Inappropriate activation of the mineralocorticoid receptor (MR) results in oxidative stress and inflammation in the heart and vasculature, leading to cardiac tissue fibrosis and dysfunction. These MR-dependent processes underpin the development of heart failure in all-cause heart failure or heart failure postmyocardial infarction. However, the therapeutic potential of current MR antagonists is limited by undesirable side effects, including hyperkalemia, gynecomastia, and impotence. Identifying cell-specific features of MR signaling may allow for the development of selective MR antagonists that provide cardiovascular protection while maintaining normal renal function.

The negative consequences of MR activation in the heart are now recognized as a direct consequence of MR signaling in cardiovascular cells rather than secondary to renal MR-dependent blood pressure increases. With the use of tissue-selective MR-null mice, this laboratory and others have shown that MR-mediated cardiac pathology arises from a series of cell-specific responses. Macrophage/microcyte MR-null mice show normal inflammatory cell recruitment but loss of both the typical early inflammatory response and tissue fibrosis and hypertension at 8 weeks after either a deoxycorticosterone (DOC)/salt or N\(^{-}\)-nitro-L-arginine methyl ester (L-NAME)/salt challenge. Studies in cardiomyocyte MR-null mice identified an essential role for the receptor in the initiation and progression of DOC/salt-induced cardiac tissue inflammation and remodeling through the regulation of chemoattractant signals and extracellular matrix turnover. An important role for cardiomyocyte MR in remodeling and functional changes postinfarct or after transaortic constriction has also been demonstrated. More recently, a novel role for

**Abstract**—Recent studies have identified novel pathological roles for mineralocorticoid receptors (MR) in specific cell types in cardiovascular disease. The mechanisms by which MR promotes inflammation and fibrosis involve multiple cell-specific events. To identify the role of MR in endothelial cells (EC-MR), the current study explored the vascular responses to aldosterone in wild-type (WT) and EC-null mice (EC-MRKO). Nitric oxide function was impaired in the thoracic aorta and mesenteric arteries of aldosterone-treated WT mice. Although endothelial nitric oxide function was equivalently impaired in the mesenteric arteries of aldosterone-treated EC-MRKO mice, endothelial function was unaffected in the aorta, suggesting a differential role for EC-MR depending on the vascular bed. Second, the contribution of EC-MR to cardiovascular inflammation, fibrosis, and hypertension was determined in WT and EC-MRKO treated with deoxycorticosterone/salt for 8 days or 8 weeks. At 8 days, loss of EC-MR prevented macrophage infiltration and the expression of proinflammatory genes in the myocardium. Increased cardiac fibrosis was not detected in either genotype at this time, mRNA levels of profibrotic genes were significantly lower in EC-MRKO mice versus WT. At 8 weeks, deoxycorticosterone/salt treatment increased macrophage recruitment and proinflammatory gene expression in WT but not in EC-MRKO. Collagen deposition and connective tissue growth factor expression were significantly reduced in EC-MRKO versus WT. Interestingly, systolic blood pressure was equivalently elevated in deoxycorticosterone/salt treated WT and EC-MRKO. Our data demonstrate that (1) EC-MR signaling contributes to vascular nitric oxide function in large conduit arteries but not in resistance vessels and (2) an independent role for EC-MR in the inflammatory and profibrotic response to deoxycorticosterone/salt. 

**Key Words:** deoxycorticosterone ■ endothelial cells ■ macrophages ■ receptors, mineralocorticoid
vascular smooth muscle cell (VSMC) MR in blood pressure control and vascular function in aging has been described; the importance of endothelial MR in obesity-induced endothelial dysfunction has also been demonstrated. Endothelial cells (EC) are actively involved in numerous pathological conditions of the cardiovascular system, including atherosclerosis, inflammation, and tissue remodeling. The vascular endothelium expresses MR, glucocorticoid receptors, and the aldosterone specificity conferring enzyme, 11β-hydroxysteroid dehydrogenase type 2, all of which play an important role in vessel wall function. Patients with primary aldosteronism exhibit a higher degree of endothelial dysfunction than patients with essential hypertension, whereas MR blockade improves endothelium-dependent vasodilation in congestive heart failure. Enhanced MR signaling in EC causes endothelial dysfunction in experimental models via generation of reactive oxygen species, a reduction in nitric oxide (NO) bioavailability, and subsequent vasodilatation. Monocyte/macrophage attachment is also promoted via MR-dependent intercellular adhesion molecule 1 (ICAM-1) expression and monocyte adhesion to the vascular endothelium.

