Arterial Stiffness

Smooth Muscle Cell Mineralocorticoid Receptors Are Mandatory for Aldosterone–Salt to Induce Vascular Stiffness

Guillaume Galmiche, Anne Pizard,* Alexandre Gueret,* Soumaya El Moghrabi, Antoine Ouvrard-Pascaud, Stefan Berger, Pascal Challande, Iris Z. Jaffe, Carlos Labat, Patrick Lacolley, Frédéric Jaisser

See Editorial Commentary, pp 442–443

Abstract—Arterial stiffness is recognized as a risk factor for many cardiovascular diseases. Aldosterone via its binding to and activation of the mineralocorticoid receptors (MRs) is a main regulator of blood pressure by controlling renal sodium reabsorption. Although both clinical and experimental data indicate that MR activation by aldosterone is involved in arterial stiffening, the molecular mechanism is not known. In addition to the kidney, MR is expressed in both endothelial and vascular smooth muscle cells (VSMCs), but the specific contribution of the VSMC MR to aldosterone-induced vascular stiffness remains to be explored. To address this question, we generated a mouse model with conditional inactivation of the MR in VSMC (MR\(^{SMKO}\)). MR\(^{SMKO}\) mice show no alteration in renal sodium handling or vascular structure, but they have decreased blood pressure when compared with control littermate mice. In vivo at baseline, large vessels of mutant mice presented with normal elastic properties, whereas carotids displayed a smaller diameter when compared with those of the control group. As expected after aldosterone/salt challenge, the arterial stiffness increased in control mice; however, it remained unchanged in MR\(^{SMKO}\) mice, without significant modification in vascular collagen/elastin ratio. Instead, we found that the fibronectin/\(\alpha_5\)-subunit integrin ratio is profoundly altered in MR\(^{SMKO}\) mice because the induction of \(\alpha_5\) expression by aldosterone/salt challenge is prevented in mice lacking VSMC MR. Altogether, our data reveal in the aldosterone/salt hypertension model that MR activation specifically in VSMC leads to the arterial stiffening by modulation of cell-matrix attachment proteins independent of major vascular structural changes. (Hypertension. 2014;63:520-526.) • Online Data Supplement

Key Words: aldosterone ● carotid arteries ● integrins ● mice, transgenic ● receptors, mineralocorticoid ● vascular stiffness

Arterial stiffness has emerged as an important marker of cardiovascular risk not only in patients with cardiovascular diseases, such as hypertension or diabetes mellitus, but also in patients with chronic kidney diseases where it contributes to the increased cardiovascular morbidity and mortality. The process of aging is also associated with increased arterial stiffness. Indeed, blood pressure (BP) involves 2 components: (1) the mean arterial pressure (MAP), a steady state component dependent on cardiac output and vascular resistance; and (2) the pulse pressure (PP), a pulsatile component dependent on arterial stiffness and pulse wave reflections. Therefore, arteries are permanently exposed to both a basal stretch (related to MAP) and a pulsatile stretch (related to PP). PP is now recognized as a major determinant of long-term outcomes. PP and cyclic stretch also contribute to atherosclerosis and coronary artery disease by modulating infiltration of the arterial wall with lipids and inflammatory cells. Effects of mechanical strain on vascular smooth muscle cells (VSMCs) migration and proliferation and modulation of superoxide anion production by the endothelium have also been proposed to contribute to cardiovascular disease progression. Thus, it has been proposed that therapies aimed at destiffening the arteries might improve cardiovascular outcomes.

The renin–angiotensin–aldosterone system plays an important role in vascular stiffening. Specifically, the mineralocorticoid hormone aldosterone has been found to contribute to arterial stiffness both in human patients and in experimental

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models. Aldosterone is made by the adrenal gland and is known to function by binding to the mineralocorticoid receptor (MR) in the kidney to regulate BP. Several studies indicated that patients with primary aldosteronism develop increased arterial wall stiffness when compared with patients with the same degree of essential hypertension supporting a BP-independent mechanism. Increased aldosterone plasma levels were associated with increased arterial stiffness that is prevented by adrenalectomy in patients with aldosterone-producing adenoma. Circulating aldosterone is also associated to aortic stiffening in normotensive overweight and obese adults. MR antagonists, such as eplerenone or spironolactone, prevent the development of vascular stiffness in several experimental models, including salt-induced vascular stiffness in diabetic rats, the classical aldosterone–salt hypertensive model, and in postischemic heart failure. The underlying mechanisms of the deleterious effects of aldosterone and the beneficial effects of MR blockers on vascular stiffness remain unclear and whether they are because of direct vascular aldosterone/MR signaling or secondary to altered salt handling needs to be explored.

