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Direct Contribution of Vascular Mineralocorticoid Receptors to Blood Pressure Regulation

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Summary

1. Hypertension is an extremely prevalent cardiovascular risk factor and current antihypertensive therapies do not adequately treat hypertension in many affected individuals. Thus a better understanding of mechanisms of hypertension could lead to novel therapies.
2. Mineralocorticoid receptors (MR) are known to regulate blood pressure by responding to aldosterone in the kidney to regulate sodium retention. Recent evidence supports a direct contribution of the vasculature to control of BP and suggests the possibility that MR antagonists may also lower blood pressure by acting on extra-renal MR.
3. This review summarizes existing research considering the role of vascular mineralocorticoid receptor (MR) in regulating vasoreactivity and blood pressure.
4. Multiple studies indicate a role for vascular MR in modulating vasoconstriction and vasorelaxation. Activation of MR in vascular endothelial and smooth muscle cells leads to increased reactive oxygen species production and decreased availability of nitric oxide, important regulators of vascular reactivity.
5. Transgenic mouse models, including an endothelial MR over-expressing mouse and a smooth muscle cell-specific MR knockout mouse, support a direct role for vascular MR in control of blood pressure.
6. This new evidence demonstrating that vascular MR directly contributes to control of vasoreactivity and blood pressure supports vascular MR, and the pathways it controls, as novel therapeutic targets to treat hypertension.

Keywords

Mineralocorticoid receptor; aldosterone; nitric oxide; reactive oxygen species; vasoreactivity; blood pressure

Introduction: Potential for extra-renal MR to regulate blood pressure

The mineralocorticoid receptor (MR) is a member of the steroid receptor family of intracellular hormone-activated receptors, known for its role in blood pressure (BP) control. MR is expressed in renal epithelial cells and classically participates in BP regulation by binding the steroid hormone aldosterone (aldo) to induce expression of genes involved in sodium retention, thereby increasing blood volume and BP.¹ Hyperaldosteronism is present in a subset of patients with essential hypertension and the prevalence of hyperaldosteronism among patients with essential hypertension increases with the severity of BP elevation, with 2% of Stage 1 (SBP 140–159 mmHg, DBP 90–99 mmHg) hypertensive patients affected, as many as 13% of Stage 3 (SBP >180 mmHg, DBP 110 mmHg) patients affected², and over 20% of patients with Resistant Hypertension attributed to hyperaldosteronism.³ MR antagonists have been used for decades as an effective treatment for hypertension in patients with both elevated and normal serum aldosterone levels. More recently, it has become clear that MRs are also expressed in non-renal tissues and data from human trials and animal models support the potential for extra-renal MR to contribute to BP control. A recent meta-analysis of MR antagonist clinical trials demonstrated that BP reduction with MR blockade does not correlate with changes in plasma potassium, a marker of renal MR activation, supporting the potential for non-renal MR to contribute to BP modulation in humans.⁴ Mice deficient in the MR in all tissues die in the neonatal period from salt wasting, which is consistent with the known role of the MR in regulating vascular volume.^{5, 6} However, mice with distal renal tubule-specific MR deficiency survive unless challenged with low-salt conditions.^{7, 8} Although MR in other parts of the nephron might compensate for the loss of distal renal tubule MR in this model, these data also support the possibility that loss of extra-renal MR could be contributing to the hypotension and mortality associated with complete MR deficiency. We and others have shown that MR is expressed in the endothelial cells (EC) and smooth muscle cells (SMC) of the human vasculature and that aldo at physiologic (1nM) and pathologic (10nM) concentrations can activate MR-mediated gene expression in these cells (reviewed in ⁹). Furthermore there is emerging evidence from molecular, cellular, and transgenic mouse studies supporting a direct role for the vasculature in control of BP.¹⁰ Thus MR in the blood vessel could directly contribute to control of BP by modulating vascular reactivity and tone. This review will summarize current understanding of how vascular MR contributes to pathways involved in vasorelaxation and vasoconstriction and recent in vivo evidence directly implicating vascular MR in control of BP.