The specific role of the EC-MR in vascular physiology and pathology, and in the setting of cardiac remodeling and hypertension, has not been directly addressed in vivo. Thus the aims of the present study are 2-fold. First, to investigate the role of EC-MR in normal vascular physiology and aldosterone-induced vascular pathology and second to determine the effect of loss of EC-MR in the initiation and development of DOC/salt-mediated cardiac pathology. We generated EC-specific MR knockout (EC-MRKO) mice and investigated responses to aldosterone treatment for 2 weeks (to assess vascular function) or DOC/salt treatment for 8 days or 8 weeks (to identify early MR-dependent inflammatory events and the development of interstitial cardiac fibrosis and remodeling).

Materials and Methods

Additional Materials and Methods are provided in the online-only Data Supplement.

EC-Specific MR Knockout Mice

All procedures involving animals were approved by the Monash University Animal Ethics and Biosafety Committee. Tie2Cre+/−, MR−/−/Tie2Cre−/−, and MR−/−/Tie2Cre−/− (wild-type [WT]) mice were used to generate MR−/−/Tie2Cre−/− (EC-MRKO) mice (Figure S1A and S1B in the online-only Data Supplement). The genomic identity of the mice was determined by polymerase chain reaction of genomic DNA (Figure S1A and S1B). The mRNA levels for MR and endothelial NO synthase (iNOS; EC marker) were assessed in CD31+ cells isolated from aorta and spleen by fluorescence-activated cell sorting. MR mRNA levels were similarly determined in splenic DNA (Figure S1A and S1B). The mRNA levels for MR and endothelial NO synthase (iNOS; EC marker) were assessed in CD31+ cells isolated from aorta and spleen by fluorescence-activated cell sorting. MR mRNA levels were similarly determined in splenic DNA (Figure S1A and S1B). The mRNA levels for MR and endothelial NO synthase (iNOS; EC marker) were assessed in CD31+ cells isolated from aorta and spleen by fluorescence-activated cell sorting. MR mRNA levels were similarly determined in splenic DNA (Figure S1A and S1B). The mRNA levels for MR and endothelial NO synthase (iNOS; EC marker) were assessed in CD31+ cells isolated from aorta and spleen by fluorescence-activated cell sorting.
samples confirmed an EC phenotype (Figure S1A–S1F). MR expression was equivalent in splenic CD11b+ macrophages isolated from EC-MRKO mice and WT mice (Figure S1E), MR expression in VSMC was also preserved (Figures S1F). EC-MRKO mice displayed a normal phenotype with standard body, heart, and kidney weights, and plasma aldosterone levels were within the expected range for mice drinking 0.9% saline NaCl/0.04% KCl (Tables S2 and S3).

EC-MR Does Not Contribute to Aldosterone-Induced Resistance Vessel Endothelial Dysfunction But Regulates Endothelial Dysfunction in the Aorta

Aldosterone for 2 weeks impaired ACh-induced relaxation in small mesenteric arteries from both WT and EC-MRKO mice compared with vehicle-treated mice (Figure 1A and 1B), suggesting that EC-MR do not contribute to endothelial dysfunction of resistance arteries. Loss of MR from ECs also reduced ACh-induced relaxation responses of small mesenteric arteries in vehicle-treated animals (Figure S4A). Relaxation responses to sodium nitroprusside and papaverine were not affected by aldosterone treatment in either genotype (Figure S2).