A primary role of for aldosterone/MR in the vasculature has been proposed because MR is expressed in both the endothelium and medial smooth muscle cells, and genetic alteration of MR expression in these cell types modulates vascular function in mouse models. McCurley et al recently demonstrated a key role of smooth muscle cell MR in the control of the vasoactive property of resistance arteries with long-term effects on BP control. These observations led us to investigate whether the MR expression in VSMC is involved in the stiffening of large arteries induced by the classical nephrectomy–aldosterone–salt hypertension model.

Methods
Methods are provided in the Methods in the online-only Data Supplement.

Results
SMC-Specific Inactivation of the MR Gene
MR expression is reduced by 48% at the mRNA level (Figure 1A) and 74% at the protein level (Figure 1B) in the whole aorta of MRSMKO mice when compared with control mice. The partial reduction is a result of the MR expression that is not affected in endothelial cells of MRSMKO mice (see below). Immunohistochemical analysis of MR expression in aortas (Figure 1C) reveals MR expression in both endothelium (black arrow) and smooth muscle (open arrow) cells in control mice, whereas the MR signal is restricted to endothelial cells in MRSMKO mice (black arrow). The MR mRNA expression was similar in brain, skeletal muscle, kidney, spleen, and liver between control mice and MRSMKO mice (Figure S1 in the online-only Data Supplement).

Renal Function
Steady-state renal Na+/K+ homeostasis was analyzed in metabolic cage studies. No difference is observed in 24-hour food or water intake, estimated creatinine clearance, urinary proteins, Na+ and K+ excretion, or urinary Na+/K+ ratio between control mice and MRSMKO mice (Table; Figure 2A and 2B). Plasma aldosterone levels were not different between control mice and MRSMKO mice (707.7 ± 56.6 pg/mL in control mice and 686.0 ± 118.8 pg/mL in MRSMKO mice; n = 6 per group). Plasma creatinine values are also similar (11.18 ± 1.62 µmol/L in control mice and 13.39 ± 0.82 µmol/L in MRSMKO mice; n = 5 per group). To evaluate the capacity of renal Na handling, an acute NaCl load challenge (Figure 2C) and a low salt diet challenge (Figure 2D) were performed. The urinary Na+ excretion is similar between control mice and MRSMKO mice in the 2 experimental settings. These data demonstrate MR-specific deletion in VSMC with no effect on renal sodium handling.

BP and Echo-Tracking Analysis
Baseline
Systolic arterial pressure measured in conscious MRSMKO mice is significantly lower than in control mice (Figure 3A), with no difference in heart rate in the 2 groups (bpm, 513 ± 27 in MRSMKO versus 570 ± 15 in control mice; n = 5–8). In isoflurane-anesthetized mice, hemodynamic parameters, estimated by echo tracking of the right carotid (CA), indicate that PP, arterial distensibility (Dist), incremental elastic modulus (Einc), and wall thickness (WS) at MAP do not differ between control mice and MRSMKO mice, with the exception of the arterial diameter at systolic and at MAP that are significantly smaller in mutant mice (Table). The Einc–WS curves in MRSMKO and control mice are shown in Figure 3B.
The mean WS within the 300- to 750-kPa range of Einc (MWS<sub>300–750</sub>) is similar in 2 groups (Table). The mean distensibility within the 80- to 116-mm Hg range of AP (MDist<sub>80–116</sub>; Table; Figure 3D) was calculated from the Dist–AP curves in MR SMKO and control mice as shown in Figure 3C. No significant difference is observed.

**Effects of Aldosterone–Salt Treatment**

Nephrectomy–aldosterone–salt (NAS) treatment significantly increases systolic arterial pressure to a similar level in conscious control mice and MR<sup>SMKO</sup> mice (Figure 3A). In isoflurane-anaesthetized mice, PP is higher and heart rate is lower with aldosterone–salt treatment when compared with those of baseline, with no significant change in arterial pressure and Diameter, Dist, Einc, and WS at MAP (Table). MR gene inactivation in VSMC did not affect the NAS-response of these parameters. NAS treatment significantly reduces the distensibility (measured by MDist<sub>80–116</sub>) but increases the stiffness (measured by MWS<sub>300–750</sub>) in control mice. However, this is not observed in MR<sup>SMKO</sup> mice (Table; Figure 3D). This indicates that NAS treatment increases arterial stiffness in control mice but not in MR<sup>SMKO</sup> mice.