Role of vascular MR in regulation of nitric oxide availability

Molecular Mechanism of Vasodilatation

Nitric oxide (NO) is a principle mediator of vasorelaxation. Endothelial cells (EC) produce NO when the enzyme endothelial nitric oxide synthase (eNOS) is activated by factors such as acetylcholine, bradykinin, or shear stress. NO enables smooth muscle cell (SMC) relaxation by paracrine mechanisms, diffusing from ECs to local SMCs where it activates soluble guanylyl cyclase (sGC) to produce cyclic guanosine monophosphate (cGMP). Increased SMC cGMP causes activation of protein kinase G (PKG), facilitating myosin dephosphorylation by myosin light chain phosphatase (MLCP) resulting in SMC relaxation

(see Figure 1).¹¹ NO availability is sensitive to reactive oxygen species (ROS) produced by vascular oxidase enzymes including NADPH oxidases.¹² Vascular ROS modulate NO availability in multiple ways: directly, by converting circulating NO to reactive intermediates that do not vasodilate and indirectly, by uncoupling eNOS so that NO is no longer produced from arginine (reviewed in ¹³). ROS are also capable of modulating relaxation by post-translational oxidative modifications of eNOS in ECs¹⁴ and of guanylate cyclase (GC) in SMCs.¹⁵ Therefore, in considering the role of vascular MR in NO availability and vasorelaxation, production of both NO and ROS is important.

Role of MR in NO and ROS production

Multiple studies confirm a role for MR in ECs and SMCs in regulation of NO and ROS production. MR has been shown in cultured ECs to regulate NO availability directly, by modulating the phosphorylation state of eNOS thereby regulating its activity¹⁶ and indirectly, via regulation of reactive oxygen species (ROS) production as previously reviewed.¹⁷ The direct effect of EC MR on eNOS activity remains controversial, with some studies demonstrating MR activation and some showing MR inhibition of eNOS activity that may depend on the source of the EC, the culture conditions, and other factors.^{16, 18} However, studies in cultured ECs show that the net effect of aldo (1–100nM) on NO^{16, 18} and ROS^{19–21} production is to decrease NO availability. Likewise, SMCs treated with aldo (1–100nM) show increased ROS production.¹⁵ Studies in both ECs and SMCs support an NADPH oxidase-mediated mechanism for the increase in ROS with aldo treatment. In human coronary artery ECs, aldo treatment upregulated mRNA expression of the NADPH oxidase subunit Nox4 in an MR-dependent manner.²¹ Several studies in vascular SMCs show that activation of MR by aldo increases ROS production via activation of NADPH oxidase through a c-Src-dependent pathway (Figure 1).^{22, 23} In a whole vessel study recently published by our laboratory, carotid arteries harvested from transgenic mice specifically lacking SMC MR showed decreased ROS production in response to angiotensin II (AngII) treatment or to aging, when compared to vessels from age-matched mice with intact SMC MR.²⁴ These data provide the first conclusive evidence that SMC MR directly contributes to ROS production in whole vessels.

Recent studies have begun to elucidate mechanisms for MR-mediated regulation of eNOS activity and NO availability. Several recent studies in rodent models of pulmonary hypertension have revealed that MR inhibition attenuates the disease phenotype.^{14, 25} In exploring the mechanism of this finding, Maron and colleagues showed that in human pulmonary artery ECs, aldo-stimulated ROS production resulted in post-translational modification of endothelin B receptor (ET(B)), a potent inhibitor of eNOS. Aldo-stimulated ROS modified cysteinyl thiols in the eNOS-activating region of ET(B) to decrease endothelin-1-stimulated eNOS activity.¹⁴ Another study in human umbilical vein endothelial cells showed that aldo treatment decreased eNOS activity by cytoskeletal rearrangement leading to dephosphorylation of the eNOS activator Akt.²⁶ Additionally, in a model of portal hypertension, MR inhibition with spironolactone significantly lowered portal BP and activated the NO pathway via downregulation of the RhoA/ROCK-2 pathway that negatively regulates eNOS (Figure 1).²⁷ Regulation of eNOS by MR is likely mediated by “non-genomic” mechanisms in which non-nuclear MR, localized to caveoli, modulates

eNOS function by activation of rapid signaling rather than “genomic” mechanisms involving gene transcription. This is supported by a study using transgenic mice lacking caveolin 1 (Cav1), an anchoring protein involved in regulation of eNOS function²⁸, which suggested that in the absence of Cav1 protein, MR exerts a pro-relaxation effect, stabilizing or stimulating eNOS.²⁹ MR in non-vascular cells may also modulate NO availability, perhaps by paracrine mechanisms. A recent study using a mouse model over-expressing human MR only in the cardiomyocyte demonstrated a NADPH oxidase-mediated, ROS- dependent, decrease in NO-mediated endothelial-dependent coronary vasorelaxation.³⁰ Taken together, in vitro studies suggest that aldo-stimulated vascular MR-dependent modulation of NO availability occurs through multiple pathways that collectively contribute to impaired vasorelaxation (Figure 1).

