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

Katelee Barrett Mueller, Shawn B. Bender, Kwangseok Hong, Yan Yang, Mark Aronovitz, Frederic Jaisser, Michael A. Hill, Iris Z. Jaffe

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±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;66:00-00. DOI: 10.1161/HYPERTENSIONAHA.115.06172.)

• Online Data Supplement

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

A mple clinical trial data reveal that mineralocorticoid receptor (MR) antagonist drugs decrease blood pressure (BP) and improve survival in systolic congestive heart failure.1-3 It is well established that MR antagonists (MRAs) prevent the hormone aldosterone from activating renal MRs, thereby decreasing sodium and water retention.4 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)5 and in intimal endothelial cells (ECs).6 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.7 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 MR8 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.9-11 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.12 In humans, MRA treatment improves brachial artery vasodilation in patients with congestive heart failure13 and improves coronary flow reserve in diabetics,14 suggesting
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. 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. 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. However, 2 groups recently deleted MR from ECs using a Tie2 promoter strategy, revealing no difference in basal BP or vasoconstriction. Using this model, Rickard et al demonstrated decreased aortic and mesenteric endothelial-dependent relaxation, whereas Schafer et al 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 and splenic leukocytes and lymph nodes contained only loxP MR, confirming lack of MR gene recombination in immune cells 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 deletion 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).

**Result**

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 (MRf/f) were bred with mice containing a Cre-recombinase transgene driven by the EC-specific VE-cadherin promoter (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 arteries 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, 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) and the expected decline in FENa 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. Increased 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.

**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.
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.14 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 2A)
EC-MR-KO vessels achieved a significantly greater maximal vasodilation than MR-intact controls in the absence of inhibitors (Figure 4B). There was equivalent preconstriction 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 \( \text{L-NAME} + \text{indomethacin} \), supporting that EC-MR-KO maintains the acetylcholine response after AngII hypertension by enhancing eNOS or COX1 production of paracrine vasodilators. After \( \text{L-NAME} \) 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,\(^8\) we assessed the specific role of EC-MR in the...
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 3×10^{-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 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 preconstricted with phenylephrine (1 µmol/L) and (B) coronary arterioles preconstricted with U46619 (0.1–0.3 µmol/L). Number of animals per group is indicated in parentheses. KO indicates knockout.

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.
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 (ETB, known to be expressed on ECs) were measured from primary mouse heart ECs. ETB 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 ETB 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...
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.9,10 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 systemic, 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±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.17 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.16 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 these 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, but that EC-MR participates in the development of endothelial dysfunction in response to cardiac risk factors including obesity14 and now also after exposure to hypertension.32 We demonstrate no difference in mesenteric or coronary vasodilation in healthy EC-MR-KO mice as Schäfer et al18 did in the aorta of Tie2-MR-KO mice. This contrasts with the study by Rickard et al.17 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 obesity19 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.24 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 dysfunction in patients with high cardiovascular risk, including those with hypertension, diabetes mellitus, and heart failure13,14,25,26 rather than in those with a healthy vasculature.27 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.28,29

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,16 whereas the Tie2-MR-KO mouse showed no difference in vasoconstriction in the aorta,18 perhaps because of vascular bed differences. However, our study is consistent with the study by Rickard et al11 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.12

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. 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 ETB receptor mRNA in mouse cardiac ECs from EC-MR-KO mice may suggest one potential mechanism. Activation of endothelial ETB receptors by endothelin-1 induces vasorelaxation that is known to counter the constrictive response in the SMCs in an NO-dependent manner. Previous work has also shown a role for MR in post-translational inactivation of ETB in cultured ECs. 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. 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). Endothelin-1 levels are also associated with decreased coronary flow reserve and decreased ejection fraction after MI. MRAs reduce mortality after MI complicated by left ventricular dysfunction and improve coronary flow reserve in patients with diabetes mellitus. 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 and in other KO mice using the VE-Cadherin promoter. 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, and MR transcrip tional activity was recently found to be modulated by estrogen. Indeed, the coronary response to endothelin-1 is modified by aging in a sex-dependent manner. 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.