**Morphology of the Tunica Media and Gene Expression in Arteries**

CA media cross-sectional area values are similar in untreated control mice and in MR<sup>SMKO</sup> mice (Table S2), with no difference in elastin and collagen content or in collagen:elastin ratio (Table S2). No difference is observed in CA fibronectin, collagen I, and in α5-, α1-, and αV-integrins mRNA expression between the 2 groups at baseline (Figure 4A). NAS treatment results in significant cardiac and renal hypertrophy with a trend toward an increase in CA media cross-sectional area that does not differ between control mice and MR<sup>SMKO</sup> mice (Table S2).

Moreover, elastin and collagen content and the collagen:elastin ratio do not significantly change with NAS in either genotype (Table S2). CA fibronectin and collagen I mRNA levels increase to a similar extent (∼2-fold; Figure 4A) after exposure of both genotypes to NAS. However, NAS treatment increases α5-integrins expression only in control mice expressing VSMC MR because this effect was abrogated in the MR<sup>SMKO</sup> mice (Figure 4A). Conversely, NAS treatment significantly decreases expression of α1-integrin only in the CA from MR<sup>SMKO</sup> mice and not in the control mice. Expression of αV-integrin was not modified in either group after NAS treatment (Figure 4A). We confirmed by Western blot in aorta that the loss of MR in VSMC prevents increased
expression of α5-integrins by the NAS, whereas αV-integrin was not changed in the mutant mice when compared with control mice (Figure 4B; Figure S3). Aorta collagen I protein level increases to a similar extent (≈2-fold; Figure 4B; Figure S3) after exposure of both genotypes to NAS, in agreement with mRNA results. Neither MR inactivation in VSMC nor NAS treatment significantly changes the angiotensin II receptor mRNA expression (Figure S2).

Discussion

Using a genetic approach to abolish MR expression specifically in smooth muscle in vivo, we showed that VSMC MR is necessary for increased arterial stiffness in response to aldosterone and high salt–induced hypertension. The physiological and pathological roles of MR still remain to be fully understood in extrarenal cells, such as cardiomyocytes, endothelial cells, VSMCs, and inflammatory cells. In the cardiovascular system, the pioneering work of Brilla et al, showing a profibrotic effect of aldosterone in the myocardium, gave new dimensions to this hormone and its receptor. Clinical trials have clearly demonstrated the therapeutic benefit of MR blockade in heart failure, leading to changes in the therapeutic guidelines. The Randomized ALdactone Evaluation Study (RALES), Eplerenone Post–Acute Myocardial Infarction

Table. Effects of NAS Treatment on Blood Pressure and Mechanical Properties of the Carotid in Anesthetized Control and MRSMKO Mice

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<td>0.43±0.03</td>
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**Values are mean±SEM; n, number of carotid arteries. DAP indicates diastolic arterial pressure; Dist, distensibility; Einc, incremental elastic modulus; HR, heart rate; MAP, mean arterial pressure; MDist 116–116, mean distensibility within the 80- to 116-mm Hg range of pressure; MR, mineralocorticoid receptor; MWS 300–750, mean wall stress within the 300- to 750-kPa range of Einc; NAS, nephrectomy–aldosterone–salt; PP, pulse pressure; and SAP, systolic arterial pressure.**

*P<0.001, †P<0.05, ‡P<0.01 vs untreated mice; §P<0.05 vs control mice.

Figure 4. Effects of NAS treatment on fibronectin, collagen I, and integrins expression in arteries from control (CTL) and MRSMKO mice. A, mRNA levels of fibronectin, Col1α, α5, α1, and αV subunits of integrins in the carotid arteries. B, Protein levels of Col1α, α5, and αV subunits of integrins in aorta (Western blots are shown in Figure S2). Data are expressed as a fold change of mean value in untreated CTL mice±SEM. *P<0.05, **P<0.01, ***P<0.001 vs untreated mice; ###P<0.001 vs CTL mice. n=5 mice per group. C, Schematic model for the role of vascular smooth muscle (VSM) MR to regulate the vascular stiffness in large arteries. ROS indicates reactive oxygen species.
Heart Failure Efficacy and Survival Study (EPHESUS), and Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF) clinical trials have demonstrated that the addition of MR antagonists to standard care markedly reduced the overall and cardiovascular mortality in patients with heart failure. More recently, attention has been focused on the vascular effects of aldosterone, not as consequences of increased BP, but rather to a primary effect of aldosterone on vascular remodeling, inflammation, or atherosclerosis.

