Deoxycorticosterone Acetate/Salt–Induced Cardiac But Not Renal Injury Is Mediated By Endothelial Mineralocorticoid Receptors Independently From Blood Pressure

Achim Lother, David Fürst, Stella Bergemann, Ralf Gilsbach, Florian Grahammer, Tobias B. Huber, Ingo Hilgendorf, Christoph Bode, Martin Moser, Lutz Hein

Abstract—Chronic kidney disease has a tremendously increasing prevalence and requires novel therapeutic approaches. Mineralocorticoid receptor (MR) antagonists have proven highly beneficial in the therapy of cardiac disease. The cellular and molecular events leading to cardiac inflammation and remodeling are proposed to be similar to those mediating renal injury. Thus, this study was designed to evaluate and directly compare the effect of MR deletion in endothelial cells on cardiac and renal injury in a model of deoxycorticosterone acetate–induced hypertension. Endothelial MR deletion ameliorated deoxycorticosterone acetate/salt–induced cardiac remodeling. This was associated with a reduced expression of the vascular cell adhesion molecule Vcam1 in MR-deficient cardiac endothelial cells. Ambulatory blood pressure telemetry revealed that the protective effect of MR deletion was independent from blood pressure. Similar to the heart, deoxycorticosterone acetate/salt–induced severe renal injury, including inflammation, fibrosis, glomerular injury, and proteinuria. However, no differences in renal injury were observed between genotypes. In conclusion, MR deletion from endothelial cells ameliorated deoxycorticosterone acetate/salt–induced cardiac inflammation and remodeling independently from alterations in blood pressure but it did not affect renal injury. These findings suggest that the anti-inflammatory mechanism mediating organ protection after endothelial cell MR deletion is specific for the heart versus the kidney. (Hypertension. 2016;67:130-138. DOI: 10.1161/HYPERTENSIONAHA.115.06530.)

Key Words: aldosterone ■ endothelial cells ■ heart failure ■ hypertension ■ kidney diseases ■ mineralocorticoid receptor

Chronic kidney disease is a growing problem in industrialized countries with high incidence and prevalence.1 Hypertension is one of the major risk factors for the development of chronic kidney disease.1 The classical action of aldosterone and the mineralocorticoid receptor (MR) in the kidney is the tubular regulation of sodium reabsorption and potassium secretion. However, the MR is not only expressed in kidney epithelial cells but also in other cell types within and outside the kidney, such as podocytes,2 renal endothelial cells,3–5 vascular smooth muscle cells,6 immune cells,2,7,8 the brain,9 or the heart.10,11

During the past 2 decades, it has been recognized that activation of the MR during disease may become maladaptive and then can directly mediate end organ damage.12 In the heart, MR activation mediates cardiac hypertrophy, fibrosis, and left ventricular dysfunction.12 The usage of mineralocorticoid receptor antagonists (MRAs) has proven highly beneficial during cardiac disease.13,14 In animal models of hypertensive nephropathy, the MRA spironolactone reduced renal injury independently from blood pressure.15–17 MRAs might also be useful to prevent end organ damage in humans with chronic kidney disease.18,19 However, the use of MRAs is considerably limited by the potentially life-threatening risk of hyperkalemia among this patient population.18,19 Therefore, it is highly desirable to understand the mechanisms driving the beneficial effect of MRAs during kidney disease to enable the development of novel selective MRAs that do not affect renal tubular function.

The hallmarks of aldosterone’s detrimental effects on the kidney are inflammation, fibrosis, glomerular injury, and proteinuria.16,20–22 There is some evidence that aldosterone-induced end organ damage in the heart and the kidney is based on similar mechanisms, particularly on an inflammatory response mediated by MR activation.12,23
cytokines and the expression of adhesive molecules in endothelial cells are major regulators of immune cell attraction and activation within many tissues. It has recently been reported that MR deletion from endothelial cells prevented macrophage infiltration of the heart and cardiac fibrosis in a mouse model of hypertension induced by the MR agonist deoxycorticosterone acetate (DOCA) in the presence of high salt. We hypothesized that this finding could be translated into chronic kidney disease treatment. Thus, the aim of this study was to directly compare the effect of endothelial MR deletion on hypertension and cardiac versus renal injury in response to DOCA/salt treatment.

