

## Endothelial Mineralocorticoid Receptors Differentially Contribute to Coronary and Mesenteric Vascular Function Without Modulating Blood Pressure

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**Abstract**—Arteriolar vasoreactivity tightly regulates tissue-specific blood flow and contributes to systemic blood pressure (BP) but becomes dysfunctional in the setting of cardiovascular disease. The mineralocorticoid receptor (MR) is known to regulate BP via the kidney and by vasoconstriction in smooth muscle cells. Although endothelial cells (EC) express MR, the contribution of EC-MR to BP and resistance vessel function remains unclear. To address this, we created a mouse with MR specifically deleted from EC (EC-MR knockout [EC-MR-KO]) but with intact leukocyte MR expression and normal renal MR function. Telemetric BP studies reveal no difference between male EC-MR-KO mice and MR-intact littermates in systolic, diastolic, circadian, or salt-sensitive BP or in the hypertensive responses to aldosterone±salt or angiotensin II±L-nitroarginine methyl ester. Vessel myography demonstrated normal vasorelaxation in mesenteric and coronary arterioles from EC-MR-KO mice. After exposure to angiotensin II–induced hypertension, impaired endothelial-dependent relaxation was prevented in EC-MR-KO mice in mesenteric vessels but not in coronary vessels. Mesenteric vessels from angiotensin II–exposed EC-MR-KO mice showed increased maximum responsiveness to acetylcholine when compared with MR-intact vessels, a difference that is lost with indomethacin+L-nitroarginine methyl ester pretreatment. These data support that EC-MR plays a role in regulating endothelial function in hypertension. Although there was no effect of EC-MR deletion on mesenteric vasoconstriction, coronary arterioles from EC-MR-KO mice showed decreased constriction to endothelin-1 and thromboxane agonist at baseline and also after exposure to hypertension. These data support that EC-MR participates in regulation of vasomotor function in a vascular bed–specific manner that is also modulated by risk factors, such as hypertension. (*Hypertension*. 2015;66:988-997. DOI: 10.1161/HYPERTENSIONAHA.115.06172.)

### • Online Data Supplement

**Key Words:** blood pressure ■ endothelial cells ■ endothelin-1 ■ hypertension ■ receptors, mineralocorticoid

A ample clinical trial data reveal that mineralocorticoid receptor (MR) antagonist drugs decrease blood pressure (BP) and improve survival in systolic congestive heart failure.<sup>1-3</sup> It is well established that MR antagonists (MRAs) prevent the hormone aldosterone from activating renal MRs, thereby decreasing sodium and water retention.<sup>4</sup> More recently, it has become clear that MR is expressed in tissues outside of the kidney including in the cardiovascular system. In the vasculature, MR is expressed in medial smooth muscle cells (SMCs)<sup>5</sup> and in intimal endothelial cells (ECs).<sup>6</sup> SMCs and ECs function in concert to regulate arteriolar diameter, thereby globally controlling vascular resistance to contribute to systemic BP and locally modulating regional tissue blood flow.<sup>7</sup> Because the benefits of MRA are disproportionately greater than their

natriuretic properties, it has been postulated that some of their beneficial effects may be mediated by inhibition of vascular MR<sup>8</sup> by mechanisms that are only beginning to be elucidated.

Recent studies in mice specifically deficient in MR in SMC have demonstrated that SMC-MR contributes directly to regulation of systemic BP and to vasoconstriction.<sup>9-11</sup> Despite substantial effort, our understanding of the specific role of EC-MR in BP control and vasoreactivity has been more elusive. In obese rats, MR inhibition improves coronary endothelial-dependent vasodilation, and MR activation in healthy rats impairs coronary endothelial-dependent vasodilation.<sup>12</sup> In humans, MRA treatment improves brachial artery vasodilation in patients with congestive heart failure<sup>13</sup> and improves coronary flow reserve in diabetics<sup>14</sup> suggesting

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a role for endothelial MR in regulating vascular function in patients with cardiovascular disease or risk factors. Early studies exploring potential MR signaling mechanisms in cultured ECs revealed disparate effects of MR activation on endothelial nitric oxide synthase (eNOS) activity with reports of both MR-dependent inhibition and activation of eNOS activity or NO production.<sup>8,15</sup> Likewise, extensive experimentation has been performed in isolated vessels with variable effects of MR activation on vasoconstriction and vasodilation that may depend on the species, vascular bed, or experimental strategy used.<sup>8</sup> Overall, the data support a role for vascular MR in vasomotor control, yet the specific role of EC-MR is unclear from such studies in which the MR is activated or inhibited in SMCs and ECs simultaneously.

To examine the role of EC-MR in BP regulation and vasoreactivity, recent studies have used transgenic animals in which MR expression was specifically modulated in the endothelium. Mice overexpressing human MR in ECs have elevated BP and increased mesenteric myogenic tone and constriction, with no change in mesenteric endothelial-dependent vasodilation.<sup>16</sup> However, 2 groups recently deleted MR from ECs using a Tie2 promoter strategy, revealing no difference in basal BP or vasoconstriction.<sup>17,18</sup> Using this model, Rickard et al<sup>17</sup> demonstrated decreased aortic and mesenteric endothelial-dependent relaxation, whereas Schafer et al<sup>18</sup> found no change in aortic endothelial function in healthy animals but protection from obesity-associated aortic endothelial dysfunction. In addition to these conflicting results, these EC-MR knockout (EC-MR-KO) mouse models are complicated by MR deletion from leukocytes because of expression of the Tie2 promoter in bone marrow-derived cells.<sup>18</sup> This has made interpretation of the specific role of EC-MR in this model difficult in light of the recent identification of a role for leukocytes in BP regulation<sup>19</sup> and a role for MR in modulating leukocyte function.<sup>20</sup>

To clarify our understanding of the role of EC-MR in BP regulation and vasoreactivity, we generated an EC-MR-KO mouse with MR specifically deleted from ECs but with intact leukocyte MR using the VE-Cadherin (VE-Cad) promoter driving Cre-recombinase. Using this model, telemetry studies were performed to examine the role of EC-MR in BP regulation under normal ambulatory conditions and in response to sodium loading/restriction and to renin-angiotensin-aldosterone system activation. The role of EC-MR in endothelial-dependent relaxation and in vasoconstriction to multiple contractile agonists was explored in mesenteric and coronary arterioles to address the potential role of EC-MR in distinct vascular beds. The contribution of EC-MR to arteriolar vasoconstriction and to endothelial dysfunction after exposure to angiotensin II (AngII) hypertension was also examined in the model.