ACh-induced relaxation was also impaired in thoracic aorta from aldosterone-treated WT mice compared with vehicle treatment (Figure 1C). In contrast to mesenteric resistance arteries, Ach responses were not impaired in aldosterone-treated EC-MRKO mice (Figure 1D), suggesting that endothelial NO dysfunction of the thoracic aorta is mediated by EC-MR. Similarly, contractile responses to the NOS inhibitor L-NAME (measure of basal endothelial NO production) were ≈40% lower in aortas from aldosterone-treated WT mice compared with vehicle-treated WT mice but were unaffected in aldosterone-treated EC-MRKO mice. Aortic responses to sodium nitroprusside or U46619 were not different between any group (Figure S3). Of note, ACh responses were also impaired in thoracic aorta from vehicle-treated EC-MRKO compared with WT (Figure S4B).

EC-MR Signaling and DOC/Salt-Mediated Cardiac Inflammation and Fibrosis at 8 Days

Cardiac responses to vehicle or DOC/salt were examined at 8 days to determine the role of EC-MR in the onset of cardiac inflammation and remodeling. The number of infiltrating CD11b+ macrophages was significantly increased in WT DOC/salt mice only (Figure 2A). Similarly, mRNA levels for CCR5 were increased in response to DOC/salt in WT but not in EC-MRKO (Figure 2B) as were mRNA values for iNOS and T-cell chemokine regulated on activation, normal T-cell expressed and secreted (Figure 2B and Table S4). No change in cardiac interstitial collagen deposition was detected for any group (Figure 2D). In contrast, DOC/salt treatment increased PAI-1 and COL-3 in both genotypes, although to a lesser extent in EC-MRKO (Figure 2F and Table S4). A significant genotype effect was detected for mRNA levels of CTGF, as well as for PAI-1, which showed a reduction in values for EC-MRKO compared with WT CON (control) (Figure 2E and 2F). Immunohistochemical analysis of CTGF protein expression showed equivalent levels in untreated WT and EC-MRKO mice (Figure S5). Moreover, DOC/salt treatment increased CTGF protein expression regardless of genotype.

EC-MR Regulates DOC/Salt-Mediated Cardiac Inflammation and Fibrosis at 8 Weeks But Not SBP

To determine the role of EC-MR in the long-term development of cardiac inflammation and fibrosis, responses to DOC/salt were determined at 8 weeks. Increased CD11b+ macrophage infiltration in response to DOC/salt treatment was observed in WT mice only (Figure 3A). In contrast to expression at 8 days, CCR5 expression did not seem to be affected by DOC/salt treatment but was lower in EC-MRKO versus WT mice (Figure 3B). Moreover, mRNA values for iNOS and regulated on activation, normal T-cell expressed and secreted did not change with treatment or genotype (Table S4).

DOC/salt treatment for 8 weeks produced a significant increase in collagen deposition in WT but not in EC-MRKO
mice (Figure 3C). Similarly, a DOC/salt-induced increase in mRNA levels for CTGF was detected in WT mice only (Figure 3D). Increased COL-3 and PAI-1 levels were equivalent in WT and EC-MRKO, indicating MR activation in other cell types (Table S4). DOC/salt treatment for 8 weeks significantly increased SBP over control regardless of genotype (P<0.05 by 2-way ANOVA; Figure 4).

DOC/salt treatment for 8 weeks increased mRNA levels for iNOS in the aorta of WT mice only. In contrast, a significant genotype effect was observed for eNOS, which showed higher mRNA values for aortas from EC-MRKO mice versus WT mice, but with no effect seen with DOC/salt treatment (Figure S6A and S6B). The genotype effect in mRNA levels of eNOS was also observed at the protein level (Figure S7). mRNA levels for placental growth factor and tumor necrosis factor α were increased by DOC/salt in aortic tissue from mice of both genotypes (data not shown), supporting MR activation in VSMC, which remains intact in EC-MRKO mice.⁷ At 8 weeks, immunostaining for ICAM-1 showed positive staining in coronary vessels of WT DOC/salt-treated mice compared with all other groups (Figure S8).