Another potential important beneficial effect of MR antagonists may be in preventing the involvement of MR in arterial stiffness and modulation of PP. Studies in patients with primary aldosteronism showed that aldosterone is associated with vascular morphological (wall thickening and carotid fibrosis) and functional (arterial stiffness) changes that are alleviated by adrenalectomy in patients with aldosterone-producing adenoma. This was independent of BP when compared with BP-matched essential hypertensive patients. In patients with hypertensive, the aldosterone:renin ratio is positively correlated with aortic PP and wave reflections and may predict the response to spironolactone as antihypertensive therapy. This correlation between aldosterone and vascular stiffness extends to other clinical conditions, such as obesity, even with normal BP. Interestingly, arterial stiffening has been strongly correlated with genetic polymorphism in the aldosterone synthase (CYP11B2) gene, in particular to the CC genotype (C-344T variant), which is associated to increased plasma aldosterone levels. Whether this polymorphism also affects local production of aldosterone in the vascular wall, for example, remains to be determined.

Animal models with high salt and aldosterone challenge have helped to identify the primary role of MR activation in arterial stiffness. However, pharmacological approaches, such as aldosterone infusion or pharmacological MR antagonism, do not distinguish between primary vascular effects, leading to vascular damage and arterial stiffness, and secondary consequences of increased arterial BP, increased salt intake, and altered global neurohormonal status. The genetic approach of using conditional cell-specific gene inactivation is necessary to gain further insights into the mechanisms that are involved. Using such an approach, inducible smooth muscle–specific MR inactivation was recently used to identify a role for SMC MR in aging- and angiotensin II–induced stiffening. Using an integrin α5β3 expression modulates the functional adaptation and remodeling of large arteries, indicating that integrins also play an important role in regulating arterial elasticity. Using an integrin α1 knockout mouse model, and wild-type mice. The decrease in α-integrins in NAS-treated MRSMKO mice may thus contribute to protection from arterial stiffening in response to NAS treatment in these mice. In light of this result, we suggest that the absence of induction of α5 expression in the carotids of MRSMKO mice, independent of change in collagen/elastin content and fibronectin regulation, prevents formation of functional matrix attachments sites, thereby attenuating arterial stiffening. The functional mechanism linking these alterations to the prevention of increased arterial stiffness in MRSMKO mice remains to be addressed, and VSMC-specific α5 knockout mice may be useful for this goal.
Perspectives
In the present study, we used an approach of specific deletion of the MR from VSMC in vivo to explore the direct role of MR in vascular stiffening after aldosterone challenge. Concomitant expression of VSMC MR, fibronectin, and integrins is necessary for aldosterone–salt to induce vascular stiffening. The pathophysiological relevance of VSMC MR has recently been established in maintaining normal BP during aging. In view of our findings, it may be concluded that the regulatory role of VSMC MR is also crucial in the function and structure of elastic arteries with implications for the adverse vascular remodeling that occurs with hypertension and with aging and contributes to cardiovascular disease.

Acknowledgments
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Disclosures
None.

References


**Novelty and Significance**

**What Is New?**

- Mineralocorticoid receptor activation specifically in vascular smooth muscle cell leads to the arterial stiffening induced by aldosterone–salt challenge, independently of blood pressure increase.
- Mineralocorticoid receptor expressed in vascular smooth muscle cell is mandatory for α5-integrin upregulation by aldosterone–salt in carotids.

**What Is Relevant?**

- The vascular smooth muscle mineralocorticoid receptor is a target for pharmacological intervention aimed at destiffening the arteries to improve cardiovascular outcomes.

**Summary**

The vascular smooth muscle cell mineralocorticoid receptor is crucial for aldosterone–salt–induced carotid stiffening.
Smooth Muscle Cell Mineralocorticoid Receptors Are Mandatory for Aldosterone–Salt to Induce Vascular Stiffness

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Supplemental materials to

Smooth muscle cell mineralocorticoid receptors are mandatory for aldosterone-salt to induce vascular stiffness

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Running title: VSMC MR and Arterial Stiffness

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**Expanded materials and methods**

All animal breeding, housing, and protocols were performed in accordance with the ethical guidelines of INSERM for the care and use of laboratory animals.

