothelium. In addition, the action of the MR on eNOS activation may be at least partially mediated by upregulation of the EnNaC upon MR activation. Indeed, specific EnNaC deletion in the endothelium showed a similar beneficial effect to endothelial MR deletion in Western diet-induced obesity on aortic stiffness and fibrosis.⁴⁸ Similarly, EnNaC deletion prevented Western diet-induced renal artery and renal EC stiffening, effects that were linked to prevention in eNOS inactivation, reduced oxidative stress, and renal perivascular fibrosis.⁴⁹

We recently showed that endothelial EnNaC knockout ameliorates kidney lesions induced by renal ischemia and leads to faster recovery of kidney oxygenation.⁵⁰ The effect of endothelial EnNaC deletion in promoting better recovery from hypoxia

may be related to the increased generation of NO, thus preventing sustained vasoconstriction and allowing better kidney perfusion. Indeed, pharmacologic ENaC inhibition in human ECs decreased eNOS phosphorylation of threonine 495, thus activating eNOS, as shown by the increased dimer vs. monomer ratio.⁵⁰ Further studies are required to identify whether this protective effect could play a role in the prevention of the transition from AKI to CKD.

Neutrophil gelatinase-associated lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL), a small circulating protein, has shown enhanced levels in a range of pathologic conditions and has been suggested as an early biomarker of renal injury or inflammation.⁵¹ NGAL is now identified as an MR target in the CV system, which contributes to the aldosterone–MR mediated profibrotic effects, through the induction of collagen-I, galectin-3, and cardiotrophin-1.^{52,53} In particular, NGAL deficiency in inflammatory cells and especially from dendritic cells prevented CV fibrosis induced by a nephrectomy–aldosterone–salt challenge.^{54,55} In the context of CKD, it was shown that NGAL is not only a marker of the disease but also plays a causative role in kidney damage because following severe nephron reduction, mice lacking NGAL had reduced kidney lesions compared with controls.⁵⁶ Similarly, NGAL gene deletion reduced tubulointerstitial fibrosis and tubular cell death induced by endoplasmic reticulum stress in proteinuric mice.⁵⁷ In an antibody-mediated nephritis model, NGAL-deficient mice showed amelioration of histology lesions and reduced proteinuria, while exogenous NGAL administration exacerbated kidney injury, proteinuria, and mortality through the promotion of inflammation and apoptosis.⁵⁸ Furthermore, we reported recently that a newly characterized pharmacologic NGAL inhibitor prevents renal tubulointerstitial fibrosis

and reduced profibrotic marker expression in a mouse model of CKD, mimicking the benefit of NGAL gene deletion.⁵¹

Nicotinamide adenine dinucleotide phosphate oxidase

Activation of the MR in non-epithelial tissues often leads to oxidative injury. Many studies have shown that MR activation in ECs increases the activity and expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, thus promoting reactive oxygen species generation and oxidative stress-induced injury.²²⁻²⁴ Similar to ECs, one of the consequences of MR activation in VSMCs is increased oxidative stress mediated by the activation and increased expression of NADPH oxidases.^{34,59,60}

Galectin-3

One of the profibrotic molecule induced by aldosterone-MR is galectin-3. It has been demonstrated that galectin-3 pharmacologic inhibition or knockout protects against renal fibrosis and renal epithelial-to-mesenchymal transition induced by aldosterone-salt treatment.⁶¹ Therefore, galectin-3 is thought to be a key molecule that mediates the profibrotic effects of aldosterone and the MR in the kidney. Whether inhibition of galectin-3 prevents aldosterone-MR mediated CKD progression remains to be explored.

Serum and glucocorticoid-induced kinase 1

Serum and glucocorticoid-induced kinase 1 (SGK-1) stimulates aldosterone-dependent sodium resorption and modulates blood pressure. A transgenic mouse model with overexpression of SGK-1 showed exacerbated albuminuria, glomerular hypertrophy, and renal fibrosis associated with altered fibrotic and inflammatory molecules.⁶² Findings suggest that enhanced SGK-1 activity is a risk factor for the development of mineralocorticoid-dependent kidney injury and the authors concluded that SGK-1 may offer a therapeutic target to delay CKD development.

CONCLUSIONS

CKD is a major global health challenge and the evidence presented here highlights the central role of MR signaling in disease progression, providing insights into the role of the MR in non-epithelial cells in the pathogenesis of CKD. Data from preclinical MR knockout models in non-epithelial cells, including podocytes, ECs, VSMCs, and myeloid cells, highlight the opportunities for targeting the effects of MR signaling as a treatment option. There is growing interest in the use of MRAs for the management of patients with CKD and the preclinical and clinical evidence to date is discussed in accompanying articles by Fodo & Luther (**Citation for accompanying review article to be included when available**) and Rossing (**Citation for accompanying review article to be included when available**). In summary, the literature shows that this is an active area of research and the identification of a number of potential targets shows promise for targeting the MR signaling in non-epithelial cells.

DISCLOSURES

Publication of this article was supported by an educational grant from Bayer AG.

TN has nothing to disclose.

SG has nothing to disclose.

FJ received research grants from AstraZeneca and Bayer SAS and honoraria from Bayer SAS, and KBP Biosciences.

JB-C has nothing to disclose.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Jo Luscombe, PhD, of Chameleon Communications International, who provided editorial assistance with funding via an educational grant from Bayer AG. The authors would also like to acknowledge Alexander Roeder, Ronny Guenther, Katja Marx, and Josephin Schoenrich, of CAST PHARMA, who designed the figures, with funding from Bayer AG. This work was supported by the Fight-HF Avenir Investment Program (ANR-15-RHUS-0004), the Fondation de Recherche sur l'Hypertension Artérielle (REIN/NgalPA - 2017/2018) and the ANR NGAL-HT (ANR-19-CE14-0013). TN was supported by a fellowship from the Uehara Memorial Foundation.