**Methods**

**Generation of MR Mutant Mice**

Deletions of the MR gene from endothelial cells (MR^{Cdh5Cre}) was achieved using a tamoxifen-inducible Cre recombinase under control of the cadherin 5 gene promoter (Cdh5Cre-ERT2) and mice carrying a conditional MR allele (MR^{Cre}). Cre-negative (MR^{lox/lox}) litters were used as wild-type controls. For cell isolation experiments, mice were crossbred with reporter mice expressing the green fluorescent protein depending on Cre activity (MR^{lox/lox}mTmG^{Cdh5Cre} or MR^{lox/lox}mTmG^{Cdh5Cre}), obtained from Jackson laboratories.

All mice were treated with 2-mg tamoxifen (20 mg/mL in sunflower oil and 10% ethanol; Sigma, Steinheim, Germany) intraperitoneally per day on 5 consecutive days at least 4 weeks before any further experiment. All animal procedures were approved by the responsible animal care committees (Regierungspaedium Freiburg, Germany) and they conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (2011).

**Isolation of Endothelial Cells and Macrophages**

Endothelial cells from MR^{lox/lox}mTmG^{Cdh5Cre} or MR^{lox/lox}mTmG^{Cdh5Cre} mice were isolated by fluorescence-activated cell sorter after enzymatic dissection. After removal of the capsule kidneys were minced and incubated in a buffer containing NaCl, 135 mmol/L; KCl, 4 mmol/L; MgCl2, 1 mmol/L; HEPES, 2.5 mmol/L; Ca2+, 50 μmol/L; collagenase B (Roche, 1 mg/mL); hyaluronidase (Sigma, 1 mg/mL); and trypsin (Sigma, 33 μg/mL) at 37°C for 30 minutes. Nuclei were labeled by incubation with Draq5 fluorescent dye (New England Biolabs) for 5 minutes at room temperature. Green fluorescent protein–positive cells (Figure 1A) were isolated from the single-cell suspension using a Biorad S3 cell sorter. Cardiac endothelial cells were isolated from single-cell suspensions using anti-CD31 magnetic beads (Miltenyi Biotech) according to the manufacturer’s instructions.

For macrophage isolation, peritoneal lavage was stained with the following antibodies (Biolegend) for flow-assisted cell sorting (BD fluorodeoxyuridine-activated cell sorter Aria II): anti-CD45.2 (clone 104), anti-CD19 (clone 1D3), anti-CD11b (clone M1/70), and anti-F4/80 (clone BM8). Peritoneal macrophages were identified as CD45.2+CD19negCD11b+ F4/80+ cells.

**DOCA/Salt Model and Hemodynamic Measurements**

Male MR^{Cdh5Cre} and MR^{lox/lox} mice underwent unilateral nephrectomy at 12 weeks of age and then received DOCA from subcutaneous pellets (DOCA, 2.5 mg/d; Innovative Research of America) and 1% NaCl with drinking water for 6 weeks as described previously (Figure 1F). Untreated mice served as controls.

Blood pressure was determined invasively by telemetry after implantation of pressure transmitters TA11PA-C10 (Data Sciences International) into the left carotid artery as previously described at 2 and 6 weeks after initiation of DOCA treatment.

**Urine and Serum Measurements**

Spot urine and blood samples were obtained after 6 weeks of DOCA treatment. Urinary and serum albumin and creatinine were measured using a fluorometric albumin test kit (Progen, PR2005) and enzymatic colorimetric creatinine kits (LT-SYS, Lehmann) after the manufacturer’s instructions.

**Histology and Immunohistochemistry**

Tissues were fixed by immersion in 4% paraformaldehyde in phosphate-buffered saline and embedded in paraffin. Cardiac and renal interstitial fibrosis was determined from Sirius red-stained cardiac or kidney cross-sections. Glomerular injury was determined from kidney cross-sections after periodic acid Schiff staining using a semiquantitative score (0, no sclerosis; 1, mild segmental sclerosis; 2, moderate segmental sclerosis; 3, severe segmental sclerosis; and 4, global sclerosis) as previously described.

Macrophage infiltration of the kidney was derived from immunohistochemistry using an anti-F4/80 antibody (rat antimouse; 1:50, No. 16911, Abcam) and peroxidase staining. F4/80+ cells were quantified per glomerulum or per field as previously described using 40-fold magnification.

**Gene Expression Analysis**

Total RNA was prepared from isolated cells or tissue samples with the RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions. Quantitative real-time polymerase chain reaction was carried out using a MX3000p qPCR system (Agilent) and gene expression was normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase (Gapdh) by the ΔΔCt method. Primer sequences are given in Table S1 in the online-only Data Supplement.