## Methods

Mice were handled in accordance with US National Institutes of Health standards, and all procedures were approved by the Institutions Animal Care and Use Committees. All experiments were conducted using male mice on the C57Bl/6 background, and EC-MR-KO mice were compared with MR-intact littermate controls. Primers are listed in Table S1 in the online-only Data Supplement. Detailed Methods are available in the online-only Data Supplement.

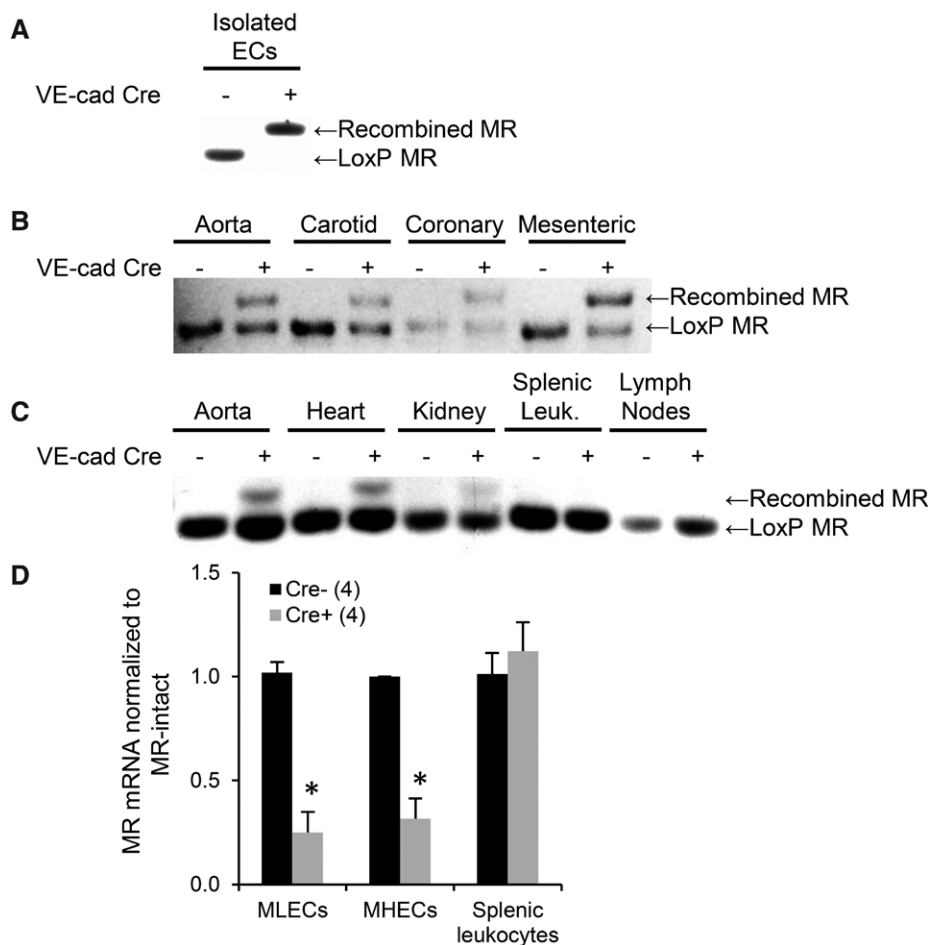
## Results

### Creating a Mouse Model With MR Specifically Deleted From ECs and Intact in Leukocytes

Mice with loxP sites flanking exons 5 and 6 of the MR gene (MR<sup>f/f</sup>)<sup>10</sup> were bred with mice containing a Cre-recombinase transgene driven by the EC-specific VE-cadherin promoter<sup>21</sup> (Cre+). EC-specific recombination of the MR gene was confirmed by polymerase chain reaction (Figure 1). Cultured ECs isolated from Cre+ mice showed complete MR DNA recombination, whereas ECs from Cre- littermates showed no MR gene recombination (Figure 1A). DNA isolated from the aorta and from carotid, coronary, and mesenteric arterioles shows MR recombination in all vessels but only from Cre+ mice (Figure 1B). DNA isolated from aorta, heart, and kidney of Cre+ mice revealed MR recombination consistent with the expected contribution of ECs in each tissue, whereas splenic leukocytes and lymph nodes contained only loxP MR, confirming lack of MR gene recombination in immune cells (Figure 1C). MR mRNA was also significantly decreased in primary cultured lung and heart ECs from Cre+ mice when compared to Cre- mice. MR mRNA expression in splenic leukocytes was unchanged in Cre+ mice (Figure 1D). These data confirm that the MR gene is specifically recombined in ECs in this mouse model with depletion of MR mRNA in ECs but not in immune cells (protein was not measured because of lack of specific mouse MR antibodies). The mice were born in Mendelian frequencies with no gross developmental differences supporting the conclusion that EC-MR is not necessary during embryological development (Table S2).