**Sources of Funding**

This work was funded by grants from the National Institutes of Health (HL095590 and HL119290 to I.Z. Jaffe), the American Heart Association (EIA18290005 to I.Z. Jaffe and PRE16920014 to K. Barrett Mueller), and the Department of Veterans Affairs BLR&D (CDA-2 BX002030 to S.B. Bender). This work was also supported by resources and the use of facilities at the Harry S Truman Memorial Veterans Hospital in Columbia, MO.

**Disclosures**

None.

**References**


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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.
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Hypertension. published online September 8, 2015;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/early/2015/09/08/HYPERTENSIONAHA.115.06172

Data Supplement (unedited) at:
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SUPPLEMENTAL MATERIAL

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

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SUPPLEMENTAL MATERIAL

Supplemental Methods:

Generation of endothelial cell-specific mineralocorticoid receptor knockout mice:

All mice were handled in accordance with US National Institutes of Health standards, and all procedures were approved by the Institutional Animal Care and Use Committees at the relevant institution. Mice lacking mineralocorticoid receptor (MR) specifically in endothelial cells (ECs) were generated by breeding mice with a loxP sites-flanking critical exons of the MR gene (MR$^{fl/fl}$) with mice containing a Cre recombinase transgene driven by the EC-specific vascular endothelial (VE)-cadherin promoter (Cre$^+$). All comparisons are made between male MR$^{fl/fl}$-VECad-Cre$^+$ (EC-MR-KO) and MR$^{fl/fl}$-VECad-Cre$^-$ (MR-intact) littermates.

Isolation of primary endothelial cells:

Mouse lung and coronary ECs were isolated as described previously. In brief, hearts and lungs were harvested from 5-8 mice at 2-4 weeks of age. Organs were chopped finely with razor blades and digested in 2mg/ml of collagenase shaking at 37°C for 30 minutes. The digested organs were mechanically dissociated by trituration, filtered through a 70µm disposable cell strainer (Becton Dickinson Labware, Bedford, MA) and centrifuged at 500 rpm for 8 minutes at 4°C. Sheep anti-rat-IgG Dynabeads were coated with anti-platelet endothelial cell adhesion molecule-1 (PECAM-1, Pharmingen) or anti-intercellular adhesion molecule 2 (ICAM-2) monoclonal antibody (Pharmingen, 2.5µg antibody/2x10^7 beads) per the manufacturer’s instructions. Beads were prepared and kept at 4°C (4x10^8 beads/ml of Dulbecco’s phosphate-buffered saline (DPBS) with 0.1% FCS without sodium azide). The cell pellet was resuspended in cold DPBS and incubated with PECAM-1-coated beads (15µl/ml of cells) at room temperature for 10 minutes with end-over-end rotation. Magnetic isolation was used to recover the bead-bound cells. The recovered cells were washed with Dulbecco’s modified eagle medium (DMEM containing 20% fetal calf serum (FCS) (DMEM-20%), resuspended in 10 ml of complete culture medium, (DMEM-20% supplemented with 100µg/ml heparin, 100µg/ml endothelial cell growth stimulant, nonessential amino acids, sodium pyruvate, L-glutamine, and antibiotics, at standard concentrations), and then plated in two gelatin-coated 65-mm dishes. After 48 hours in culture, media was removed and filtered through a Steriflip strainer. Media was replaced every two days with 50% conditioned, sterile-filtered medium plus 50% complete culture medium. When cells reached 75 to 85% confluence, they were detached with warm trypsin-ethyleneediaminetetraacetic acid (Life technologies), pelleted and resuspended in DPBS and sorted again with ICAM-2-coated beads (15µl/ml of cells). Bead-bound cells were washed and plated in complete culture medium and passaged further at a1:3 ratio. Monolayers of mouse lung endothelial cell (MLEC) isolates and mouse coronary endothelial cell (MCEC) isolates were used at passages 2 to 4 for experiments.