**Statistics**

All results are presented as mean±SEM. Data were analyzed by 2-way ANOVA and Bonferroni post-tests for multivariant comparison or Student’s t test, respectively, using GraphPad Prism 4.3 (GraphPad Software).

**Results**

**Generation and Validation of Endothelial Cell MR-Deficient Mice**

Selective deletion of the MR gene in endothelial cells was achieved using a tamoxifen-inducible Cre/IoxP system under control of the cadherin 5 promoter (Figure 1A). Renal endothelial cells were isolated from MR^{lox/lox} and MR^{Cdh5Cre} kidneys by fluorescence-activated cell sorter (Figure 1B–1C). In isolated renal endothelial cells, MR mRNA expression was reduced by >80% in MR^{lox/lox} compared with MR^{Cdh5Cre} mice (n=3 per group, P<0.01; Figure 1D). Matching results were obtained for isolated cardiac endothelial cells (Figure S1), which is comparable with previous studies using a similar model. In contrast, MR mRNA expression in total kidney lysates (Figure 1E) was similar in both genotypes.

mRNA expression of MR and M1/M2 activation marker genes in peritoneal macrophages was determined to exclude unspecific effects of the Cdh5-controlled MR deletion on macrophages and proved to be similar in both, MR^{Cdh5Cre} and MR^{lox/lox} mice (Figure S2).

**DOCA/Salt-Induced Hypertension**

Blood pressure was determined by telemetric blood pressure recording in untreated MR^{lox/lox} and MR^{Cdh5Cre} mice and during 6 weeks after unilateral nephrectomy and treatment with DOCA/salt (Figure 1F and 2). Circadian rhythm was present
in all groups (Figure 2A–2C). In CTRL mice, no differences in blood pressure were detected between both genotypes (n=3 per group; Figure 2D–2F). After 2 weeks of DOCA treatment, blood pressure (Figure 2D–2F) was increased to a similar extent in MR wild-type (mean 129.9±8.5 versus CTRL 97.8±3.9 mm Hg; P<0.01) and MR Cdh5Cre mice (mean 133.8±14.6 versus CTRL 104.1±5.7 mm Hg; P<0.01, n=3–5 per group, Figure 2D–2F). After 6 weeks of DOCA treatment, blood pressure further increased without differences between both genotypes (Figure 2D–2F).

Cardiac Remodeling After DOCA/Salt Treatment

After 6 weeks, cardiac hypertrophy induced by DOCA/salt treatment was significantly reduced in MR Cdh5Cre when compared with MR wild-type mice (Figure 3A; Table S2). MR-deficient mice were protected from an increase in lung weight as a marker of pulmonary congestion after DOCA/salt treatment (MR wild-type 203.3±13.6 versus CTRL 173.0±7.5 mg, P<0.05, MR Cdh5Cre 174.3±4.0 versus CTRL 160.4±3.8 mg, n.s.; Table S2).

Interstitial fibrosis and collagen 1α1 mRNA expression were significantly increased in hearts from MR wild-type but not MR Cdh5Cre mice (Figure 3B–3C). This was associated with an upregulation of vascular cell adhesion molecule 1 (Vcam1) mRNA expression after DOCA/salt treatment in MR wild-type but not in MR-deficient endothelial cells (Figure 3D).

DOCA/Salt Leads to Glomerular Injury and Proteinuria

After unilateral nephrectomy and treatment with DOCA/salt, the organ weight of the remaining kidney was increased significantly in MR wild-type (447.4±19.1 versus CTRL 198.7±13.3 mg; P<0.001) and MR Cdh5Cre mice (461.1±19.2 versus CTRL 198.8±5.0 mg; P<0.001) without differences.
between both genotypes (n=10–13 per group; Figure 4A; Table S2). At baseline, urinary protein excretion was similar in MR<sup>Cdh5Cre</sup> and MR<sup>wild-type</sup> mice (Figure 4B). DOCA/salt treatment lead to a marked albuminuria in both the genotypes (Figure 4B; n=5–6 per group). Histological analysis revealed severe kidney damage with glomerular sclerosis (Figure 4C). Glomerular injury as determined by scoring increased by 1.75 points in MR<sup>wild-type</sup> (n=5–7; P<0.001) or 1.72 points in MR<sup>Cdh5Cre</sup> mice (n=5–8; P<0.001; Figure 4D), respectively.