### EC-MR Does Not Contribute to Basal, Diurnal, Salt-Sensitive, or Renin-Angiotensin-Aldosterone System-Regulated BP

To clarify the role of EC-MR in BP control, EC-MR-KO and MR-intact littermates were implanted with radiotelemetry devices for continuous ambulatory BP measurements. Specific deletion of EC-MR did not affect systolic or diastolic BP, mean arterial pressure, heart rate, or activity level over the 24-hour cycle (Figure 2A; Figure S1). Systolic BP was unchanged from 4 to 8 months of age in EC-MR-KO mice (Figure 2B). Because MR in the kidney contributes to BP control in response to varying sodium conditions,<sup>22</sup> salt sensitivity of BP was assessed. The modest BP decrease with low sodium intake and the increase with high sodium intake was equivalent in both genotypes (Figure 2C) with no change in serum and urine electrolytes and food and water consumption (Table S2). Fractional excretion of sodium (FENa<sup>+</sup>) and the expected decline in FENa<sup>+</sup> on a low sodium diet were the same in both genotypes, thereby confirming intact renal MR function in EC-MR-KO mice (Figure 2D). We next investigated whether EC-MR participates in the BP response to renin-angiotensin-aldosterone system activation, a common contributor to hypertension.<sup>22</sup> BP increased to a similar extent in EC-MR-KO and MR-intact mice in response to aldosterone infusion with a further increase with addition of 1% sodium in the drinking water as did the response to AngII infusion,



**Figure 1.** A mouse model with mineralocorticoid receptor (MR) deleted specifically from endothelial cell (EC) and not from leukocytes. **A–C**, MR genomic DNA was amplified with primers specific for the LoxP MR or recombined MR. **A**, The MR gene is completely recombined in primary cultured mouse ECs only from Cre+ mice. **B**, MR recombination occurs in Cre+ aorta, carotid, coronary, and mesenteric vessels. **C**, MR recombination occurs only in EC-containing tissues in VE-cad-Cre+ mice and not in lymph nodes or splenic leukocytes (Leuk). **D**, MR mRNA is reduced in primary ECs cultured from mouse lungs (mouse lung endothelial cells [MLECs]) and hearts (MHECs) but not in leukocytes from EC-MR-knockout mice. Number of animals per group is indicated in parentheses. \* $P < 0.05$  vs MR-intact.

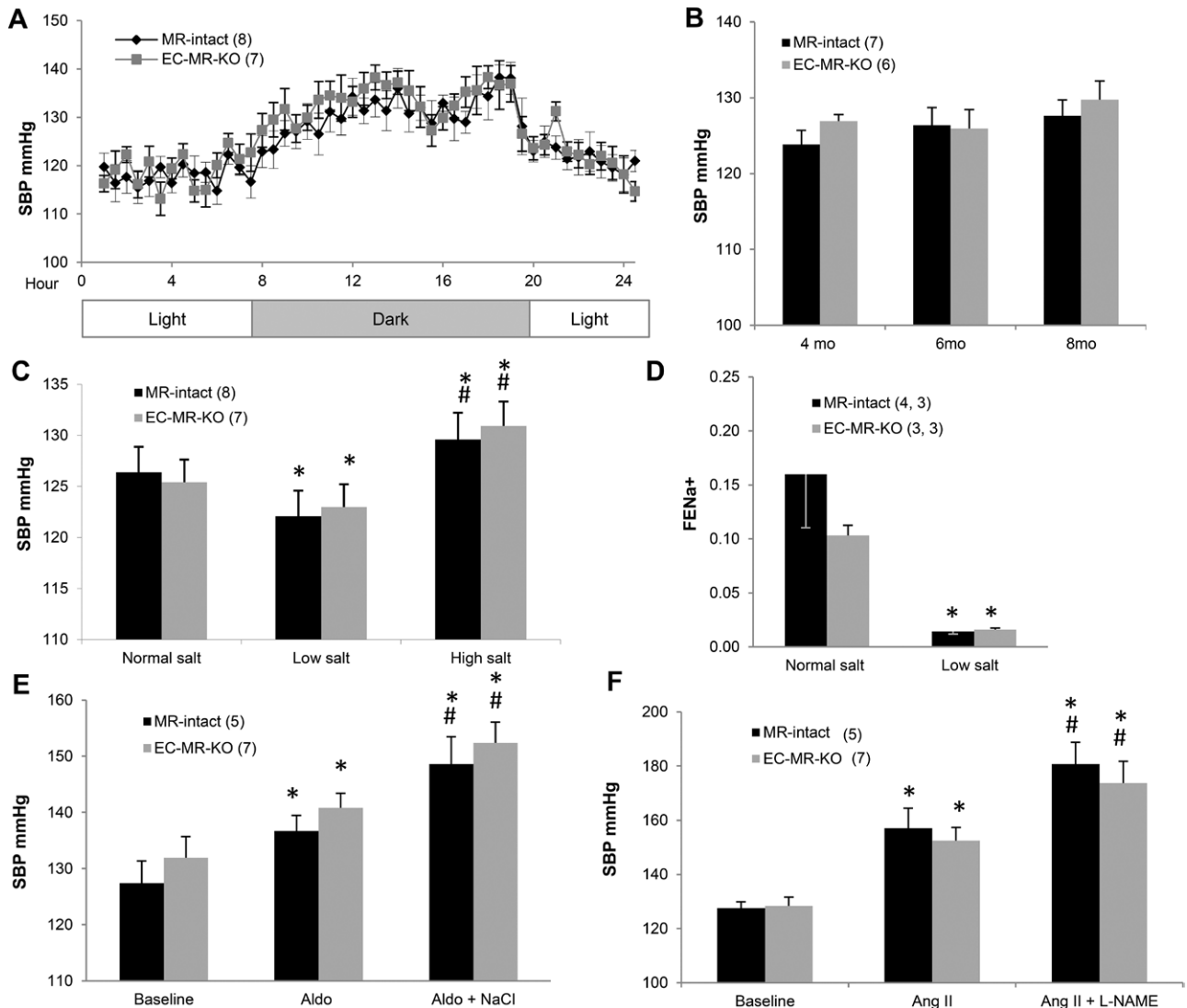
which is also further enhanced by the addition of the eNOS inhibitor L-nitroarginine methyl ester (L-NAME) (Figure 2E and 2F).

### EC-MR Deletion Does Not Affect Basal Mesenteric or Coronary Arteriolar Vasodilation

To address conflicting findings on the role of EC-MR in vasodilation, we examined endothelium-independent vasodilation to sodium nitroprusside and endothelium-dependent relaxation to acetylcholine in mesenteric and coronary arterioles. These vessels were chosen because mesenteric vasoreactivity contributes to regional blood flow and systemic vascular resistance, and coronary vascular function is critical to coronary flow reserve, an important predictor of cardiovascular outcomes that was recently found to be modulated by MRA treatment.<sup>14</sup> Wire myography revealed no difference in endothelium-independent or endothelium-dependent vasodilation in mesenteric or coronary arterioles from EC-MR-KO mice when compared with those from MR-intact littermates (Figure 3).