**Generation of SMC-specific MR inactivation**

Floxed MR mice (MR$^{fl/fl}$) were crossed with transgenic mice expressing Cre recombinase under the control of the regulatory elements of the mouse SM22alpha gene (SM22-Cre mice) to generate MR$^{SMKO}$ (MR$^{fl/fl}$;SM22-Cre) mice. Mice were born at Mendelian frequencies. For all studies, male MR$^{fl/fl}$ (control/CTL) and MR$^{SMKO}$ mice were phenotyped at age 4-5 months. To assess the chronic effects of the uninephrectomy-aldosterone-salt treatment (thereafter referred as NAS) on vascular function, mice were uninephrectomized at 4 month of age and were given vehicle or aldosterone (200µg/day) via osmotic minipumps (Alzet, Durect Corporation, CA, USA) with high sodium (1% NaCl) in the drinking water for 4 weeks.

**Blood Pressure and Echo-tracking Measurements**

Systolic arterial pressure (SAP) and heart rate (HR) were measured in conscious animals using a tail-cuff sphygmomanometer as described previously. Mice were gently handled for four days (including blank measurements) before determination of blood pressure, to avoid stress. Simultaneous recording of arterial diameter (left carotid artery) and blood pressure (right carotid artery) in isoflurane-anesthetized mice were done as previously described. Arterial diameter measurement was obtained by using an ultrasonic echo-tracking device (Diarad-00, Asulab SA). The relationship between the pressure (P) and the lumen cross-sectional area (LCSA) was fitted with the model of Tardy et al. by using an arctangent function and three optimal fit parameters ($\alpha$, $\beta$, $\gamma$) as follows:

\[
LCSA = \alpha \left( \frac{\pi}{2} + \tan^{-1}\left( \frac{P - \beta}{\gamma} \right) \right)
\]

Carotid cross-sectional distensibility (Dist), a derivative of this function, was used to assess the global elastic behaviour of the artery. Circumferential wall stress (WS) and incremental elastic modulus (Einc), which characterises the intrinsic mechanical properties of the wall material, were calculated with the above-mentioned parameters. Dist, WS and Einc are given by the following equations:

\[
\text{Dist}(P) = -\frac{1}{LCSA} \times \frac{\delta LCSA}{\delta P}
\]

\[
WS = \frac{2LCSA \times P}{MCSA}
\]

\[
Einc = \frac{3}{\text{Dist}} \left( 1 + \frac{LCSA}{MCSA} \right)
\]
where the media cross sectional area (MCSA) was determined by histomorphometry. The reproducibility was ±1% (inter-observer coefficient of variation of carotid diameter). The mechanical parameters measured at mean arterial pressure assesses arterial function at operating physiological pressure whereas Einc/WS curves explore intrinsic properties of the wall material, independent of pressure levels.

**Histological Procedures**

Histological studies of the arteries were performed on carotid arteries (CA) fixed in situ with 10% buffered formalin at a constant pressure of 100 mmHg for 40 min to provide conditions of fixation close to the physiological in situ state of the vessel. For morphological analysis, all arterial samples were embedded in paraffin and 5 µm sections were stained with Sirius red for collagen and Orcein for elastic fibres. Composition of the arterial wall and mean cross sectional area (MCSA) were determined by computer-directed image analysis as described previously. Immunochemical analysis of the MR expression was performed as described previously using a 6G1 monoclonal antibody (kindly provided by C. Gomez-Sanchez, Division of Endocrinology, University of Mississippi Medical Center, Jackson, MS, USA). Non-specific signal was assessed by omitting the primary antibody. Image acquisition was made on a Leica DM4000 microscope (Leica, Wetzlar, Germany).

**Western blotting analysis**

Western blot analysis was performed on protein collected from the aorta from control and MR^SMKO^ mice that were immediately homogenized after dissection using a 1% SDS homogenisation buffer with protease and phosphatase inhibitors (Roche Diagnostics, Meylan, France). Extracts were centrifuged at 13,200 rpm for 20 minutes at 4°C and was immediately frozen or diluted with Laemmli buffer (2x, Sigma-Aldrich, St Quentin Fallavier, France) and boiled for 5 min. Twenty micrograms of total proteins were loaded on 4–15% Mini-PROTEAN® TGX™ precast polyacrylamide gels (Bio-Rad, Marnes-la-Coquette, France), then transferred to PVDF membranes. Western blotting analyses were performed as described by Gomez-Sanchez et al.