Renal Inflammation and Fibrosis After DOCA/Salt Treatment

Renal interstitial fibrosis as determined from Sirius red-stained kidney sections was increased in MR<sup>wild-type</sup> (2.4±0.2 versus CTRL 0.1±0.1; P<0.001) and MR<sup>Cdh5Cre</sup> mice (2.3±0.2 versus CTRL 0.1±0.1; P<0.001) without differences between both genotypes (n=7–9 per group; Figure 5A–5B). Similar results were obtained for mRNA expression of the collagen 3a1 gene (Col3a1, Figure 5C) as a fibrosis marker gene.

DOCA/salt treatment led to an accumulation of macrophages in the kidneys of MR<sup>wild-type</sup> (14.0±2.0 versus CTRL 4.8±0.9 per field; n=6–8 per group; P<0.01) and MR<sup>Cdh5Cre</sup> mice (11.4±1.6 versus CTRL 2.9±0.8%; n=6–8 per group; P<0.01; Figure 5D). Similar results were obtained for expression of the macrophage marker gene F4/80 in total kidney lysates (Figure 5E). mRNA expression of the proinflammatory vascular (Vcam1, Figure 5F) in kidneys from MR<sup>Cdh5Cre</sup> and MR<sup>wild-type</sup> mice increased after DOCA/salt treatment to similar levels in both the genotypes.

Discussion

The main findings of this study are (1) that MR deletion from endothelial cells ameliorates DOCA/salt-induced cardiac...
injury while (2) hypertension and (3) glomerular injury, proteinuria, and renal fibrosis in response to DOCA/salt are independent from endothelial MR expression. These findings suggest that different mechanisms are involved in the protective effect of MR antagonism in heart versus kidney diseases.

**Blood Pressure Regulation**

Aldosterone increases blood pressure by altering fluid and sodium balance via activation of the MR in renal epithelial cells, leading to enhanced sodium reabsorption. However, during the past years it became more and more clear that aldosterone also acts directly on the vasculature...
and vascular MR might as well be involved in blood pressure regulation.

In our model, MR deletion from endothelial cells did not affect blood pressure at baseline or in response to DOCA/salt. This is in line with the results from previously published experiments when hypertension induced by DOCA, aldosterone, angiotensin II, or L-NAME was independent from endothelial MR deletion. In contrast, overexpression of the MR in endothelial cells lead to a moderate increase in blood pressure in untreated mice. We conclude that MR expression in endothelial cells at a physiological level does not significantly contribute to blood pressure regulation. However, when MR expression is upregulated during disease, it might become more relevant for endothelial effects on blood pressure as demonstrated by experimental MR overexpression.

Which other cell types or mechanisms might be involved in the aldosterone effect on vascular reactivity and blood pressure? MR in vascular smooth muscle cells aggravates the vascular response to angiotensin II and the development of hypertension in aged mice. MR deletion from vascular smooth muscle cells ameliorates arterial stiffening and vascular remodeling after aldosterone treatment. Interestingly, after MR deletion from macrophages contradictory findings including aggravation or prevention of hypertensive responses were reported. In addition, MR in the central nervous system controls salt appetite and neurohumoral activity and can thus regulate blood pressure at baseline and during disease. Finally, aldosterone exerts part of its effects on the vasculature rapidly via the G protein–coupled estrogen receptor independently of the MR. However, there are contradictory findings with respect to G protein–coupled estrogen receptor activation mediating vasoconstriction or vasodilatation and its role in aldosterone-induced hypertension needs to be defined.

MR Deletion From Endothelial Cells Ameliorates Cardiac Remodeling

Endothelial cell MR deletion ameliorates cardiac remodeling and pulmonary congestion in response to DOCA/salt. Importantly, this protective effect is independent from
differences in blood pressure. DOCA/salt increased the expression of vascular cell adhesion molecule Vcam1 in wild-type endothelial cells, which was fully prevented by MR deletion. Aldosterone or DOCA treatment has been associated with an increased expression of adhesion molecules in the heart.\textsuperscript{47,48} Previously, we now demonstrate that is directly mediated via MR in endothelial cells. Vcam1 is central for leukocyte adhesion and recruitment of blood monocytes to the heart,\textsuperscript{47,48} which subsequently promote inflammation and contribute to detrimental cardiac remodeling.\textsuperscript{49} MR deletion from monocytes/macrophages potently inhibits cardiac inflammation and remodeling in response to DOCA/salt treatment.\textsuperscript{28} Pressure overload\textsuperscript{50} or L-NAME, and angiotensin II treatment.\textsuperscript{7} We show here that the prevention of vascular inflammation exerts a similar beneficial effect on the heart.