### EC-MR Modulates Mesenteric Endothelial-Dependent Vasodilation in the Setting of Angiotensin II-Induced Hypertension

Pressure myography was also used to assess endothelial-dependent vasodilation in EC-MR-KO mice when compared with that in controls at baseline and after exposure to 2 weeks of AngII hypertension (Figure 4). Differences in the COX1- and eNOS-dependent component (as measured after indomethacin and L-NAME inhibition) and in the endothelium-derived hyperpolarizing factor (EDHF)-dependent component (inhibited by SKCa channel inhibitor Apamin and IKCa channel inhibitor 1-[(2-chlorophenyl)diphenylmethyl]-1H-pyrazole [TRAM34]) were also examined. Figure 4A confirms in pressurized vessels that in healthy animals, EC-MR does not contribute to vasodilation to sodium nitroprusside or acetylcholine. Moreover, no difference was observed between the genotypes in the non-EDHF or EDHF contributions to vasodilation (Figure 4A) or in individual COX1- and eNOS-dependent components (Figure S2). However, after 14-day infusion with a pressor dose of AngII (same dose as in Figure



**Figure 2.** Endothelial cell-mineralocorticoid receptor (EC-MR) does not contribute to basal, salt-sensitive, aldosterone/salt-enhanced, or angiotensin-enhanced blood pressure (BP). **A**, Twenty-four-hour ambulatory blood pressure in healthy 6-month-old mice. **B**, Average systolic blood pressure (SBP) at 4, 6, and 8 mo of age in MR-intact and EC-MR-knockout (KO) mice. **C**, Average SBP in 5- to 6-month-old mice on days 3 to 5 of normal diet, low-salt diet, or high-salt diet. \* $P < 0.05$  vs normal diet; # $P < 0.05$  vs low-salt diet. **D**, Fractional excretion of sodium (FENa+) on normal diet and low-salt diet. \* $P < 0.05$  vs normal diet. **E** and **F**, SBP assessment in hypertension models in 7- to 8-month-old mice. **E**, SBP on day 4 of aldosterone infusion, day 9 of aldosterone + 1% NaCl in water. \* $P < 0.05$  vs baseline; # $P < 0.05$  vs aldosterone alone. **F**, BP on day 7 of angiotensin II (AngII) infusion; and on day 14 of AngII + L-nitroarginine methyl ester (L-NAME). \* $P < 0.05$  vs baseline; # $P < 0.05$  vs AngII alone. Number of animals per group is indicated in parentheses.

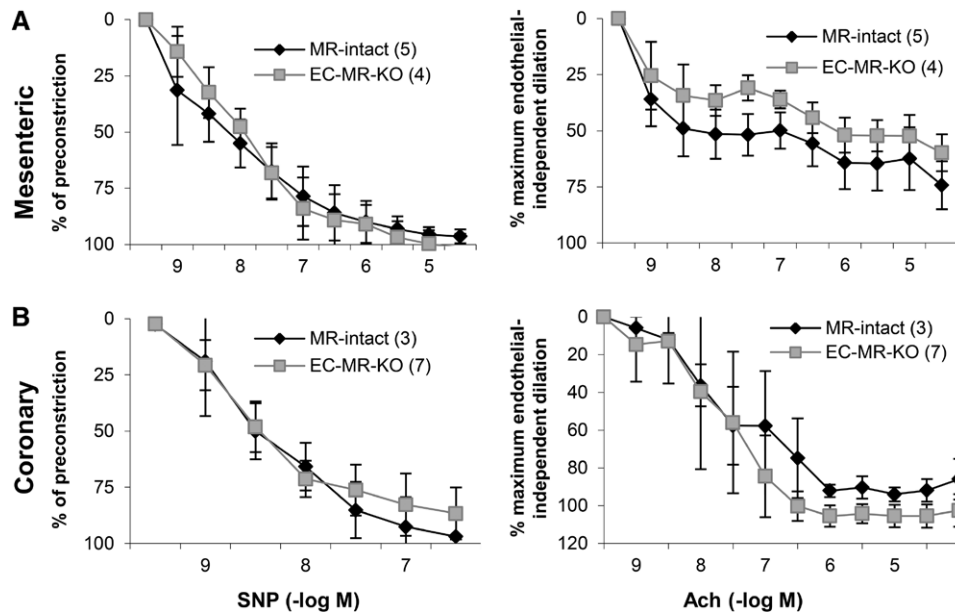
2F), EC-MR-KO vessels achieved a significantly greater maximal vasodilation than MR-intact controls in the absence of inhibitors (Figure 4B). There was equivalent precontraction to phenylephrine (Figure S3) with no difference between genotypes in endothelial-independent vasodilation (Figure 4B). The improvement in endothelial function in EC-MR-KO mesenteric vessels was eliminated by pretreatment with L-NAME + indomethacin, supporting that EC-MR-KO maintains the acetylcholine response after AngII hypertension by enhancing eNOS or COX1 production of paracrine vasodilators. After L-NAME and indomethacin pretreatment, EC-MR-KO vessels displayed decreased dilation to  $10^{-7}$  M acetylcholine, suggesting a difference in EDHF. Addition of Apamin + TRAM34 eliminated vasodilation to acetylcholine in EC-MR-KO vessels, supporting that this is indeed because

of an EDHF-mediated mechanism. Interestingly, in the MR-intact mice, there was a residual vasodilation with all 4 inhibitors that may represent an additional EC-MR-regulated mechanism of endothelial-dependent vasodilation that is activated in response to AngII hypertension.

Contrary to the mesenteric vessels, after exposure to AngII hypertension, coronary endothelium-dependent vasodilation was not different between genotypes (Figure S4), supporting vascular bed-specific differences in the role of EC-MR in hypertension-induced alterations in endothelial function.