**RNA extraction and real-time PCR**

Total RNA was extracted from carotid arteries by using the TRIZOL® reagent (Life Technologies Corporation, Carlsbad, CA, USA), according to manufacturer protocol. The reverse transcription of mRNA (100ng) was performed with Superscript II reverse transcriptase KIT (Life Technologies Corporation, Carlsbad, CA, USA). Transcript levels of genes were analyzed by real-time PCR (fluorescence detection of SYBR Green) in an iCycler iQ apparatus (Bio-Rad). For each sample, mRNA levels were normalized the geometric mean of the amount of two housekeeping genes, 18S and hypoxanthine guanine phosphoribosyl transferase (HPRT). The analyzed genes and their specific primer sequences are listed in Table S3.

**Renal function**

Metabolic studies were performed on mice kept in individual cages (Phymep Marty Technology, Paris, France) for 1 week, and urine samples were collected under mineral oil. Blood samples were collected from the tail into heparinized microtubes (Microvette CB; Starstedt, Germany). Plasma aldosterone levels were measured by radioimmunoaassay (Diagnostics Products, LA Garenne-Colombes, France). Urinary and plasma creatinine concentrations were determined by enzymatic method (Thermo Fischer Scientific, Finland), according to the manufacturer’s protocol. Urinary sodium, potassium and urea concentrations were determined with an automatic analyser, as described previously.
Steady-state study
Mice in metabolic cages had access to standard food (0.3% sodium, A03; SAFE, Epinay, France) and tap water for 2 days before the beginning of urine collection. Urine was collected every other day for 3 days.

Acute NaCl load
Mice in metabolic cages had free access to standard food (0.3% sodium) and tap water for 2 days before the beginning of urine collection. Urine was collected before and 3, 6 and 24 hours after an acute NaCl load (525 µmol of NaCl as oral gavage). Urine was collected before and 3, 6 and 24 hours after an acute NaCl load (525 µmol of NaCl as oral gavage).

Low salt diet
Mice in metabolic cages had free access to standard food (0.3% sodium) and tap water for 2 days. Then, the low salt diet (0.1% sodium; SAFE, Epinay, France) was provided and urine was collected at 5, 24, 29 and 48 hours.

Statistical analysis
All values are expressed as mean ± SEM. mRNA/protein expression and renal function values were analysed using unpaired Student t tests. We compared the Distensibility-arterial pressure curves (Dist-AP curves) and the incremental elastic modulus/Wall Stress curves (Einc/WS curves) using the median values of the common range of either arterial pressure for distensibility or Einc for wall stress. Arterial mechanical and structural parameters were analysed using 2-ways ANOVA, and the Holm-Sidak post hoc test was used for intergroup comparisons. Renal Na handling experiments were analysed using 1-way ANOVA on Ranks, and the Dunn’s post hoc test was used for intergroup comparisons. Differences were considered significant at values of P<0.05.
Supplemental references
## Tables and supporting information