Our results confirm the finding from a published study of endothelial MR deletion using a Tie2-driven Cre recombinase.\textsuperscript{5} It had been criticized\textsuperscript{51} that the Tie2-Cre used for that and other studies\textsuperscript{52} leads to recombination in macrophages as well,\textsuperscript{5,52} which makes it difficult to determine endothelial- from macrophage-specific effects. LysM-Cre mediated MR deletion prevents macrophage infiltration of the heart and triggered a switch toward the protective M2 phenotype in MR-deficient macrophages.\textsuperscript{7} We did not observe any differences in MR or M1/M2 marker gene expression in macrophages from MR\textsuperscript{53} mice, which underlines that MR deletion was specific for endothelial cells in our model.

**DOCA/Salt-Induced Kidney Injury is Independent From Endothelial MR**

The mechanisms mediating the organ damage after aldosterone treatment have been assumed to be similar in the heart and the kidney.\textsuperscript{53} MR activation leads to an upregulation of proinflammatory cytokines and profibrotic proteins, such as transforming growth factor $\beta$ or plasminogen activator inhibitor 1.\textsuperscript{12} Similar to the heart MR deletion from macrophages using an LysM-Cre mouse model prevents glomerular injury and renal fibrosis and reduces proteinuria in a mouse model of antilglomerular basement membrane glomerulonephritis.\textsuperscript{5} MR-deficient mice show less expression of proinflammatory and profibrotic proteins and macrophage accumulation in the kidney is markedly reduced.\textsuperscript{2} Pharmacological\textsuperscript{54} or genetic\textsuperscript{53,56} inhibition of the inflammatory response ameliorates organ damage during DOCA/salt treatment, too. Although MR activation in macrophages is clearly detrimental,\textsuperscript{2} it remains uncertain whether it is the initial trigger or a secondary event aggravating renal injury. Other LysM-positive myeloid cells such as neutrophils also express MR\textsuperscript{1} and might be involved in the protective effect of MR deletion when interacting with macrophages or endothelial cells. Renal endothelial cells produce vasoactive substances such as nitric oxide or endothelin-1 and act as modulators of the immune system via the expression of cytokines or adhesion molecules.\textsuperscript{57} In this study, unilateral nephrectomy and treatment with DOCA/salt induced renal organ damage, including inflammation, renal fibrosis, glomerular injury, and proteinuria as expected.\textsuperscript{16,20,30,54} However, this was independent from endothelial MR deletion.

**Renal versus Cardiovascular inflammatory Response to MR Activation**

Despite considerable overlap, there seems to be some significant difference between MR effects in renal versus cardiac endothelial cells. Macrophage infiltration of the kidney after DOCA/salt treatment in MR\textsuperscript{Cdh5Cre} was similar to MR\textsuperscript{tubCre} mice. In contrast to the heart, MR ablation from kidney endothelial cells did not alter the expression of Vcam1 in the kidney. In a previous study, spironolactone or blood pressure normalization both improved renal injury in response to DOCA/salt treatment.\textsuperscript{16} Interestingly, blood pressure normalization but not MR inhibition prevented the upregulation of Vcam1 in that study,\textsuperscript{16} suggesting that mechanical force rather than MR activation induces Vcam1 expression in the kidney. In fact renal endothelial cells are specialized cells that show some distinct features, for example, glomerular endothelial cells form a discontinuous layer to allow filtration,\textsuperscript{58} and several differences in the expression of surface antigens between vascular and glomerular endothelial cells that might influence leukocyte adhesion and transmigration have been described.\textsuperscript{59}

**Cellular Basis of Kidney Fibrosis**

Human kidney biopsies from patients with proteinuria showed accumulation of macrophages and increased expression of proinflammatory cytokines, such as interleukin 6 or transforming growth factor $\beta$.\textsuperscript{59} Of note, MR expression correlated with inflammation in these biopsies but was predominantly enhanced in vascular smooth muscle and not in endothelial cells.\textsuperscript{55} It has been discussed whether tubulointerstitial fibroblasts, glomerular mesangial cells, or podocytes might be the initiators of aldosterone-induced renal injury.\textsuperscript{60} Anyhow, specific MR deletion from podocytes did not prove to be beneficial\textsuperscript{2} and this questions needs to be solved.