### EC-MR Differentially Modulates Vasoconstriction in a Vascular Bed-Specific Fashion

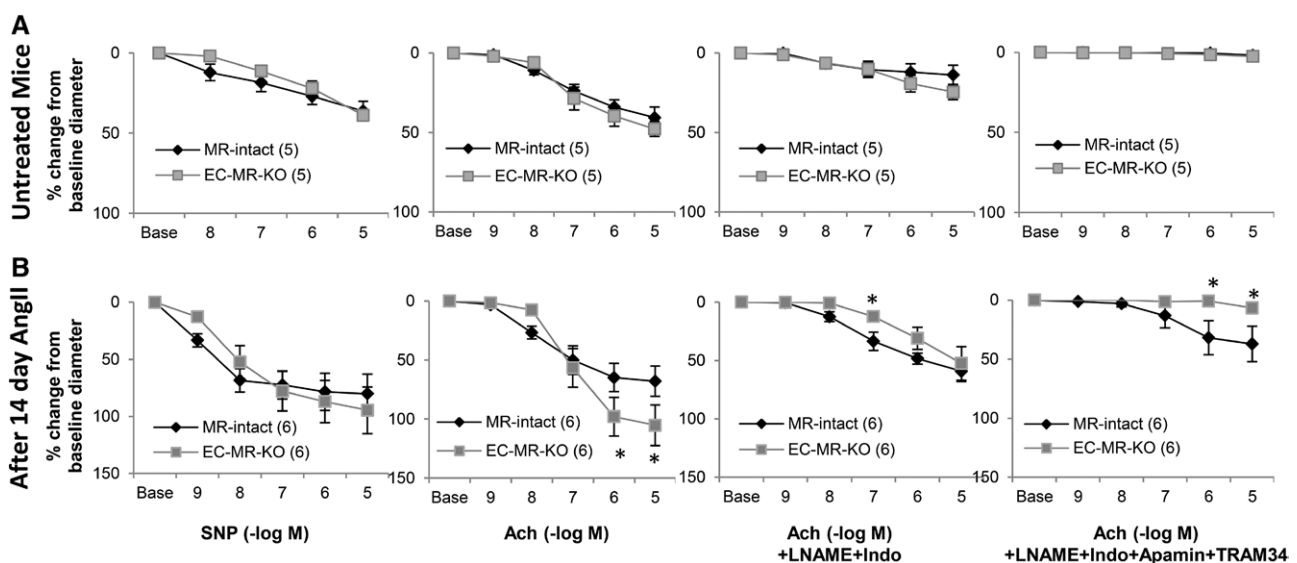
To clarify conflicting findings for the role of EC-MR in vasoconstriction,<sup>8</sup> we assessed the specific role of EC-MR in the



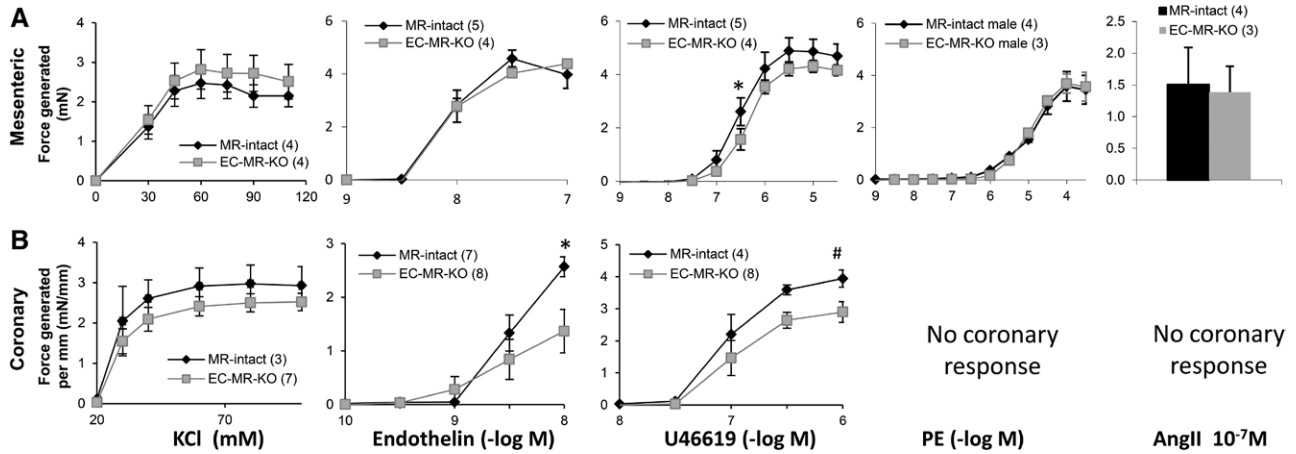
**Figure 3.** Endothelial cell-mineralocorticoid receptor (EC-MR) does not contribute to mesenteric or coronary vasodilation. Sodium nitroprusside (SNP) and acetylcholine (Ach) dose-response curves were assessed by wire myography in (A) mesenteric arterioles precontracted with phenylephrine (1  $\mu\text{mol/L}$ ) and (B) coronary arterioles precontracted with U46619 (0.1–0.3  $\mu\text{mol/L}$ ). Number of animals per group is indicated in parentheses. KO indicates knockout.

responses of mesenteric and coronary arterioles to various contractile agonists. In mesenteric vessels, there was no difference in constriction between EC-MR-KO and MR-intact controls to potassium chloride, endothelin-1, phenylephrine, or AngII with a decrease in constriction only to the thromboxane agonist U46619 in EC-MR-KO at  $3 \times 10^{-7}$  M (Figure 5A). Mesenteric myogenic constriction in response to increasing intraluminal pressure was also measured, revealing no significant differences between EC-MR-KO and MR-intact

mesenteric vessels in the level of myogenic tone (Figure S5), consistent with the lack of a difference in BP. Interestingly, coronary vessels from EC-MR-KO mice showed a decrease in constriction to U46619 (26% decrease at a dose of  $10^{-6}$  M) and a pronounced decrease in constriction to endothelin-1 (49% decrease at  $10^{-8}$  M) when compared with vessels from MR-intact littermates (Figure 5B). These data support the concept that EC-MR differentially contributes to vasoconstriction depending on the vascular bed and on the contractile stimulus.