Aldosterone Treatment Increases ICAM-1 and CTGF mRNA Levels in HUVECs

HUVECs incubated with 10 nmol/L aldosterone for 5 hours showed a significant increase in ICAM-1 and CTGF mRNA levels that was blocked by preincubation with 1 μmol/L spironolactone. These data are consistent with the previously reported role of EC-MR in the regulation of the monocyte adhesion and extravasation factor ICAM-1⁶ and demonstrate a role for the EC-MR in regulation of the inflammatory/fibrotic marker CTGF in the vascular cells (Figure S9A and S9B).

Discussion

Our data demonstrate that aldosterone-induced endothelial dysfunction is dependent on intact MR signaling in the aorta but not in small resistance-like mesenteric vessels. EC-MR also seem to play an important role in basal, NO-dependent vascular function. We also show that EC-MR play a key role in DOC/salt-mediated cardiovascular inflammation and fibrosis but not blood pressure regulation, via regulation of macrophage recruitment. Reduced cardiac tissue macrophage numbers at early and late time points were accompanied by a reduction in proinflammatory and profibrotic markers and vascular expression of ICAM-1 and CTGF. Taken together, this study illustrates a unique role for the EC-MR in aldosterone-induced endothelial dysfunction and in DOC/salt-mediated cardiac tissue fibrosis and inflammation. This cardiac protection is predominantly via regulation of inflammatory cell infiltrate, which supports our previous demonstration of the central role of macrophages in cardiovascular remodeling.⁵

Figure 2. Markers of cardiac inflammation and fibrosis at 8 days. A, Quantification of CD11b+ macrophage infiltration. Gene expression of inflammatory cytokines, (B) C-C chemokine receptor type 5 (CCR5), and (C) inducible nitric oxide synthase (iNOS). D, Cardiac fibrosis at 8 days, gene expression for fibrotic markers. E, Connective tissue growth factor (CTGF) and (F) plasminogen activator inhibitor-1 (PAI-1). All data are analyzed by 2-way ANOVA, with results displayed below each figure, and by Tukey multiple comparison post tests. *P<0.05 denotes significant differences. Mean±SEM; n=7 to 9. CON indicates control; EC-MRKO CON, endothelial cell MR-null mice given 0.9% saline to drink; EC-MRKO DOC, endothelial cell MR-null mice given deoxycorticosterone plus 0.9% saline to drink; WT CON, wild-type mice given 0.9% saline to drink; and WT DOC, wild-type mice given deoxycorticosterone plus 0.9% saline to drink.
EC-MR and Endothelial Dysfunction

The mechanisms that contribute to the differential vascular relaxation responses observed in EC-MRKO mice remain to be determined but may reflect underlying differences between vessel beds to contributing factors such as sheer stress and chronic sodium loading.18 Several clinical and experimental animal studies show that aldosterone can cause vascular dysfunction, and although the mechanisms responsible are largely undefined, aldosterone-dependent production of reactive oxygen species, a reduction in NO bioavailability, and thus of vasodilatation may play a role.19 Consistent with these studies, we now show that in mice, aldosterone treatment causes marked impairment of endothelial NO function in small resistance-like mesenteric arteries. The major new finding from these experiments was that MR-dependent impairment of endothelial NO function in these arteries occurs in both WT and EC-MRKO mice. These data suggest that EC-MR are unlikely to play a role in MR-dependent endothelial dysfunction in resistance arteries and are consistent with equivalent pressor response to DOC/salt in both genotypes. In contrast, endothelial NO function was significantly impaired by aldosterone treatment in aortas from WT but not EC-MRKO mice, supporting a role for the MR in endothelial dysfunction in larger conduit arteries as has been recently shown.12 Conduit arteries are not major contributors to systemic blood pressure. However, in the presence of hypertension, they are subjected to shear stress and turbulent flow, which together with macrophage recruitment contribute to endothelial damage and can lead to atherosclerosis.20 Blockade of MR-dependent endothelial damage may thus provide additional protection in large vessel or coronary vascular disease regardless of etiology.12 In the EC-MRKO mice, loss of DOC/salt induction of iNOS expression, a significant increase in eNOS expression plus a lack of response to L-NAME support a role for NO signaling in MR-mediated vascular function and dysfunction. Both gene and protein levels of eNOS were significantly increased in untreated EC-MRKO mice. This finding may reflect a compensatory

