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Cardiomyocyte Mineralocorticoid Receptors Are Essential for Deoxycorticosterone/Salt-Mediated Inflammation and Cardiac Fibrosis

Amanda J. Rickard, James Morgan, Laura A. Bienvenu, Elizabeth K. Fletcher, Greg A. Cranston, Jimmy Z. Shen, Melissa E. Reichelt, Lea M. Delbridge, Morag J. Young

Abstract**—**Because the role of mineralocorticoid receptors in specific cell types in cardiac remodeling remains unknown, we have compared cardiac responses with deoxycorticosterone/salt in cardiomyocyte mineralocorticoid receptor-null (MyoMRKO) and wild-type (WT) mice at 8 days and 8 weeks. No differences in cardiac function between untreated WT and MyoMRKO mice were found, whereas profibrotic markers were reduced in MyoMRKO hearts at baseline. At 8 days, MyoMRKO showed monocyte/macrophage recruitment equivalent to WT mice in response to deoxycorticosterone/salt but a suppression of markers of fibrosis compared with WT. At 8 weeks, MyoMRKO mice showed no deoxycorticosterone/salt-induced increase in inflammatory cell infiltration and collagen deposition or in proinflammatory gene expression. Although some profibrotic markers were equivalently increased in both genotypes, MyoMRKO mice also showed increased baseline levels of mRNA and protein for the transforming growth factor-β/connective tissue growth factor inhibitor decorin compared with WT that was accompanied by higher levels of matrix metalloproteinase 2/matrix metalloproteinase 9 activity. These data point to a direct role for cardiomyocyte mineralocorticoid receptor in both deoxycorticosterone/salt-induced tissue inflammation and remodeling and suggest potential mechanisms for the cardioprotective effects of selective mineralocorticoid receptor blockade in cardiomyocytes that may involve regulation of matrix metalloproteinase 2/matrix metalloproteinase 9 activity and the transforming growth factor-β-connective tissue growth factor profibrotic pathway. **(*Hypertension*. 2012;60:1443-1450.)**

• **Online Data Supplement**

Key Words: mineralocorticoid receptor ■ deoxycorticosterone ■ cardiac fibrosis ■ inflammation ■ remodeling

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Clinical and experimental studies have demonstrated the therapeutic potential of mineralocorticoid receptor (MR) antagonists, although hyperkalemia, gynecomastia, and erectile dysfunction limit their widespread use.[1] Identifying tissue-specific features of MR signaling that can be exploited for the rational design of cardiovascular-selective MR antagonists may offer cardiovascular protection without compromising renal function.

We and others have shown that MR signaling in the context of high salt (the deoxycorticosterone [DOC]/salt model) leads to inflammation, fibrosis, and ultimately cardiac dysfunction.[2] Such remodeling is a direct cardiovascular effect of MR activation rather than secondary to elevated blood pressure.[3] Recent studies have identified novel roles for MR signaling in monocytes/macrophages[4] and endothelial cells[5] in the development of cardiac fibrosis.

Cardiomyocytes express both functional MR and glucocorticoid receptors, but not 11β-hydroxysteroid dehydrogenase 2, thus MR in cardiomyocytes normally act as high-affinity receptors for glucocorticoids.[6] In contrast, in epithelial cells (and vascular smooth muscle cells), glucocorticoids do not activate MR because of enzymatic modification by 11β-hydroxysteroid dehydrogenase 2.[7] Cardiomyocyte overexpression of MR causes cardiac arrhythmias by 2 months of age, but no change in fibrosis, inflammation, or apoptosis.[8] More recent studies using cardiomyocyte MR-null mice show a differential role for cardiomyocyte MRs in remodeling and functional changes postinfarct[9] and after transaortic constriction.[10] Although loss of cardiomyocyte MR improved infarct healing, scar thickness, and cardiac function in this model, cardiomyocyte MR-null mice were not protected from tissue remodeling, suggesting that the role of cardiomyocyte MR in the progress of cardiac disease may be dependent on the specific cardiac insult.