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TABLE

Table 1. Overview of mediators of cellular injury in non-epithelial cells: Evidence from preclinical models of MR deficiency

Non-epithelial cell type	Mediator of cellular injury	Role of mediator	Impact of mediator	Reference
Podocytes				
	Rac1	• Involved in the activation and modulation of MR activity	• Elevated Rac1 activity leads to development of podocyte lesions and severe albuminuria	Shibata <i>et al.</i> 2008 ¹⁰
	NLRP3 inflammasome	• Mediates podocyte injury induced by aldosterone	• Activation of the NLRP3 inflammasome promotes aldosterone-induced podocyte apoptosis	Bai <i>et al.</i> 2017 ¹⁵
Endothelial cells				
	MMP-2	• Cleaves syndecan-4 from the endothelial cell surface	• A damaged endothelial glycocalyx can lead to glomerular albumin leakage	Butler <i>et al.</i> 2019 ³⁰
Vascular smooth muscle cells				

Rac1	<ul style="list-style-type: none"> • Induces production of reactive oxygen species 	<ul style="list-style-type: none"> • Induces sulfenic acid modification on the endothelin-B receptor preventing the ability to stimulate eNOS and NO production 	Barrera-Chimal <i>et al.</i> 2017 ²⁶
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Inflammatory cells

MR inhibition of IL-4 receptor signaling	<ul style="list-style-type: none"> • Plays a role in macrophage polarization 	<ul style="list-style-type: none"> • MR activation decreases IL-4 receptor signaling, resulting in enhanced macrophage polarization to an M1 (pro-inflammatory macrophage) phenotype 	Barrera-Chimal <i>et al.</i> 2018 ³⁷ Barrera-Chimal <i>et al.</i> 2019 ⁴⁰
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Fibroblasts

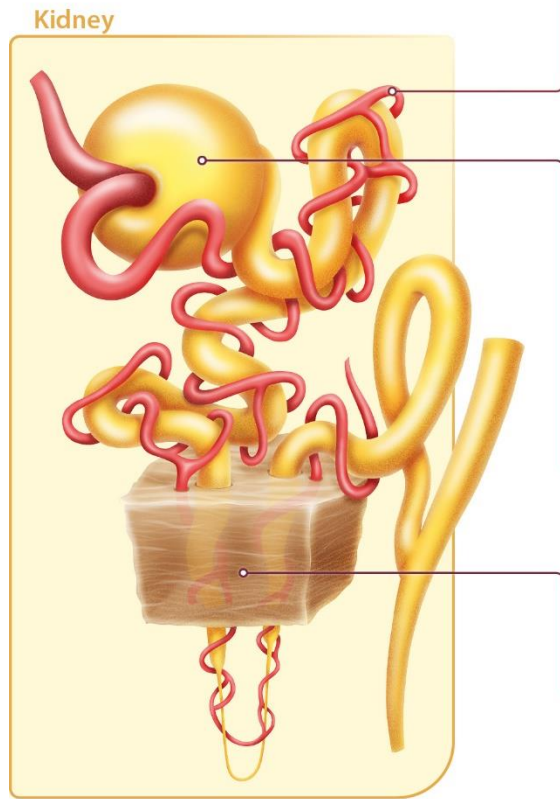
Fibronectin	<ul style="list-style-type: none"> • Involved in chronic cellular activation and tissue remodeling 	<ul style="list-style-type: none"> • Aldosterone stimulates fibronectin synthesis, which can lead to fibrosis 	Chen 2013 ⁴¹ Bowers <i>et al.</i> 2019 ⁶³
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eNOS, endothelial nitric oxide synthase; IL-4, interleukin-4; MMP-2, matrix metalloproteinase-2; MR, mineralocorticoid receptor;

NLRP3, NOD-like receptor family, pyrin domain containing 3; NO, nitric oxide; Rac1, Ras-related C3 botulinum toxin substrate 1.

FIGURE

Figure 1. Potential mediators of cellular injury following MR overactivation in non-epithelial cells leading to vascular dysfunction, inflammation and fibrosis.



Vasculature



Vascular smooth muscle cell MR
 Rac1, Nox2, Itga5, calcium channel, calcification genes, SGK-1



Endothelial cell MR
 MMP-2, Leukocyte adhesion: ICAM-1, VCAM-1, E-selectin, EnNaC, eNOS, Nox4, SGK-1



Fibroblast MR
 Fibronectin, Nox2, CTGF, galectin-3, SGK-1



Myeloid cell MR
 Macrophage polarization

Glomerulus



Podocyte MR
 Rac1, NLRP3 inflammasome

Interstitium



Fibroblast MR
 Fibronectin, Nox2, CTGF, galectin-3, SGK-1



Myeloid cell MR
 IL-4 signaling inhibition, macrophage polarization, NGAL, CCL5

CCL5, chemokine (C-C motif) ligand 5; CTGF, connective tissue growth factor; ENaC, endothelial epithelial sodium channel; eNOS, endothelial nitric oxide synthase; ICAM-1, intercellular adhesion molecule 1; Itg α 5, Integrin subunit alpha 5; MMP-2, matrix metalloproteinase-2; MR, mineralocorticoid receptor; NGAL, neutrophil gelatinase-associated lipocalin; NLRP3, NOD-like receptor family, pyrin domain containing 3; NOX, NADPH oxidase; Rac1, Rac Family Small GTPase 1; SGK-1, serum and glucocorticoid-regulated kinase 1; VCAM-1, vascular cell adhesion molecule 1.