PCR analysis of Nr3c2 genomic DNA:

Aorta, carotid, mesenteric artery, coronary artery, heart, kidney, and lymph nodes were isolated from EC-MR-KO and MR-intact littermates and frozen in liquid nitrogen. Splenic leukocytes were isolated by grinding of whole spleens through a 70µm filter followed by
treatment with Red Blood Cell Lysing Buffer Hybri-Max (Sigma). DNA was extracted from each tissue, from cultured ECs, or isolated leukocytes with the DNeasy kit (Qiagen) and PCR was performed as described using a combination of three primers listed in Supplemental Table 1.

Quantitative RT-PCR:

RNA was extracted and reverse transcribed with an RNeasy mini kit (Qiagen), and quantitative RT-PCR was performed with gene-specific primers as previously described. C<sub>T</sub> values were normalized to β2-microglobulin (B2MG) and mRNA levels in EC-MR-KO samples were expressed as a percentage of those in MR-intact samples. Specific primers for quantitative RT-PCR are listed in Supplemental Table S1.

Blood pressure measurement by telemetry:

For all surgical procedures, mice were anesthetized using 2.5% isofluorane gas. All blood pressure studies were performed using implantable blood pressure transmitters (Data Sciences International, TA11PA-C10) with \( n = 4–8 \) mice per group and blood pressure was recorded for 60 seconds every 30 min as previously described. Mice were maintained on a 12 hour light-dark cycle with normal chow (0.3% NaCl; Harlan diet TD8604) and water available ad libitum. For salt challenges, mice with telemetric devices were fed a low-salt diet (0.02% NaCl; Harlan diet TD90228) or a high-salt diet (6% NaCl; Harlan diet TD90230) for 5 days with normal chow washout for 7 days in between, and blood pressure was averaged on days 3–5 of each diet. For aldosterone and salt administration, osmotic minipumps were implanted (Alzet) to infuse aldosterone (Sigma) at 240 μg kg<sup>−1</sup> d<sup>−1</sup>, for 2 weeks. BP was measured on Day 4. On Day 5, 1% NaCl was added to the drinking water followed by BP measurement on Day 9. For AngII administration, osmotic minipumps were implanted (Alzet) to infuse AngII (Sigma) at 800 ng kg<sup>−1</sup> min<sup>−1</sup> for 2 weeks. BP was measured on Day 7. After 1 week, L-NAME (0.2mg/ml, Sigma) was added to the drinking water and BP was measured on Day 14.

Serum and urine chemistries:

Serum aldosterone was measured by radioimmunoassay (MP Biomedicals). Twenty-four–hour urine and simultaneous serum samples were collected from mice fed normal (0.3%) or low-salt chow in metabolic cages. Electrolytes were quantified (IDEXX Preclinical Services) and fractional excretion of sodium (FENa<sup>+</sup>) was calculated: FENa<sup>+</sup> = (serum Cr × urine Na) / (serum Na × urine Cr) × 100.

Wire myography:

Acetylcholine (Ach), indomethacin, L-N<sup>⁶</sup>-nitroarginine methyl ester (L-NAME), phenylephrine (PE), and sodium nitroprusside (SNP) were purchased from Sigma-Aldrich (St. Louis, Mo, USA). Endothelin-1 and thromboxane A2 analog U46619 were purchased from Tocris (Bristol, UK). Chemical agents were dissolved in physiologic saline solution (PSS)/Kreb’s buffers or dimethyl sulfoxide (only for U46619) based on solubility. Vessel segments (endothelium intact, 1.5–2 mm in length) were micro-dissected and mounted for isometric tension recordings (Danish Myo Technology, Model 610, Aarhus, Denmark) and data was analyzed using PowerLab software (AD Instruments) under conditions optimized for responsiveness of each vascular bed as
described below. Ring segments were then equilibrated for 30 minutes. The vessels were maintained in PSS gassed (95% O₂ and 5% CO₂) to maintain pH (7.4) at 37°C.