In vivo rodent models support a role for MR in regulating the balance of NO and ROS that correlates with MR regulation of blood pressure, providing indirect evidence that vascular MR may contribute to BP control by this mechanism. In models of mineralocorticoid-induced hypertension, systemic MR activation was associated with increased ROS along with the rise in BP, both of which were inhibited by the MR antagonist eplerenone.^{31–33} Furthermore treatment with an inhibitor of NADPH-oxidase, one of the key enzymes involved in vascular ROS production, prevented mineralocorticoid induced vascular ROS formation and attenuated the associated BP increase.³⁴

In clinical studies of patients with cardiovascular disease, there is also some support for a contribution of MR to the generation of oxidative stress and regulation of vasorelaxation. A clinical trial of the MR antagonist spironolactone in patients with congestive heart failure showed improved EC-dependent vasodilation in patients randomized to MR antagonist treatment.³⁵ In a trial of patients with chronic kidney disease, MR antagonist treatment resulted in decreased urinary isoprostanes, a measure of global oxidative stress.³⁶ These findings suggest MR antagonism in high-risk individuals increases vascular NO availability and improves endothelial vasodilatory function.

Vascular MR in vessel reactivity and tone

Vascular SMCs modulate vasoreactivity by relaxing in response to NO and other vasodilators, and contracting in response to contractile agonists. SMC also contract in response to elevated intraluminal pressure, a property called myogenic tone. Recently the concept has emerged that by contributing to total vascular resistance, vascular reactivity and tone may directly contribute to systemic BP.¹⁰ Aldo-induced SMC ROS production was recently shown in vitro to directly modify guanylate cyclase by cysteinyl thiol oxidation resulting in diminished responsiveness of the guanylate cyclase to NO¹⁵ that would be expected to prevent vasorelaxation. Additional studies have sought to determine the role of vascular MR in vasoreactivity using ex vivo contraction and relaxation studies, with some conflicting findings. In ex vivo vessel studies, the direct effect of aldo on the vessel varied widely, potentially due to differences in species, vascular bed, aldo dose and timing (reviewed in¹⁷). However, virtually all studies showed the effect of aldo to be inhibited by ex vivo MR antagonist treatment, directly implicating vascular MR (reviewed in³⁷). One study found that when aldo was infused directly into the vessel lumen to contact the

endothelium, it resulted in relaxation, but when it was added to the surrounding bath to target SMC, it caused contraction.³⁸ Other studies show that intact vessels treated with aldosterone relax, while vessels denuded of their endothelium contract in response to aldosterone.^{38, 39} These findings support the possibility that EC MR and SMC MR elicit opposite vasoreactivity responses in the context of the whole blood vessels.

Additional studies in animal vessels reveal a role for vascular MR in modulating the response to other vasoconstricting agents in a manner that may depend on the specific vascular bed. One study in rabbit renal arterioles showed decreased KCl-mediated vasoconstriction with aldosterone treatment.⁴⁰ Another study in rat aortae found EC-dependent attenuation of phenylephrine (PE)-mediated vasoconstriction with aldosterone treatment.⁴¹ Both of these pro-relaxation effects were inhibited by the MR antagonist spironolactone. However, the vasoreactivity of resistance vessels, rather than large conduit vessels such as the aorta, is thought to be more relevant for the modulation of systemic blood pressure. Mesenteric resistance vessels from a mouse model over-expressing MR in the endothelial cells contracted more strongly to PE, KCl, and a thromboxane analog than vessels from wild-type mice.⁴² This supports the potential for enhanced activation of EC MR to directly contribute to vascular contractile responses; however, it is possible that these findings are due to the effects of supraphysiologic levels of MR in this model. Future development of a transgenic mouse with endothelial cell-specific MR deletion would allow direct investigation of the role of endogenous EC MR in vasoconstriction as well as vasorelaxation.

The recent development of a mouse model in which SMC MR is specifically deleted in adulthood by tamoxifen induction (SMC-MR-KO) has revealed a role for SMC MR in regulating vascular contraction, particularly with aging.¹⁷ In young mice, mesenteric resistance vessel contraction to AngII was diminished in SMC-MR-KO mice while the contractile response to KCl and thromboxane was unaffected. With aging, resistance vessels from control mice developed augmented agonist-induced contraction compared to vessels from young mice but this enhanced contraction with aging was absent in vessels from SMC-MR-KO mice. These resistance arteries from aged SMC MR deficient mice had similar structure and stiffness as those from control mice, but they developed significantly less spontaneous myogenic tone, supporting the notion that SMC MR contributes to overall vascular tone in aged mice independent of structural changes in the vasculature. Voltage-gated L-type calcium channels participate in the development of myogenic tone and the response to contractile agonists. The messenger RNA expression of the Cav1.2 subunit and the activity of the L-type calcium channel was substantially reduced in vessels from SMC-MR-KO mice. These findings support a direct role for SMC MR in regulating vascular calcium channel expression and activity, an important mediator of vascular contraction and myogenic tone that could directly contribute to blood pressure regulation by non-renal mechanisms (Figure 2).