**Figure 4.** Endothelial cell-mineralocorticoid receptor (EC-MR) deletion modulates mesenteric endothelial-dependent relaxation after exposure to angiotensin II (AngII)-induced hypertension. Mesenteric arterioles were isolated from untreated mice (A) or mice exposed to 14-day AngII infusion (B), and the change in vessel diameter after preconstriction with phenylephrine (100 nmol/L) was assessed by pressure myography. Relaxation-response curves are shown to sodium nitroprusside (SNP), acetylcholine (Ach), Ach+L-nitroarginine methyl ester (L-NAME)+indomethacin (Indo), or Ach+L-NAME+Indo+Apamin+1-[(2-chlorophenyl)diphenylmethyl]-1H-pyrazole (TRAM34). Number of animals per group is indicated in parentheses. \* $P < 0.05$  vs MR-intact KO indicates knockout.



**Figure 5.** Endothelial cell-mineralocorticoid receptor (EC-MR) differentially regulates vasoconstriction depending on the vascular bed and the contractile agonist. Dose-response curves for contractile force were recorded by wire myography in (A) mesenteric arterioles in response to potassium chloride (KCl), endothelin-1, the thromboxane agonist U46619, phenylephrine (PE), or a single dose of AngII; and in (B) coronary arterioles (normalized to length of the vessel segment) to KCl, endothelin-1, and U46619. No graph is included for PE and AngII as coronary arterioles did not respond to those agonists. Number of animals per group is indicated in parentheses. \* $P < 0.05$ , # $P < 0.005$  vs EC-MR-knockout (KO).

### EC-MR Also Contributes to Coronary Vasoconstriction to Endothelin-1 and Thromboxane After Hypertension and to Expression of Endothelin-B Receptor in Coronary ECs

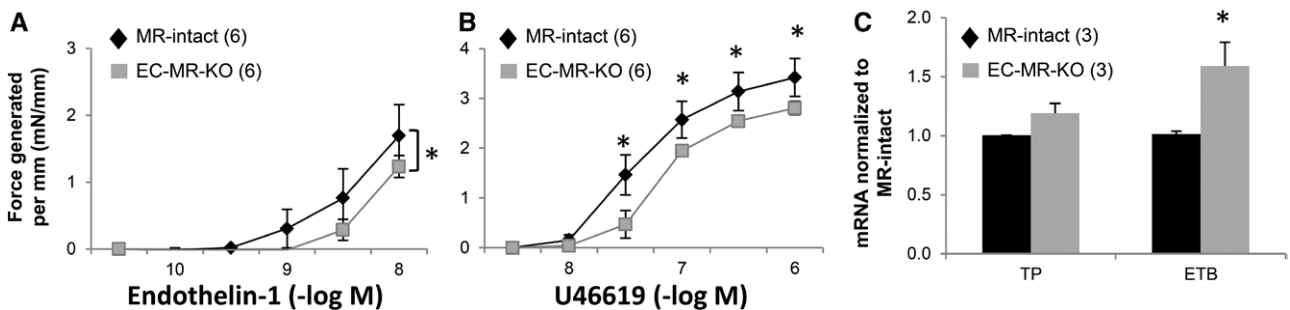
Coronary vasoconstriction to endothelin-1 and thromboxane was also measured after 14-day exposure to AngII hypertension. The decreased coronary contractile response to endothelin-1 and thromboxane in EC-MR-KO mice persisted after exposure to hypertension and was significant over a greater range of agonist concentrations after exposure to AngII (Figure 6A and 6B).

Several potential mechanisms for decreased coronary vasoconstriction in EC-MR-KO mice were considered. The contractile differences were not because of alterations in coronary vasodilation at baseline (Figure 3) or after AngII exposure (Figure S4). SMC L-type calcium channels are required for vascular constriction; however, single cell patch-clamp recordings in freshly dispersed coronary (and mesenteric) SMC revealed no difference in L-type calcium channel function in SMCs from EC-MR-KO mice

(Figure S6). mRNA expression levels of the thromboxane receptor and of the vasodilatory endothelin-B receptor ( $ET_B$ ; known to be expressed on ECs) were measured from primary mouse heart ECs.  $ET_B$  expression was significantly increased (59%) in coronary ECs from EC-MR-KO when compared with coronary ECs from MR-intact mice (Figure 6C), supporting the possibility that EC-MR contributes to coronary vasoconstriction in response to endothelin-1 by regulating coronary endothelial  $ET_B$  expression. Thromboxane receptor mRNA was unchanged in coronary ECs from EC-MR-KO mice, suggesting a different mechanism for the enhanced coronary constriction to U46619 in mice deficient in EC-MR.

### Discussion

In summary, we developed a truly EC-specific MR-KO mouse model with intact immune cell MR to clarify the specific role of EC-MR in BP regulation and vascular function. Using this model, we demonstrated that (1) EC-MR deletion does not alter renal sodium handling, basal BP, or BP after diverse hypertensive stimuli, (2) EC-MR does not contribute



**Figure 6.** Decreased coronary vasoconstriction to endothelin-1 and U46619 after hypertension and decreased endothelial cell (EC) endothelin-B receptor mRNA in EC-mineralocorticoid receptor (MR)-knockout (KO) mice. Coronary vasoconstriction was assessed by wire myography after 14-day angiotensin II (AngII) infusion to a dose escalation of (A) endothelin-1 and (B) thromboxane agonist U46619. \* $P < 0.05$  vs MR-intact. C, Expression of the thromboxane receptor (TP) and the endothelin-B receptor (ETB) was measured in mRNA isolated from mouse cardiac endothelial cells. Number of animals or experiments is indicated in parentheses. \* $P < 0.01$  vs MR-intact.

to basal endothelium-dependent relaxation in mesenteric or coronary arterioles; (3) EC-MR deletion improves mesenteric (but not coronary) endothelial function after exposure to AngII hypertension, and (4) EC-MR differentially regulates vasoconstriction in distinct vascular beds, enhancing the responsiveness of coronary arterioles to endothelin-1 and thromboxane both at baseline and after AngII hypertension. Overall, these studies provide substantial new data supporting that EC-MR does not contribute to BP regulation in mice under basal and most experimental conditions. Rather, these data uncover a role for EC-MR in regulation of vasoconstriction and in modulation of endothelial function after hypertension that is vascular bed specific and may explain some of the seemingly conflicting findings in previous studies. These results also support new therapeutic opportunities for targeting pathways critical to maintenance of tissue-specific perfusion, particularly to the heart, in the setting of cardiovascular disease.