| Figure 3. Markers of cardiac inflammation and fibrosis at 8 weeks. Treatment groups as for Figure 2. A, Quantification of CD11b+ macrophage infiltration. B, Gene expression of inflammatory cytokine C-C chemokine receptor type 5 (CCR5). C, Cardiac fibrosis at 8 days. D, Gene expression for fibrotic marker connective tissue growth factor (CTGF). All data are analyzed by 2-way ANOVA, with results displayed below each figure, and by Tukey multiple comparison post-tests. *P<0.05 denotes significant differences. Mean±SEM; n=7 to 9. DOC indicates deoxycorticosterone; EC-MRKO, endothelial cell–specific mineralocorticoid receptor knockout; NS, nonsignificant; and WT, wild type. |

| Figure 4. Systolic blood pressure at 8 weeks. Treatment groups as for Figure 1. Data are analyzed by 2-way ANOVA, with results displayed below each figure, and by Tukey multiple comparison post-tests. *P<0.05 denotes significant differences. Mean±SEM; n=7 to 9. EC-MRKO indicates endothelial cell–specific mineralocorticoid receptor knockout; NS, nonsignificant; and WT, wild type. |
mechanism whereby under normal condition eNOS is inhibited by EC-MR and suggests that EC-MR signaling may regulate expression/activity of eNOS and hence vascular function.

Our findings are compatible with the conclusion that endothelial MR receptors (1) are not critical for mediating impairment of endothelial function by aldosterone treatment in the mesenteric artery, but (2) these receptors may be important mediators of aldosterone-induced aortic endothelial dysfunction. However, we note that responses to Ach were smaller, particularly in the aorta, from vehicle-treated EC-MRKO versus WT mice. No such difference was noted by Schafer et al., and we currently do not have any explanation for this difference in baseline endothelial function observed in our study. Moreover, this difference in baseline function may have limited the ability of aldosterone to cause further endothelial impairment in aortas of EC-MRKO mice as occurred in WT mice.

**EC-MR and Blood Pressure Regulation**

As noted above, DOC/salt-mediated SBP elevation was not abrogated by loss of MR in EC, demonstrating that MR activation in other tissues make a substantial contribution to long-term blood pressure regulation and verifying that MR-dependent tissue fibrosis and inflammation is independent of blood pressure. DOC/salt-mediated hypertension occurs through several tissue-specific MR-mediated mechanisms including renal epithelial MR activation and sodium/water retention, central MR activation and increased sympathetic nerve activity, and via VSMC activation. Although EC regulate vascular tone through the synthesis and release of vasoactive mediators to the underlying VSMC, MR in EC regulate vascular tone through the synthesis and release of vasoactive mediators to the underlying VSMC,23 MR in EC and cardiomyocytes independently promotes inflammation in the vessel wall that in turn regulates EC function and may be sufficient to increase blood pressure. In support of this notion, a recent study described an important role for EC-MR in obesity-induced endothelial dysfunction. Diet-induced obesity and hence an elevated inflammatory status lead to MR-dependent EC dysfunction in aortic rings, responses that were equivalent to aldosterone infusion for 2 weeks; resistance vessels were not, however, examined. It is important to note that the present study differed from previous reports in that there is a blunted vasodilatory response in the aorta (and mesenteric vessels) of EC-MRKO mice. It is not clear these studies differ, but given the increased baseline and lack of response to L-NAME, these changes may be dependent on NO signaling. Of note, VSMC MR-null mice also show enhanced vasodilation to Ach compared with WT mice and suggests that VSMC MR can also modulate endothelial function.