The aims of the present study were to explore the role of cardiomyocyte MR in the initiation and development of cardiac inflammation and fibrosis, in particular in the regulation of the transforming growth factor-β (TGFβ)/connective tissue

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From the Prince Henry's Institute of Medical Research, Clayton, Victoria, Australia (A.J.R., J.M., L.A.B., E.K.F., G.A.C., J.S., M.J.Y.); Departments of Physiology (A.J.R., M.J.Y.) and Medicine (J.S., M.J.Y.), Monash University, Melbourne, Victoria, Australia; Institut National de la Santé et de la Recherche Médicale, Paris Cardiovascular Research Centre, Paris, France (A.J.R.); University Paris Descartes, Sorbonne Paris Cité, Paris, France (A.J.R.); Department of Physiology, Melbourne University, Melbourne, Victoria, Australia (L.A.B., M.E.R., L.M.D.).

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Correspondence to Dr Morag J. Young, Prince Henry's Institute of Medical Research, PO Box 5152, Clayton 3168, Victoria, Australia. E-mail morag.young@princehenrys.org

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growth factor (CTGF) profibrotic pathway. The present study was designed to determine the proinflammatory (membrane cofactor protein-1, C-C chemokine receptor type 5 [CCR5]), oxidative stress (NOX2, p22phox), and profibrotic (TGFβ, CTGF, fibronectin, collagen III [Col III]) cardiac responses in wild-type (WT) and cardiomyocyte MR-null (MyoMRKO) mice, given DOC/salt and investigated at both the initiation of tissue injury (8 days) and once fibrosis is established (8 weeks). We also performed microarray analysis for the identification of additional important inflammatory and fibrotic signaling pathways that may contribute to the translation of cardiomyocyte MR activation into tissue remodeling via known or novel pathways.

Materials and Methods

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

Cardiomyocyte-Specific MR Knockout Mice

All procedures involving animals were approved by the Monash University Animal Ethics and Biosafety Committees. The presence of the $MR^{flox/flox}$ and MLC2v Cre transgene was determined by polymerase chain reaction analysis of genomic DNA from tail tips with the primers listed in Table S1 of the online-only Data Supplement (Figure S1A and S1B). MR expression in cardiomyocytes was determined by immunohistochemical analysis and Western blot as previously described.[4]

Male $MR^{flox/flox}$ and $MR^{flox/flox}/MLC2v^{Cre+/-}$ mice (25–30 g) were uninephrectomized and given vehicle or DOC (Sigma-Aldrich), administered via a subcutaneous pellet (7 mg of pellets/3 weeks), and maintained on standard chow plus 0.9% NaCl plus 0.4% KCl solution for 8 days or 8 weeks. Systolic blood pressure (SBP) was measured by tail-cuff plethysmography at weeks 4 and 8, as previously described (IITC Life Science).[4]

Radioimmunoassay for Hormone Levels

Aldosterone, corticosterone, angiotensin II, and atrial naturietic peptide were determined in serum by standard radioimmunoassay according to the manufacturers' instructions (MP Biomedicals).

Assessment of Ex Vivo Heart Function

WT and MyoMRKO mice given vehicle or DOC/salt for 8 weeks (final age, 16 weeks) were anesthetized with sodium pentobarbitone (70 mg/kg, IP). A thoracotomy was performed, and hearts excised into ice-cold perfusion fluid for cannulation and perfusion on a Langendorff perfusion system (expanded Methods in the online-only Data Supplement).

Histological and Immunohistochemical Analyses

Cardiac fibrosis was quantified by Picrosirius red staining (5-μm sections) and digital analysis of systematically sampled sections as previously described.[4] Cardiomyocyte cross-sectional area was determined in hemotoxylin and eosin–stained sections, with 80 to 100 cells sampled per heart (n=7 hearts per treatment group) and the cross-sectional area determined directly using Analysis LS Research software (Olympus).

Inflammatory cell infiltration was determined by CD11b immunostaining (Abcam). Immunostaining was also performed for TGFβ1 (1:300; Santa Cruz, Biotechnology Inc), CTGF (1:100; Abcam), fibronectin (1:1000; Santa Cruz Biotechnology), Col III (1:300; Abcam), and decorin (1:100; R&D Bioscience, Sapphire Bioscience Pty Ltd) and the relevant negative IgG used as control. Immunostaining was assessed by 2 investigator blinded to the identity of the samples using a semiquantitative scoring system of 0, +, ++, and +++, where 0 is no staining and +++ is strong staining. Between-investigator variability was calculated to be ≈15%.