This study provides substantial clarification of the role of EC-MR in BP control in the context of the existing literature. It is clear that MR in the kidney contributes to BP regulation, and it has recently been demonstrated by 2 groups that MR in SMC also contributes to BP regulation by modulating vascular tone.<sup>9,10</sup> This study provides the first data using telemetry as the gold standard approach to BP measurement in a model in which MR is deleted from ECs. From these data, we conclude that EC-MR does not contribute to systolic, diastolic, mean, diurnal, or salt-sensitive hypertension. Furthermore, EC-MR deletion does not alter the response to typical hypertensive stimuli including mineralocorticoid excess±salt or AngII±L-NAME. This is consistent with the previously published Tie2-Cre EC-MR-KO model in which BP was measured by tail cuff and was not different in Cre+ animals at baseline or after uninephrectomy/deoxycorticosterone acetate/salt treatment.<sup>17</sup> The finding that EC-MR deletion does not modulate BP contrasts with the substantially elevated BP in the transgenic mouse overexpressing human MR in the endothelium.<sup>16</sup> Although it is possible that the hypertension in the overexpression model is because of supraphysiologic levels of MR expression or a functional difference between human and mouse MR, it is also possible that there are conditions in which MR is substantially upregulated in the endothelium (such as aging or heart failure) in which EC-MR might play an enhanced role in BP control, and additional studies are needed to explore those possibilities.

These results also add to a growing body of literature supporting the idea that EC-MR may have a minimal (or even vasodilatory) role in vessels under basal conditions,<sup>8</sup> but that EC-MR participates in the development of endothelial dysfunction in response to cardiac risk factors including obesity<sup>18</sup> and now also after exposure to hypertension.<sup>23</sup> We demonstrate no difference in mesenteric or coronary vasodilation in healthy EC-MR-KO mice as Schafer et al<sup>18</sup> did in the aorta of Tie2-MR-KO mice. This contrasts with the study by Rickard et al,<sup>17</sup> showing decreased aortic and mesenteric endothelial-dependent relaxation in Tie2-MR-KO mice. The difference in mesenteric vasodilation in the Rickard study could be because of the Tie2-Cre model if immune cell MR

contributes to mesenteric vascular function, or to technical variations in how the vessel studies were performed. However, after exposure to risk factors, emerging data support a model in which EC-MR contributes to changes in endothelial function induced by obesity<sup>18</sup> and hypertension. The enhanced vasodilation in EC-MR-KO mesenteric vessels after AngII-induced hypertension was eliminated by pretreatment with L-NAME+indomethacin, supporting that EC-MR activation in the setting of AngII-induced hypertension may inhibit the production of NO or prostacyclin. The potential decrease in the EDHF contribution to relaxation in EC-MR-KO vessels is consistent with studies showing that EC-MR regulates the vasodilatory potassium channel component of EDHF, SKCa.<sup>24</sup> The presence of residual vasodilation after treatment with L-NAME+indomethacin+apamin+TRAM34 only in MR-intact mice suggests the possibility of an unidentified EC-MR-dependent vasodilator component that is activated by AngII hypertension. Additional studies are needed to determine the detailed role and mechanism by which EC-MR regulates each component of vasodilation not only in this AngII hypertension model but also with other hypertensive stimuli including high sodium or other doses of AngII or aldosterone, or with other cardiovascular stresses such as obesity, diabetes mellitus, or heart failure.

In this study, EC-MR contributed to endothelial dysfunction in response to AngII without a difference in BP. Although endothelial dysfunction is not always associated with changes in BP, it is an independent risk factor for cardiovascular disease, and therapies that improve endothelial function, also improve outcomes. Although the specific role of EC-MR is difficult to determine in humans, clinical data support that MR activation may play a greater role in endothelial function in patients with high cardiovascular risk, including those with hypertension, diabetes mellitus, and heart failure<sup>13,14,25,26</sup> rather than in those with a healthy vasculature.<sup>27</sup> Thus, MR antagonism may provide clinical benefit in the setting of cardiovascular risk factors, such as hypertension or obesity, by interfering with the development of endothelial dysfunction, an early step in the development of atherosclerosis that also has negative prognostic implications.<sup>28,29</sup>

This study further demonstrates that EC-MR contributes to vasoconstriction in a manner that depends on the vascular bed examined and on the contractile agonist tested. This finding might explain what seem to be conflicting results in the literature. For example, the EC-MR-overexpressing mouse showed enhanced mesenteric vasoconstriction,<sup>16</sup> whereas the Tie2-MR-KO mouse showed no difference in vasoconstriction in the aorta,<sup>18</sup> perhaps because of vascular bed differences. However, our study is consistent with the study by Rickard et al<sup>17</sup> as EC-MR-deletion had no effect on mesenteric constriction in both studies. In addition, this is the first study to explore the specific role of EC-MR in coronary function, and our data revealing a role for EC-MR in coronary vasoconstriction to endothelin-1 and thromboxane might explain the recent finding that MRAs reverse coronary vascular dysfunction in a rat model of obesity.<sup>12</sup>