**EC-MR and Cardiac Tissue Fibrosis**

A key finding in the present study was the lack of a fibrotic response at 8 weeks to DOC/salt administration in mice null for EC-MR. These data were paralleled by reduced expression of the profibrotic genes COL-3 and PAI-1 at the early, 8-day, time point and of CTGF at both 8 days and 8 weeks. CTGF is a matrix protein that has important roles in cell adhesion, migration, and proliferation and is expressed in both cardiomyocytes and the vessel wall. Taken together with our demonstration of CTGF regulation by aldosterone in HUVECs and our previous demonstration that DOC/salt-induced CTGF expression is independent of cardiomyocyte MR activation, our data suggest that MR regulation of CTGF expression is predominantly, but not exclusively, in the vessel wall. However, it should be noted that elevated CTGF alone does not increase fibrosis and induction of CTGF by aldosterone is also not sufficient to increase fibrosis. These observations may be because of the short time course of treatment (1 week versus 8 weeks) or reflect the hypothesis that MR activation has a permissive role in promoting fibrosis and requires other stimuli, for example, that MR-stimulated profibrotic genes require an enhanced sodium intake or inflammatory cytokine expression to promote cardiac fibrosis.

COL-3 expression was also markedly reduced in our study at 8 days, suggesting that endothelial MR regulate early expression of the profibrotic factor. However, mRNA levels of COL-3 and PAI-1 were equivalent between genotypes at 8 weeks, data that is again consistent with our studies in cardiomyocyte MR-null mice and supportive of MR regulation in other cell types (eg, VSMC). Hemodynamic stress can also contribute a proinflammatory response in the vasculature. Given that SBP was elevated in both WT and EC-MRKO mice, this may have contributed to the activation of the EC, resulting in the stimulation of profibrotic mediators via alternative mechanisms. Together, these data show an early reduction in profibrotic signaling that is associated with significant reductions in tissue fibrosis and suggest that EC-MR regulation of CTGF may play an important early mechanistic, or permissive, role in driving fibrosis in addition to the aldosterone-mediated, MR-dependent regulation of CTGF in cardiomyocytes that has recently been described.

**EC-MR and Cardiac Tissue Inflammation**

We have clearly demonstrated that EC-MR are critical for the recruitment of macrophages to the heart at both 8 days and 8 weeks in the DOC/salt model of cardiac fibrosis. The reduced inflammatory cell infiltrate was accompanied by downregulation of mRNA levels of CCR5 at 8 days and 8 weeks, reflecting this loss of macrophage recruitment signals. Interestingly, these data are equivalent to data obtained for cardiomyocyte MR-null mice, which likely reflect the similar lack of inflammatory cell infiltrate in the 2 models. We did not find any changes in MCP-1 (monocyte chemoattractant protein-1) mRNA levels at either time point (data not shown), suggesting that the MR is regulating other key macrophage recruitment or cell adhesion signals (eg, CCR5 and ICAM-1). MR-dependent ICAM-1 expression has been previously identified in vivo and in vitro to contribute directly to leukocyte adhesion in response to aldosterone.

In the present study, the DOC/salt-mediated increases in vascular ICAM-1 expression were attenuated by loss of MR signaling in ECs, and in cultured EC, aldosterone regulated ICAM-1 mRNA levels. Along with the reduced DOC/salt-induced macrophage infiltration in EC-MRKO mice, these findings highlight a potential mechanism by which the MR in EC contributes to DOC/salt-mediated cardiac pathology.
It is important to note that the Tie2 promoter, used to delete MR expression in EC, is also present in myeloid cells. We previously described a key role for macrophage MR signaling in basal monocyte/macrophage function and in DOC/salt-induced cardiac remodeling and blood pressure regulation. Interestingly MR levels were not reduced in splenic macrophages despite the expression of Tie2 in a subset of these cells. Moreover, there are clear differences between EC-MRKO and macrophage-specific MRKO mice; DOC/salt-mediated macrophages recruitment is not affected by specific deletion of MR in macrophages, on the contrary EC-MRKO mice do not recruit macrophages, highlighting a key role for the MR in DOC/salt-induced macrophage infiltration.