Illumina BeadChip Expression Array

An Illumina BeadChip expression array was used to identify oxidative stress, inflammatory, and profibrotic genes not previously characterized in the DOC/salt model and those potentially regulated by cardiomyocyte MR. Gene expression profiles were determined with Illumina BeadChip expression arrays (MouseRef-8 version 2.0 Expression BeadChip; Illumina Inc) performed at the Australian Genome Research Facility on pooled mRNA from each treatment group and genotype. Genes identified by differential expression on microarray and those previously associated with DOC/salt inflammation and tissue remodeling were confirmed by quantitative polymerase chain reaction using primers listed in Table S1.

Matrix Metalloproteinase 2/Matrix Metalloproteinase 9 Activity

Tissue was homogenized with a Qiagen Tissue Lyser and assayed with a Molecular Probes EnzChek Gelatinase/Collagenase Assay Kit (Invitrogen). Total protein was determined with the Pierce BCA Protein Assay Kit (Thermo Scientific) and a total of 70 μg per sample were used. Briefly, samples were made up to a total volume of 200 μL with reaction buffer and 10 μL of DQ fluorescent gelatin, the substrate for gelatinases matrix metalloproteinase (MMP) 2 and MMP9. Twenty micromoles per liter of EDTA was added to separate samples and inhibited MMP2/MMP9 activity by 50% (data not shown). Readings were recorded on the Agilereader (Agilent) with a 515-nm filter at 1, 2, and 24 hours.

Statistical Analyses

All data sets were analyzed by 2-way ANOVA and Bonferroni post hoc test (GraphPad Prism version 5.0a, GraphPad Software). Normal distribution was confirmed by the D'Agostino-Pearson normality test before ANOVA. The mean difference was considered significant at $P<0.05$. All data are reported as mean±SEM and n=8 to 10 for treatment groups; for semiquantitative analysis of immunostaining, n=5 to 6 per group used.

Results

Cardiomyocyte MR-Null Mice

$MLC2v^{Cre/+}$, $MR^{flox/-}/MLC2v^{Cre/+}$, and $MR^{flox/flox}$ (WT) mice were used to breed $MR^{flox/flox}/MLC2v^{Cre/+}$(MyoMRKO) mice (Figure S1C and S1D); they showed normal fertility. Deletion of the loxP-flanked genomic region of MR resulted in loss of MR expression in cardiomyocytes (Figure S1A and S1B). MyoMRKO mice had a normal phenotype with normal body, heart, and kidney weights; baseline SBP; and plasma aldosterone, corticosterone, and angiotensin II levels within the expected range for mice drinking 0.9% saline NaCl/0.04% KCl (Tables S2 and S3).

Cardiomyocyte MR Signaling, Tissue Inflammation, and Remodeling at 8 Days

Tissue Remodeling

Cardiac responses were examined at 8 days to determine whether loss of cardiomyocyte MR influenced the onset of cardiac fibrosis. DOC/salt treatment modestly increased cardiac interstitial collagen deposition above control in WT mice only (Figure 1A). No significant change in MMP2/MMP9 activity was found at this time (Figure 1B), but a significant genotype effect was detected for the increased MMP/tissue inhibitors of metalloproteinase 1 mRNA ratio in MyoMRKO tissue versus WT (Figure 1C). The small change in fibrosis detected at 8 days of treatment was absent in MyoMRKO mice.

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A panel of markers for cardiac oxidative stress, inflammation, and remodeling plus novel targets identified from microarray analysis were examined by quantitative polymerase chain reaction (Tables S5 and S6). Gene data sets have been deposited on the online Gene Expression Omnibus. Genes from the microarray selected for further analysis were chosen for their role in oxidative stress, inflammation, and fibrosis. Genes showing significant changes are highlighted and show basal mRNA levels for plasminogen activation inhibitor 1, vascular endothelial cell growth factor A (VEGFa), and VEGF receptor 2 that were significantly lower than untreated WT mice (WT CON) in MyoMRKO hearts (Figure 1D and 1E and Table S6). mRNA levels for plasminogen activation inhibitor 1 (Figure 1D) and Per2 increased in response to DOC/salt in WT but not MyoMRKO hearts (Figure 1F). In contrast, mRNA for Col III and fibronectin was equivalently increased by DOC/salt in WT and MyoMRKO mice (Table S6). The lack of a fibrotic response in MyoMRKO mice is thus accompanied by a suppression of some, but not all, markers of fibrosis and tissue remodeling.