Studies examining the impact of aldosterone treatment on vascular responses in healthy human subjects are contradictory with most studies demonstrating a constrictive effect^{43–45} while others show relaxation.⁴⁶ However studies in patients with underlying cardiovascular disease consistently support the idea that MR activation promotes vascular constriction with increased systemic vascular resistance and reduced forearm blood flow^{47, 48} while MR

antagonism improves endothelium-dependent vasodilation.^{35, 49, 50} The entirety of these studies suggests that in patients with conditions associated with endothelial dysfunction and vascular disease, aldosterone-mediated MR activation triggers vasoconstriction, while in people with healthy vessels the effects of vascular MR activation may be variable according to factors such as the vascular bed examined, co-morbid conditions, and treatment timing (reviewed in ⁵¹). A recent clinical trial offers insight into additional mechanisms by which MR antagonism may influence vascular function. Geriatric patients with essential hypertension were randomized to spironolactone or hydrochlorothiazide. Both groups experienced comparable reductions in BP, but the spiro-treated group demonstrated attenuated sympathetic nervous system activation as evidenced by reduced release of norepinephrine, a potent vasoconstrictor.⁵² Because sympathetic nervous system activation is thought to be involved in the pathogenesis of essential hypertension, any role MR antagonists play in mitigating that activation in the vasculature could be of significant clinical benefit and further investigation of the role of vascular MR in modulating sympathetic nervous system activation might identify additional novel mechanisms.⁵³

Direct contribution of vascular MR to systemic blood pressure

As described above, animal and human data support the notion that alterations in renal function may not completely account for the impact of MR in regulation of blood pressure and a great deal of progress has been made in understanding the mechanisms by which vascular MR contributes to vascular relaxation, contraction and myogenic tone. However, whether vascular MRs contribute directly to blood pressure control cannot be determined by in vitro or ex vivo vessel studies. New studies utilizing vascular-specific transgenic mouse models have begun to elucidate the direct role of vascular MR in BP control in vivo.

Using a conditional tetracycline-inducible system, Nguyen Dinh and colleagues created a mouse with endothelium-specific over-expression of human MR (MR-EC) that can be turned off with administration of doxycycline.⁴² MR-EC mice have elevated systolic and diastolic BP compared to controls that is normalized by doxycycline, with no difference in renal sodium transport, heart rate, or heart weight. To assess whether exposure to high endothelial MR levels in early development caused this BP difference, mice were given doxycycline during development and for the first 3 months of life and then doxycycline was withdrawn. With EC MR over-expression beginning in adulthood, BP rose significantly in the MR-EC mice. Thus endothelial over-expression of human MR causes a BP elevation that is independent of perturbations in renal sodium handling or developmental effects of EC MR over-expression. MR-EC mice also showed greater BP rise in response to infusion of AngII or endothelin-1 compared to controls, consistent with ex vivo vessel data that supports a role for EC MR in potentiating vascular responses to contractile agonists. Further studies in endothelial-specific MR deficient mice will help to clarify the role of endogenous EC MR in the regulation of blood pressure.

The mouse model with tamoxifen-inducible, SMC-specific deletion of MR (SMC-MR-KO) has further extended our knowledge of the role of vascular MR in blood pressure control.¹⁷ In this model, MR is deleted specifically in SMC in adult mice by tamoxifen injection, again avoiding potential developmental effects of SMC MR on vascular function. As it does in

humans, BP rises with age in control, tamoxifen-treated SMC MR-intact mice but this age-associated rise in BP is lost in SMC-MR-KO mice. The lower BP in aged SMC-MR-KO mice is independent of sodium loading conditions and renal MR function is intact in these mice. Vascular AngII signaling is known to be enhanced with aging in humans and in rodents and is thought to contribute to aging-associated BP elevation.⁵⁴ AngII is known to promote vasoconstriction and hypertension by NADPH-oxidase-induced ROS production and by activation of Rho signaling and calcium channels to activate myosin light chain kinase (MLCK, Figure 2)). Previous studies have suggested that angiotensin type 1 receptors (AT₁R) cross talk with SMC MR during AngII stimulation in vitro.^{55–59} In SMC-MR-KO mice, AngII-induced hypertension was attenuated in young mice and completely prevented in aged mice. As described above, mesenteric resistance vessel contraction to AngII was abrogated in SMC-MR-KO mice as was Ang II–stimulated ROS production in the vascular wall. Thus, SMC MR contributes to Ang II-mediated vascular oxidative stress, contraction, and hypertension, particularly in the aging vasculature, demonstrating the in vivo relevance of cross talk between SMC MR and AT₁ receptors. Overall, studies in the SMC-MR-KO mice demonstrate a direct contribution of SMC MR to blood pressure control in aged mice via modulation of SMC ROS production, AngII signaling and calcium channel function (Figure 2).