Mesenteric myography:

Mesenteric arterioles were brought to a resting tension of 2 millinewtons (mN) in tissue baths containing warmed (37 °C), aerated (95% O₂, 5% CO₂) PSS containing (in mM): 130 sodium chloride (NaCl), 4.7 potassium chloride (KCl), 1.17 magnesium sulfate (MgSO₄), 0.03 ethylenediaminetetraacetic acid (EDTA), 1.6 calcium chloride (CaCl₂), 14.9 sodium bicarbonate (NaHCO₃), 1.18 potassium phosphate (KH₂PO₄) and 5.5 glucose. Administration of 1 μM phenylephrine (PE) was used to test arteriolar viability, and the presence of intact endothelium was confirmed by acetylcholine (Ach, 1 μM)-induced relaxation of a half-maximal PE-induced contraction. Vasoconstrictor responses were assessed to PE (10⁻⁹-10⁻⁴M), endothelin-1(10⁻⁹-10⁻⁷M), U46619 (10⁻⁹-10⁻⁵M), KCl (30-120mM), and AngII (10⁻⁷M). For relaxation studies, vessels were pre-constricted with 1 μM PE, a dose to which there was no constrictor difference, before administration of Ach (10⁻⁹-10⁻⁵M) and SNP (10⁻⁹-10⁻⁷M).

Coronary myography:

Coronary arterioles were brought to a tension equivalent to 90% of the diameter of the vessel at a transmural pressure of 90 mmHg as previously described in tissue baths of warmed (37 °C), aerated (95% O₂, 5% CO₂), PSS containing (in mM): 118.99 NaCl; 4.69 KCl; 1.17 MgSO₄; 0.03 EDTA; 2.5 CaCl₂; 25 NaHCO₃; 1.18 KH₂PO₄; 5.5 glucose. Vessel viability was subsequently assessed by exposure to 80mM KCl. Vasoconstrictor responses were assessed to endothelin-1 (10⁻¹⁰-10⁻⁸M), U46619 (10⁻⁸-10⁻⁶ M), and KCl (20-100mM). Preliminary studies showed that mouse coronary arterioles were not reactive to PE or AngII so full experiments were not performed with those agonists. For relaxation studies, vessels were pre-constricted with U46619 (0.1-0.3 µM), a dose to which there was no constrictor difference, before administration of Ach (10⁻⁹-10⁻⁴M) and SNP(10⁻⁹-10⁻⁷M). Coronary constriction data is shown as developed tension (force/mm) to account for variability of coronary vessel length due to variable branching.

Mesenteric vessel pressure myography:

Mesenteric tissues were excised from anesthetized MR-intact or EC-MR-KO mice and transferred to a cooled chamber (4°C) containing dissection solution (in mM): NaCl 140; KCl 5.6; MgSO₄ 1; NaH₂PO₄ 1.2; EDTA 0.02; sodium pyruvate 2; glucose 5; MOPS 3; albumin 0.1 mg/ml (USB Corporation, Cleveland, OH, USA); and pH adjusted to 7.3 with NaOH at room temperature. Segments (approximately 1–2 mm in length) of second-order mesenteric arterioles were micro-dissected (4°C), cannulated onto glass micropipettes, secured using 11-0 monofilament suture, and mounted in a 7 ml chamber of a cannulation stage as previously described. The cannulated arterioles were filled with a modified Kreb’s buffer containing (in mM): 111 NaCl; 4.9 KCl; 1.2 MgSO₄; 2.5 CaCl₂; 25.7 NaHCO₃; 1.2 KH₂PO₄; 11.5 glucose; 10 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES); and pH adjusted to 7.3 with NaOH at room temperature. The cannulation stage was positioned on an inverted microscope and superfused (4 ml/min) with Kreb’s buffer. Vessels were initially pressurized to 70 mmHg in the absence of luminal flow by connecting the inflow pipettes to a height-adjustable fluid reservoir. Length of the cannulated vessels was adjusted by stretching segment
length with increasing intraluminal pressure (30–110 mmHg). Optimal length was
determined by verifying that high intraluminal pressure (e.g. 110 mmHg) did not result in
lateral bowing of the cannulated vessels. Vessels were warmed to 36–37°C during a 60-
min equilibration period and allowed to develop spontaneous myogenic tone, which was
usually 15–25% of the maximal passive diameters in mice mesenteric arterioles.
Changes in vessel diameter were observed and measured using video microscopy and
a video-based caliper system. Intraluminal pressures were elevated from 10 to 110
mmHg in 20 mmHg steps to test myogenic tone and obtain pressure-diameter
relationships. Changes in diameter in response to (1) phenylephrine (1 nM–10 µM), (2)
SNP (10 nM–10 µM), and (3) Ach (1 nM–10 µM) were monitored at 70 mmHg. After the
assessment of SNP-dose response curves, phenylephrine was superfused as a
preconstrictor at a dose of 100nM, a dose to which there was no constrictor difference,
and Ach-concentration response curves were examined with the pre-constricted
mesenteric arterioles in the absence or presence of L-NAME, indomethacin, L-
NAME+indomethacin, NAME+indomethacin+Apamin+TRAM34. SNP (5µM) was applied
into the mesenteric arterioles after treatment with the two inhibitors to ensure that
vascular smooth muscle cells function had not been compromised by the experimental
procedure.

Electrophysiological recordings:
Mouse left anterior descending artery and mesenteric (2nd and 3rd order) arteriolar
myocytes were isolated as previously described. Whole cell currents of L-type calcium
channel (LTCC) were recorded using a standard whole-cell patch clamp technique.
Cells were superfused with physiological saline solution (PSS) containing (in mM): 138
tetraethylammonium chloride (TEA-Cl), 0.1 CaCl₂, 1 MgCl₂, 5 KCl, 10 HEPES, 10
Glucose, 20 barium chloride (BaCl₂), pH 7.35 (Osm ≈300 Osm/L). Pipette solution (in
mM):120 CsCl, 10 TEA-Cl, 10 EGTA, 1MgCl₂, 15 HEPES, 5 Na₂-ATP, 0.5 Tris-GTP, 0.1
CaCl₂, pH 7.2. LTCC I-V curves were obtained by using a holding potential of -70 mV,
with step changes in potential from -60 to +50 mV, duration of 400 ms. Raw current
values were normalized to cell capacitance and expressed as current density (pA
pF⁻¹).

Reagents: BayK8644 (Sigma-Aldrich) and nifedipine (Sigma-Aldrich) were
dissolved in 100% ethanol; final concentrations were Bay K 8644, 2 µM
and nifedipine, 1 µM.

Statistics:
Within-group differences were assessed with two-factor analysis of variance (ANOVA)
or repeated-measures ANOVA (telemetry and mesenteric vessel contraction studies)
with Student-Newman-Keuls post-test. P<0.05 was considered significant.
Supplemental Reference List


**Supplemental Tables:**

Table S1: Mouse primers for qRT-PCR.