Inflammatory Responses

We detected a significant increase in infiltrating monocytes/macrophage number in hearts from both WT and MyoMRKO mice (Figure 2A), consistent with the previously reported primary vascular inflammatory response to DOC/salt administration.[4,11] DOC/salt treatment for 8 days significantly increased mRNA levels for T-cell chemoattractant CCR5 and nicotinamide adenine dinucleotide phosphate oxidase subunit NOX2 in WT but not MyoMRKO mice (Figure 2B and 2C). mRNA levels for nicotinamide adenine dinucleotide phosphate oxidase subunit p22phox were lower in untreated MyoMRKO hearts than for WT CON (Figure 2D). These data show lowered oxidative markers and a loss of an innate inflammatory response in MyoMRKO compared with WT mice after DOC/salt treatment.

Cardiomyocyte MR Regulate Cardiac Inflammation and Remodeling at 8 Weeks

Tissue Remodeling

Neither genotype nor DOC/salt treatment significantly altered cardiac or renal weight relative to body weight (P=0.7 versus WT CON), nor cardiomyocyte cross-sectional area (Table S3). A marked increase in tissue fibrosis was seen after 8 weeks of DOC/salt administration in WT hearts, but there was a striking lack of fibrosis in DOC/salt-treated MyoMRKO mice (Figure 3A and Figure S2A). MMP2/MMP9 activity was lower in WT DOC compared with WT CON, whereas activity in MyoMRKO DOC was equivalent to WT CON (Figure 3B). The panel of fibrotic and inflammatory markers was also assessed at 8 weeks and showed increased CTGF, fibronectin, Col III, and TGFβ mRNA levels by DOC/salt treatment in both WT and MyoMRKO mice at 8 weeks (Figure 4A through 4C and 4E). The TGFβ1-inhibitory peptide decorin, identified in the microarray screen, was increased by DOC/salt in both genotypes and at baseline in MyoMRKO mice (Figure 4D). In contrast, baseline values for TGFβ1 and integrin-β1 mRNA were significantly lower in MyoMRKO hearts compared with WT CON (Figure 4E and 4F). VEGFa

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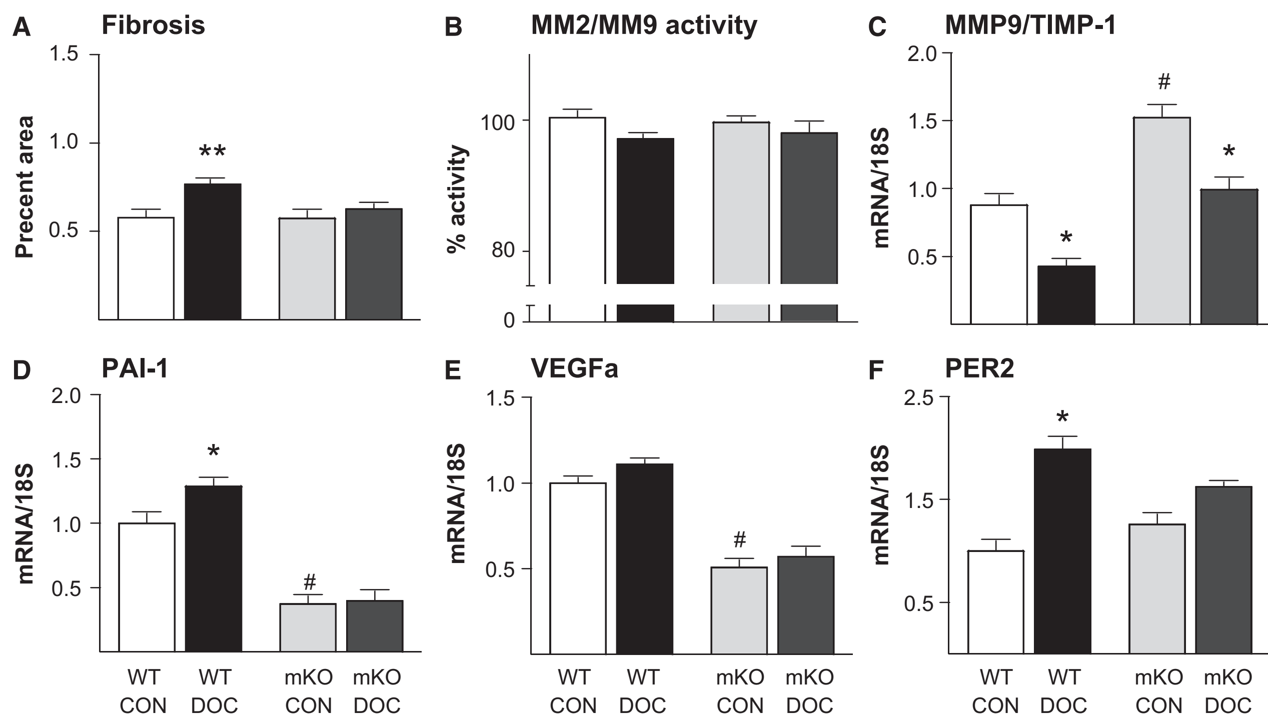