The mechanism by which EC-MR exerts site-specific effects on vascular function remains to be explored. ECs from different locations in the vasculature have distinct biochemical

and biomechanical forces to which they are exposed resulting in unique mRNA transcriptomes and epigenetic profiles.<sup>30</sup> Thus, potential mechanisms may include vascular bed-specific differences in expression of MR itself, of other components of the renin-angiotensin-aldosterone system, of other transcription factors and cofactors that interact with the MR, and of downstream mediators of contractile signaling pathways in distinct EC populations. Intact endothelial-independent vasodilation and coronary SMC calcium channel function suggest that SMC function is intact in EC-MR-KO mice. The finding that decreased endothelin-induced coronary vasoconstriction is associated with increased ET<sub>B</sub> receptor mRNA in mouse cardiac ECs from EC-MR-KO mice may suggest one potential mechanism. Activation of endothelial ET<sub>B</sub> receptors by endothelin-1 induces vasorelaxation that is known to counter the constrictive response in the SMCs in an NO-dependent manner.<sup>31</sup> Previous work has also shown a role for MR in post-translational inactivation of ET<sub>B</sub> in cultured ECs.<sup>32</sup> However, the lack of a change in thromboxane receptor mRNA despite a change in thromboxane-induced constriction demonstrates that additional mechanisms remain to be elucidated.

The finding of decreased constriction to endothelin-1 and thromboxane in coronary arteries from EC-MR-KO mice has important clinical implications. Thromboxane is released by activated platelets in the coronary arteries during acute myocardial infarction (MI) thereby contributing to vasoconstriction at the site of plaque rupture that exacerbates myocardial necrosis. Thromboxane-mediated vasoconstriction has been implicated in the morbidity of acute MI, heart failure, diabetes mellitus, and disorders of primary vasospasm such as migraine headaches.<sup>33</sup> In patients presenting with acute MI, higher circulating thromboxane A2 or endothelin-1 levels correlate with worse outcomes, including increased risk of poor myocardial perfusion after percutaneous coronary intervention (no reflow).<sup>34,35</sup> Endothelin-1 levels are also associated with decreased coronary flow reserve and decreased ejection fraction after MI.<sup>36</sup> MRAs reduce mortality after MI complicated by left ventricular dysfunction<sup>2</sup> and improve coronary flow reserve in patients with diabetes mellitus.<sup>14</sup> Thus, clinical studies suggest that exaggerated thromboxane and endothelin-1 signaling worsen outcomes after MI and that MR antagonism is protective. Data from this study support a novel explanation for the benefits of MR antagonism in coronary artery disease by demonstrating that EC-MR deletion attenuates thromboxane and endothelin-1-mediated coronary vasoconstriction.

There are several important limitations to this study. First, the lack of a specific mouse MR antibody restricts our ability to compare EC-MR protein levels in different beds or to show loss of MR protein from ECs. Instead, we confirmed MR DNA recombination in multiple vascular beds and MR DNA recombination and loss of MR mRNA in primary ECs cultured from EC-MR-KO mice. MR mRNA expression is reduced 75% in cultured EC-MR-KO ECs, consistent with reductions seen in mRNA expression in other endothelial-targeted MR-KO mice<sup>18</sup> and in other KO mice using the VE-Cadherin promoter.<sup>37</sup> Whether this is because of contaminating SMC in the EC cultures or from incomplete MR deletion from ECs is difficult to discern, but incomplete recombination would

only underestimate the role of EC-MR in vascular function. Because we identified a vascular bed-specific role for EC-MR in the mesentery and coronary microcirculation, another important limitation is that the results cannot be generalized to other vascular beds. Additional studies are needed to understand the role of EC-MR in regulating blood flow to other critical tissues, by examining cerebral, carotid, renal, and skeletal muscle microvessels in this model. Throughout the study, EC-MR-KO mice and MR-intact littermates were compared under identical conditions; however, direct comparisons cannot be made between healthy mice and those treated with AngII because the studies were not performed simultaneously (not littermates) and the untreated mice did not undergo osmotic minipump implantation surgery. Finally, for practical reasons, all experiments were performed only in male mice. There is substantial evidence that male and female mice and humans differ in their vascular function and BP responses,<sup>38,39</sup> and MR transcriptional activity was recently found to be modulated by estrogen.<sup>40</sup> Indeed, the coronary response to endothelin-1 is modified by aging in a sex-dependent manner.<sup>41</sup> Thus, additional studies are needed to characterize the role of EC-MR in female mice.

## Perspectives

MRAs decrease BP and mortality in cardiovascular disease. Over the past decade, it has become clear that MR contributes to cardiovascular function and disease in multiple tissues in addition to the kidney. This study used a unique mouse with EC-specific MR deletion with intact leukocyte MR to demonstrate that EC-MR does not contribute to BP regulation. EC-MR does contribute to vasoconstriction, specifically in the coronary arteries in response to endothelin-1 and thromboxane. This study suggests that inhibition of EC-MR may contribute to the mechanism by which MRAs improve coronary vascular function in diabetic patients and in animal models of obesity. Furthermore, although EC-MR does not play a role in endothelial function in healthy vessels, it contributes to mesenteric endothelial dysfunction in the setting of AngII hypertension. Thus, although EC-MR does not seem to contribute to BP regulation, EC-MR differentially regulates vasoconstriction depending on the vascular bed and modulates vasodilation in the context of cardiovascular risk factors with important clinical implications.

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## Disclosures

None.

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## Novelty and Significance

### What Is New?

- Using a novel mouse with the mineralocorticoid receptor (MR) specifically deleted from endothelial cells (and not leukocytes), this study demonstrates that endothelial cell-MR does not play a role in basal blood pressure control or in salt-sensitive or renin–angiotensin–aldosterone system–driven hypertension. This study reveals that endothelial cell-MR contributes to mesenteric vasodilation after exposure to angiotensin II hypertension and to regulation of coronary but not mesenteric vasoconstriction.

### What Is Relevant?

- These results reveal vascular bed– and disease-specific roles for endothelial cell-MR that may explain conflicting findings in the literature.

Moreover, the results suggest that one mechanism by which MR antagonists improve cardiovascular outcomes is by inhibiting endothelial cell-MR, thereby preventing coronary vasoconstriction in patients with cardiovascular disease.

### Summary

Endothelial MR does not contribute to blood pressure regulation but does contribute to coronary vasoconstriction and to hypertension-induced mesenteric endothelial dysfunction.