<table>
<thead>
<tr>
<th>MR genotyping primers</th>
<th>MR-4</th>
<th>MR-5</th>
<th>MR-3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCA CTT GTA TCG GCA ATA CAG TTT AGT GTC</td>
<td>CAC ATT GCA TGG GGA CAA CTG ACT TC</td>
<td>CTG TGA TGC GCT CGG AAA CGG</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Mouse qRT-PCR primers</th>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Beta-2 microglobulin</td>
<td>GCT ATC CAG AAA ACC CCT CAA</td>
<td>CAT GTC TCG ATC CCA GTA GAC GGT</td>
</tr>
<tr>
<td></td>
<td>Nr3C2 (MR)</td>
<td>GAA GAG CCC CTC TGT TTG CAG</td>
<td>TCC TTG AGT GAT GGG ACT GTG</td>
</tr>
<tr>
<td></td>
<td>Thromboxane A2 receptor (TP)</td>
<td>GTGGTCTTTGGGGCTCATATTC</td>
<td>CCCACGAGCTGAACCATCAT</td>
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<tr>
<td></td>
<td>Endothelin-B receptor (ET&lt;sub&gt;B&lt;/sub&gt;)</td>
<td>GTGGCTTCTTGGGGTATGG</td>
<td>TCTTAGTGGGGTGCGTCATTA</td>
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</table>
Table S2. Baseline characteristics of EC-MR-KO mice.
Male EC-MR-KO mice and MR-intact littermates were used between the ages of 12 and 20 weeks. N for each genotype is indicated to the right. There are no significant differences in baseline characteristics between MR-intact and EC-MR-KO mice.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MR-intact</th>
<th>n</th>
<th>EC-MR-KO</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>tibia length (mm)</td>
<td>18.14 (±0.1)</td>
<td>7</td>
<td>18.1 (±0.09)</td>
<td>7</td>
</tr>
<tr>
<td>body weight (g)</td>
<td>30.32 (±1.76)</td>
<td>7</td>
<td>29.2 (±0.68)</td>
<td>7</td>
</tr>
<tr>
<td>kidney weight (g)</td>
<td>0.17 (±0.01)</td>
<td>7</td>
<td>0.15 (±0.02)</td>
<td>7</td>
</tr>
<tr>
<td>kidney length (mm)</td>
<td>9.97 (±0.25)</td>
<td>7</td>
<td>10.0 (±0.44)</td>
<td>7</td>
</tr>
<tr>
<td>serum aldosterone (pg/ml)</td>
<td>2.65 (±0.22)</td>
<td>4</td>
<td>3.04 (±0.58)</td>
<td>4</td>
</tr>
</tbody>
</table>

**Electrolytes**

**Normal sodium diet**

<table>
<thead>
<tr>
<th>Serum</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>sodium (mEq/L)</td>
<td>152 (±1.5)</td>
<td>3</td>
<td>148.3 (±1.85)</td>
<td>3</td>
</tr>
<tr>
<td>potassium (mEq/L)</td>
<td>5 (±0.32)</td>
<td>3</td>
<td>5.05 (±0.11)</td>
<td>3</td>
</tr>
<tr>
<td>creatinine (mg/dL)</td>
<td>0.23 (±0.12)</td>
<td>3</td>
<td>0.10 (±0.01)</td>
<td>3</td>
</tr>
<tr>
<td>blood urea nitrogen (mg/dL)</td>
<td>35 (±2.73)</td>
<td>3</td>
<td>31 (±4.62)</td>
<td>3</td>
</tr>
</tbody>
</table>

**Urine**

| sodium (mEq/L)             | 207.7 (±51.47)     | 3   | 226.13 (±42.38)    | 3   |
| potassium (mEq/L)          | >200               | 3   | >200               | 3   |
| creatinine (mg/dL)         | 122.13 (±42.78)    | 3   | 154.6 (±45.35)     | 3   |
| urea nitrogen (mg/dL)      | >250               | 3   | >250               | 3   |

**Low sodium diet**

<table>
<thead>
<tr>
<th>Serum</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>sodium (mEq/L)</td>
<td>149 (±0.56)</td>
<td>3</td>
<td>152 (±2.1)</td>
<td>3</td>
</tr>
<tr>
<td>potassium (mEq/L)</td>
<td>4.65 (±.68)</td>
<td>3</td>
<td>5.1 (±1.04)</td>
<td>3</td>
</tr>
<tr>
<td>creatinine (mg/dL)</td>
<td>0.1 (±9.8x10-18)</td>
<td>3</td>
<td>0.13 (±.03)</td>
<td>3</td>
</tr>
<tr>
<td>blood urea nitrogen (mg/dL)</td>
<td>40.8 (±2.9)</td>
<td>3</td>
<td>37.7 (±4.63)</td>
<td>3</td>
</tr>
</tbody>
</table>