Figure 1. Markers of cardiac fibrosis and remodeling at 8 days. **A**, Cardiac fibrosis at 8 days. **B**, Matrix metalloproteinase (MMP) 2/MMP9 activity in whole heart tissue. **C**, Ratio of MMP9/tissue inhibitors of metalloproteinase (TIMP)-1 mRNA. **D** through **F**, mRNA expression of profibrotic/cardiac remodeling markers PAI-1, vascular endothelial cell growth factor A (VEGFa), and Per2 normalized to 18S rRNA. *P<0.05 vs WT CON; **P<0.05 vs mKO CON, #P<0.05 WT vs mKO. Mean±SEM; n=8 to 10. WT CON indicates untreated wild-type mice; WT DOC, wild-type mice treated with deoxycorticosterone (DOC); mKO CON, untreated cardiomyocyte-specific mineralocorticoid receptor (MR)-null mice; mKO DOC, cardiomyocyte-specific MR-null mice treated with DOC.

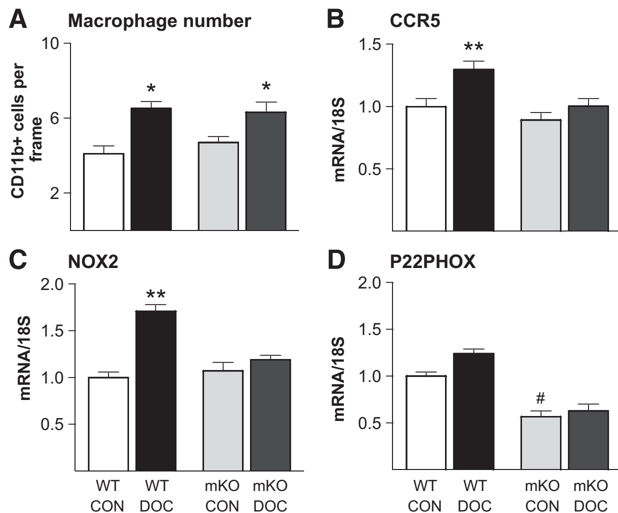

Figure 2. Marker of cardiac inflammation at 8 days. Treatment groups as for Figure 1. Deoxycorticosterone (DOC)/salt treatment for 8 days increased (**A**) CD11b+ monocytes/macrophages in both genotypes and (**B**) C-C chemokine receptor type 5 (CCR5) and (**C**) NOX mRNA levels in wild-type (WT) but not mKO hearts. **D**, mRNA values for p22phox were significantly higher in untreated WT vs mKO hearts. **P*<0.05 vs untreated mice; #*P*<0.05 WT vs mKO. Mean±SEM; n=8 to 10. WT CON indicates untreated wild-type mice; WT DOC, wild-type mice treated with deoxycorticosterone (DOC); mKO CON, untreated cardiomyocyte-specific MR-null mice; mKO DOC, cardiomyocyte-specific MR-null mice treated with DOC.

mRNA was significantly elevated by DOC/salt in WT hearts only, whereas values for VEGF receptor 2 did not reach significance (Figure 4G and 4H).