### Table S1. Urinary electrolytes in control and MR$^{SMKO}$ mice

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<td>29.9±2.5</td>
<td>32.7±3.5</td>
</tr>
<tr>
<td>K/creatinine</td>
<td>72.4±5.4</td>
<td>82.0±6.0</td>
</tr>
<tr>
<td>Na/K</td>
<td>0.40±0.01</td>
<td>0.34±0.03</td>
</tr>
<tr>
<td>Urea (mM)</td>
<td>10.22±0.44</td>
<td>11.12±0.28</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Sodium, potassium and creatinine excretion rates were measured in metabolic cages on 24h urine collection; Na, sodium; K, potassium. $n=10$/group.
Table S2. Morphometric parameters and histology of the CA in control and MR\textsuperscript{SMKO} mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CTL</th>
<th>MR\textsuperscript{SMKO}</th>
<th>CTL NAS</th>
<th>MR\textsuperscript{SMKO} NAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphometrics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>body weight (g)</td>
<td>28.6 ± 0.2</td>
<td>28.0 ± 0.8</td>
<td>27.4 ± 0.5</td>
<td>28.3 ± 0.6</td>
</tr>
<tr>
<td>kidney weight (mg/mm)</td>
<td>7.7 ± 0.6</td>
<td>9.0 ± 0.4</td>
<td>18.9 ± 0.5†</td>
<td>20.9 ± 1.7†</td>
</tr>
<tr>
<td>heart weight (mg/mm)</td>
<td>6.9 ± 0.2</td>
<td>7.6 ± 0.6</td>
<td>8.7 ± 0.2*</td>
<td>9.4 ± 0.7*</td>
</tr>
<tr>
<td>Histology of the CA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCSA (mm\textsuperscript{2} 10\textsuperscript{-3})</td>
<td>23.5 ± 1.8</td>
<td>26.1 ± 2.0</td>
<td>28.0 ± 2.2</td>
<td>32.2 ± 3.4</td>
</tr>
<tr>
<td>Thickness (mm 10-3)</td>
<td>45.2 ± 3.0</td>
<td>49.5 ± 2.1</td>
<td>47.1 ± 3.3</td>
<td>53.2 ± 4.8</td>
</tr>
<tr>
<td>Elastin content (mm\textsuperscript{2} 10\textsuperscript{-3})</td>
<td>10.4 ± 1.3</td>
<td>12.3 ± 0.7</td>
<td>10.6 ± 0.9</td>
<td>13.3 ± 2.0</td>
</tr>
<tr>
<td>Collagen content (mm\textsuperscript{2} 10\textsuperscript{-3})</td>
<td>8.8 ± 1.9</td>
<td>8.2 ± 0.8</td>
<td>8.3 ± 1.6</td>
<td>9.5 ± 2.4</td>
</tr>
<tr>
<td>Collagen/elastin ratio</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SEM. n=5-8/group. *P<0.01, †P<0.001 vs. untreated mice.
<table>
<thead>
<tr>
<th>Target mRNA</th>
<th>Primer sequences (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>18SL</td>
<td>cgccgctagaggtgaaatcc</td>
</tr>
<tr>
<td>18SF</td>
<td>tctggcaaatgccttgc</td>
</tr>
<tr>
<td>AT1RL</td>
<td>gactggatgatgctgtggtg</td>
</tr>
<tr>
<td>AT1RF</td>
<td>ctgaggttggtctgtgagt</td>
</tr>
<tr>
<td>Fn1L</td>
<td>aaggtcgggaagagtttgtt</td>
</tr>
<tr>
<td>Fn1R</td>
<td>cctgttaagggtcaagcat</td>
</tr>
<tr>
<td>HPRTL</td>
<td>cagggccagcttattgtgat</td>
</tr>
<tr>
<td>HPRTR</td>
<td>ttcgcgtcacttttagccttt</td>
</tr>
<tr>
<td>Itga1L</td>
<td>caaatgagcctggaaccaat</td>
</tr>
<tr>
<td>Itga1R</td>
<td>ccatccacgtggatttt</td>
</tr>
<tr>
<td>Itga5L</td>
<td>caagttgacaggactcagca</td>
</tr>
<tr>
<td>Itga5R</td>
<td>ggtctctgatcacaactca</td>
</tr>
<tr>
<td>ItgaVL</td>
<td>gggacaggggagaaaggtc</td>
</tr>
<tr>
<td>ItgaVR</td>
<td>gatcacaagcacaaggtg</td>
</tr>
<tr>
<td>MRL</td>
<td>ccagaagaggggaccacata</td>
</tr>
<tr>
<td>MRR</td>
<td>ggaattgtctgtgaccctgtc</td>
</tr>
</tbody>
</table>
Figure and supporting information

Figure S1. Quantitative polymerase chain reaction analysis of MR mRNA expression in tissues from MR^{SMKO} mice compared to control mice. MR quantifications are normalized to 18S and HPRT mRNA. The ratios of MR^{SMKO} versus control (set to 1) are presented for each tissue. The error bars represent SEM; n = 6/group.
Figure S2. Quantitative polymerase chain reaction analysis of Angiotensin II receptor (AT1R) mRNA expression in carotid artery of MR\textsuperscript{SMKO} mice compared to control mice, in basal condition and after NAS challenge. AT1R quantifications are normalized to 18S and HPRT mRNA. The ratios of MR\textsuperscript{SMKO} versus control (set to 1) are presented for each group. The error bars represent SEM; n = 5/group.
Figure S3. Western blot analyses of $\alpha_5$ and $\alpha_V$ subunit integrins (membrane A) and Collagen I (membrane B) in aorta from control and MR$^{\text{SMKO}}$ mice in basal condition and after NAS challenge.