In terms of CCR5, previous findings indicated that CCR5 deficiency alone does not reduce end-organ damage or hypertension in DOC/salt- or angiotensin II–dependent hypertension. Given that macrophage recruitment and CCL2 (chemokine [C-C motif] ligand 2) expression are maintained in CCR5-deficient animals, these data support the key role for overall loss of macrophage numbers in the heart as the primary mediator of the protective effects seen in EC-MRKO mice. DOC/salt-induced iNOS mRNA expression was similarly lost in EC-MRKO mice at 8 days in whole heart and in the aortic wall at 8 weeks in the current study. iNOS is expressed in both EC and macrophages and produces NO as an acute defense mechanism in injured cells. Although the loss of iNOS expression in the aortic wall suggests a direct role for the MR in regulating vascular iNOS levels, the fall in cardiac iNOS may also reflect in part reduced tissue macrophage numbers. Our data thus support an important role for EC-MR in facilitating the tissue inflammatory response by regulating the inflammatory cell content of the heart.

Baseline Responses to EC-MR Deletion
The present study highlights a key role for EC-MR in DOC-induced inflammation and monocyte recruitment to the heart; tissue resident monocyte/macrophage numbers were not affected in EC-MRKO mice, supporting a specific role for EC-MR in monocyte/macrophage recruitment during DOC/salt-mediated vascular inflammation. Moreover, in contrast to our previous studies in cardiomyocyte and macrophage MR-null mice, expression of only a few genes showed a modest but significant genotype effect, iNOS, CTGF, and PAI-1. We further showed that CTGF is directly regulated by aldosterone in HUVECs at 3 hours, supporting primary regulation by the MR. As noted above, aldosterone stimulation of cardiomyocyte MR has also recently been shown to regulate CTGF expression. CTGF derived from other cells types may play a similar permissive role in the overall fibrotic response.

Perspectives
Clinical studies have demonstrated cardiac protection with MR antagonists in heart failure. The MR regulates leukocyte adhesion to EC and a proinflammatory macrophage phenotype; our data show that the EC-MR regulates macrophage recruitment in the DOC/salt model of cardiovascular inflammation and fibrosis. This early tissue response to DOC/salt is suppressed in MR-null EC, which translates into a loss of tissue inflammation and fibrosis at 8 weeks. Our data also highlight a specific role for EC-MR in the regulation of CTGF, iNOS, and T-cell cytokine expression, which together result in a net reduction in tissue fibrosis. Whether selective loss of EC-MR is protective in other models of cardiac fibrosis, in particular aldosterone-independent models or those that specifically target the endothelium such as L-NAME treatment, remains to be seen. It is important to note, however, that clinical benefits of MR blockade are equally observed in those patients with elevated plasma aldosterone and those with low or normal aldosterone. Given the reduction in iNOS expression in the aorta and the clear protection from endothelial dysfunction, it may be that selective endothelial MR blockade may be of particular benefit in the setting of atherosclerosis, particularly when associated with hypertension.

References
Novelty and Significance

What Is New?

- Endothelial cell mineralocorticoid receptor (MR) signaling is necessary for macrophage recruitment in the deoxycorticosterone/salt treatment model.
- Recruited macrophages are essential in MR-dependent injury regardless of intact MR signaling in other cardiovascular cell types.
- Endothelial cell MR signaling regulates acetylcholine-mediated endothelial cell relaxation in large conduit arteries (ie, aorta) but not second order resistance vessels (ie, mesenteric arteries).
- MR signaling in endothelial cells does not play a role in deoxycorticosterone/salt blood pressure regulation.

What Is Relevant?

- Blocking macrophage infiltration into tissues confers protection from MR-mediated cardiac fibrosis and inflammation.
- Therapeutic targeting of endothelial cell MR may limit macrophage recruitment to cardiac and vascular tissues and thereby provide protection for cardiac remodeling and atherosclerosis, for example.
- These data also support the importance of targeting MR signaling in macrophages as a novel therapeutic strategy in cardiovascular disease.

Summary

Endothelial cells play a distinct and important role in MR-mediated inflammatory and remodeling processes, which underlie the pathogenesis of MR-mediated cardiovascular disease.
Endothelial Cell Mineralocorticoid Receptors Regulate Deoxycorticosterone/Salt-Mediated Cardiac Remodeling and Vascular Reactivity But Not Blood Pressure
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