Conclusions

Hypertension is an extremely common cardiovascular risk factor, particularly in the elderly. Systolic blood pressure rises in humans older than 40 years and hypertension occurs in over two-thirds of people over age 60. Elevated blood pressure correlates with increased risk of heart attack, stroke and progression to heart and kidney failure. Current therapies are insufficient to control blood pressure in one third of hypertensives and in almost half of elderly patients and thus a better understanding of the mechanisms could lead to novel therapies.^{60, 61} In addition to its critical role in regulating sodium handling in the kidney, MR is also expressed in EC and SMC of the vasculature. Ample data support that vascular MR contributes to NO availability and ROS production thereby inhibiting vascular relaxation, at least in the setting of vascular disease. In addition, SMC MR plays a role in vascular contraction and myogenic tone, likely by regulation of vascular ROS production and L-type calcium channel activity. In vivo, increased expression of EC MR resulted in hypertension and SMC MR deletion prevented aging-associated blood pressure elevation and many manifestations of cardiovascular aging, both without changes in kidney function. These findings suggest that the development of MR antagonists with selectivity for vascular MR or the targeting of vascular MR downstream targets might have beneficial effects on blood pressure control and cardiovascular morbidity and mortality in hypertensive patients, particularly in the aging population.

Abbreviations

aldo	aldosterone
AngII	Angiotensin II

AT1R	Angiotension II Type 1 receptor
BP	blood pressure
cGMP	cyclic guanosine monophosphate
c-Src	cellular sarcoma
Cav1	Caveolin 1
EC	endothelial cell
eNOS	endothelial nitric oxide synthase
ET(B)	endothelin B receptor
ET1	endothelin 1
KCl	potassium chloride
LTCC	L-type calcium channels
MR	mineralocorticoid receptor
MR-EC	endothelial MR overexpressing mouse
MLCK	Myosin light chain kinase
MLCP	Myosin light chain phosphatase
NADPH	nicotinamide adenine dinucleotide phosphate
NO	nitric oxide
PE	phenylephrine
PKG	protein kinase G
RhoA	Ras homolog gene family, member A
ROCK2	Rho-associated protein kinase 2
ROS	reactive oxygen species
sGC	soluble guanylyl cyclase
SMC	smooth muscle cell
SMC-MR-KO	smooth muscle cell-specific MR knockout

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Figure 1. Mechanisms of vascular relaxation and the role of vascular mineralocorticoid receptors (MR)

In endothelial cells (EC), endothelial nitric oxide synthase (eNOS) is activated by phosphorylation in response to relaxation stimuli (achetylcholine (Ach), bradykinin, shear stress) to produce nitric oxide (NO). NO diffuses to local smooth muscle cells (SMC) where it activates soluble guanylyl cyclase (sGC) to produce cyclic guanosine monophosphate (cGMP) that activates protein kinase G (PKG), facilitating myosin dephosphorylation by myosin light chain phosphatase (MLCP) resulting in SMC relaxation. Vascular MR inhibits vasorelaxation by promoting reactive oxygen species (ROS) production from NADPH oxidases (NADPH Ox) and by other mechanisms.

Figure 2. Potential roles for smooth muscle cell (SMC) mineralocorticoid receptors (MR) in the mechanisms of vascular contraction and myogenic tone generation

Contractile agonists including phenylephrine (PE), endothelin 1 (ET1) and angiotensin II (AngII) induce SMC contraction by activation of Rho signaling and L-type calcium channels (LTCC) that induce myosin phosphorylation by activating myosin light chain kinase (MLCK). AngII also enhances vascular contraction by promoting reactive oxygen species (ROS) production from NADPH oxidases (NADPH Ox). ROS further activate L-type calcium channels, Rho signaling, and MLCK function. SMC MR regulates vascular expression of the LTCC, Cav1.2 and mediates AngII-induced ROS production thereby contributing to blood pressure regulation, particularly with aging.