We hypothesized that endothelial cells could directly contribute to kidney fibrosis induced by aldosterone. Yet, fibrosis and expression of fibrosis marker genes is not altered by MR deletion from endothelial cells. Beside the proinflammatory role for MR in macrophages discussed above, other cell types might be involved in the development of renal fibrosis. Aldosterone has been shown to induce epithelial-to-mesenchymal transition of renal proximal tubular cells in vitro\textsuperscript{61} and in vivo.\textsuperscript{62} Another source of renal fibrosis are FOXD1-lineage pericytes, which can transform into myofibroblasts.\textsuperscript{63} Both cell types are unique to the kidney versus the cardiovascular system and might, therefore, represent an alternative mechanism of aldosterone mediated end organ damage that is independent from endothelial cells.

**Perspectives**

In conclusion, MR deletion from endothelial cells ameliorated DOCA/salt-induced cardiac but not renal injury. The protective effect on the heart is independent from alterations in blood pressure. These findings suggest that endothelial MR activation mediates diverse cellular and molecular mechanisms in DOCA/salt-induced cardiac versus renal injury.

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Disclosures

None.

References


Endothelial Mineralocorticoid Receptors

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Novelty and Significance

What is New?

- This is the first study that directly compares the effect of endothelial mineralocorticoid receptor deletion on cardiac versus renal injury in response to deoxycorticosterone acetate/salt treatment.
- Cardiac but not renal injury was ameliorated by endothelial mineralocorticoid receptor deletion independent from alterations in blood pressure.

What is Relevant?

- We provide evidence that the beneficial effect on cardiac remodeling is specifically mediated by mineralocorticoid receptor deletion from endothelial cells.

- We identified Vcam1 as a molecular marker that is specific for the beneficial effect of endothelial mineralocorticoid receptor deletion on cardiac versus renal remodeling.

Summary

This study shows that mineralocorticoid receptor deletion from endothelial cells protects against deoxycorticosterone acetate/salt-induced cardiac injury but not hypertension and kidney injury.
Deoxycorticosterone Acetate/Salt–Induced Cardiac But Not Renal Injury Is Mediated By Endothelial Mineralocorticoid Receptors Independently From Blood Pressure

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SUPPLEMENTAL MATERIAL

DOCA/SALT-INDUCED CARDIAC BUT NOT RENAL INJURY IS MEDIATED BY ENDOTHELIAL MINERALOCORTICOID RECEPTORS INDEPENDENTLY FROM BLOOD PRESSURE

Short title: Endothelial mineralocorticoid receptors

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Supplemental table S1: Primer sequences

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**Supplemental table S2:** Body weight and organ weights in MR\textsuperscript{wildtype} and MR\textsuperscript{Cdh5Cre} mice

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<td>Kidney weight (mg)</td>
<td>198.2 ± 13.3</td>
<td>198.8 ± 5.0</td>
<td>447.4 ± 19.1†</td>
<td>461.1 ± 19.2†</td>
</tr>
<tr>
<td>Kidney / body weight (mg/g)</td>
<td>6.6 ± 0.3</td>
<td>6.8 ± 0.1</td>
<td>15.5 ± 0.7‡</td>
<td>15.9 ± 0.6‡</td>
</tr>
<tr>
<td>Kidney weight / tibia length (mg/mm)</td>
<td>10.3 ± 0.6</td>
<td>10.5 ± 0.3</td>
<td>23.3 ± 1.0‡</td>
<td>23.7 ± 0.9‡</td>
</tr>
</tbody>
</table>

*P<0.05, †P<0.01, ‡P<0.001 vs. CTRL; §P<0.05 vs. MR\textsuperscript{wildtype}. 
Supplemental figures:

Supplemental figure S1:

Supplemental figure S1: MR expression in cardiac endothelial cells. MR mRNA expression as determined by qRT-PCR from isolated cardiac endothelial cells.

*P<0.05, n = 5 per group. Means ± SEM.
Supplemental figure S2: Macrophage characterization. Peritoneal macrophages from MR<sup>野生型</sup> and MR<sup>Cdh5Cre</sup> were identified as CD45.2+ CD19neg CD11b+ F4/80+ cells and isolated from peritoneal lavage (A). Macrophage MR (B) and M1/M2 marker gene mRNA expression (C) as derived from qRT-PCR. n = 3-4 per group. Means ± SEM.