**Urine**

| sodium (mEq/L)             | 21.65±(6)          | 3   | 17.08 (±1.7)       | 3   |
| potassium (mEq/L)          | >200               | 3   | >200               | 3   |
| creatinine (mg/dL)         | 122.13 (±42.78)    | 3   | 154.6 (±45.35)     | 3   |
| urea nitrogen (mg/dL)      | >250               | 3   | >250               | 3   |

**Food & water consumption**

**Normal sodium diet**

| Food (g)                   | 3.1 (±0.86)        | 4   | 2.62 (±0.47)       | 4   |
| Water (ml)                 | 4.75 (±0.55)       | 4   | 4.88 (±1.0)        | 4   |

**Low sodium diet**

| Food (g)                   | 2.3 (±0.29)        | 4   | 2.6 (±0.32)        | 4   |
| Water (ml)                 | 5.87 (±0.41)       | 4   | 5.97 (±0.35)       | 4   |
S1. EC-MR deletion does not affect diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate or activity levels. Mice were 6 months of age for all measurements. Number of mice for each measurement are listed in parenthesis.
S2. EC-MR does not contribute to prostacyclin and nitric oxide components of endothelial-dependent vasorelaxation in mesenteric vessels from untreated mice. Dose relaxation curve for acetylcholine alone (A) or following pretreatment with indomethacin (Cox1 inhibitor) (B), L-NAME (e-NOS inhibitor) (C), or indomethacin plus L-NAME (D) was measured by pressure myography in vessels pre-constricted with PE.
S3. EC-MR deletion does not affect phenylephrine-induced vasoconstriction in mesenteric vessels exposed to 14 days of AngII. Mesenteric vessels from mice treated with 14 days AngII were pressurized to 70 mm Hg and the change in diameter was measured via pressure myography to increasing concentrations of phenylephrine (PE) was quantified. N = 6/group.
S4. EC-MR deletion does not affect coronary arteriolar vasorelaxation following 14 days Ang II infusion. Coronary arteriolar relaxation was measured via wire myography to a dose-escalation of (A) Sodium nitroprusside (SNP) and to (B) Achetylcholine (Ach) after pre-constriction with U46619. N = 6 mice/group.
S5. EC-MR deletion does not affect mesenteric myogenic tone response at baseline or after 14 day AngII-infusion. (A) Passive and active diameters of cannulated mesenteric arterioles from healthy mice (passive diameter, p=.273; active diameter, p=.478) or from mice infused with AngII for 14 days (passive diameter, p=.594; active diameter, p=.346) over a range of intraluminal pressures. (B) Average spontaneous myogenic tone at 70 mm Hg is calculated as the percentage decrease in active lumen diameter from passive diameter for each mouse in A. There is no significant difference in spontaneous myogenic tone under either condition (untreated, p=.356; AngII-treated, p=.057). Number of animals is indicated in parentheses.
S6. EC-MR deletion does not affect coronary or mesenteric smooth muscle cell L-type calcium channel function. L-type calcium channel (LTCC) current was measured in freshly dispersed smooth muscle cells from MR-intact and EC-MR-KO (A) Coronary vessels (MR-intact: n=4 mice, 14 cells; EC-MR-KO: n=4 mice, 15 cells) and (B) Mesenteric vessels. (MR-intact: n=7 mice, 31 cells; EC-MR-KO: n=9 mice, 37 cells). LTCC agonist: BayK8644 (2uM), LTCC inhibitor: nifedipine (1uM). There is no significant difference in LTCC current between MR-intact and EC-MR-KO SMCs.