Fibronectin was detected in all hearts in interstitial and perivascular spaces with expression higher in both control and DOC-treated WT mice compared with MyoMRKO mice (Table S4 and Figure S3A). CTGF expression was detected in cardiomyocytes and the vessel wall and increased in response to DOC/salt in WT hearts only (Table S4 and Figure S3B). Vascular expression of TGFβ was not seen in MyoMRKO hearts, but was increased in hearts from DOC/salt-treated WT mice (Table S4 and Figure S4A). Similar

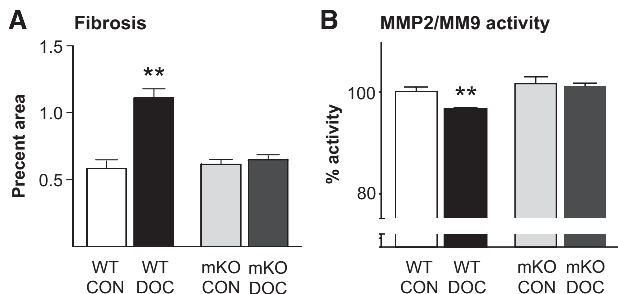

Figure 3. Cardiac fibrosis and matrix metalloproteinase (MMP) 2/MMP9 activity at 8 weeks. Treatment groups are as for Figure 1. **A**, Cardiac fibrosis and (**B**) MMP2/MMP9 activity in whole heart tissue at 8 weeks of deoxycorticosterone (DOC)/salt treatment. **P*<0.05 vs untreated mice. Mean±SEM; n=8 to 10. WT CON indicates untreated wild-type mice; WT DOC, wild-type mice treated with deoxycorticosterone (DOC); mKO CON, untreated cardiomyocyte-specific MR-null mice; mKO DOC, cardiomyocyte-specific MR-null mice treated with DOC.

to CTGF staining, Col III levels were increase WT mice only by DOC/salt treatment (Table S4 and Figure S4B). Decorin expression was detected in perivascular and interstitial regions in cardiac tissue in all mice and was higher in MyoMRKO hearts regardless of treatment (Table S4 and Figure S5). VEGFa was detected in vessel wall of both genotypes, whereas immune cell expression of CTGF, TGFβ1, and VEGFa showed no change with either genotype or treatment (data not shown).

At 8 weeks of DOC/salt, 3 patterns of responses were found for fibrotic marker expression: (1) suppression at baseline and in response to DOC/salt in MyoMRKO versus WT mice (ie, TGFβ1, integrin β1, and VEGFa); (2) equivalent upregulation in both genotypes with DOC/salt (CTGF, fibronectin, and Col III); and (3) increased basal and DOC/salt-induced expression of antifibrotic factors (decorin) in MyoMRKO versus WT mice. Upregulation of several profibrotic markers at the mRNA level was not reflected in protein expression in MyoMRKO mice.

Inflammatory Responses

A marked increase in tissue monocytes/macrophages was detected at 8 weeks of DOC/salt treatment in WT but not MyoMRKO mice (Figure 5A and Figure S2B). Similarly, increased CD45+ leukocyte and CD8+ T-cell numbers were seen in WT mice only with DOC/salt treatment (Figure 5B and 5C). Consistent with these data, baseline mRNA levels were lower in MyoMRKO CON versus WT CON for the chemoattractant proteins membrane cofactor protein-1 and CCR5, monocyte differentiation antigen CD14, and B-cell/T-cell costimulatory antigen CD81 (Figure 5D through 5G). Although mRNA levels were lower in MyoMRKO mice, a significant treatment effect was still detected for membrane cofactor protein-1 mRNA (Figure 5D) but not for CCR5, CD14, and CD81. These data show that loss of cardiomyocyte MR blocks the full inflammatory response to DOC/salt. A difference in inflammatory cell number at 8 days versus 8 weeks reflects the importance of vascular inflammation for early macrophage recruitment and the normal life span (6–16 days) of macrophages.

Cardiomyocyte MR Modulates Ex Vivo Cardiac Function but Not Blood Pressure Responses to DOC/Salt Treatment

Cardiac function was determined at 8 weeks in vehicle and DOC/salt-treated WT and MyoMRKO hearts instrumented on a Langendorff apparatus under aerobic perfusion conditions. Left ventricular–developed pressure was not different between vehicle-treated mice of both genotypes and was not altered by pacing hearts at 420 beats per minute (Table S7). DOC/salt treatment in WT, but not MyoMRKO, mice was associated with a significant increase in developed pressure, also apparent in paced hearts. Rates of cardiac contraction and relaxation assessed by a 2-tailed *t* test were also increased in WT mice given DOC/salt only (*P*=0.011 and *P*=0.012; Table S7). Heart rate and coronary flow (data not shown) were equivalent in all groups. These data demonstrate that loss of MR specifically in cardiomyocytes does not alter baseline cardiac function but that treatment with DOC/salt for 8 weeks promotes a positive inotropic state that is absent in MyoMRKO mice.

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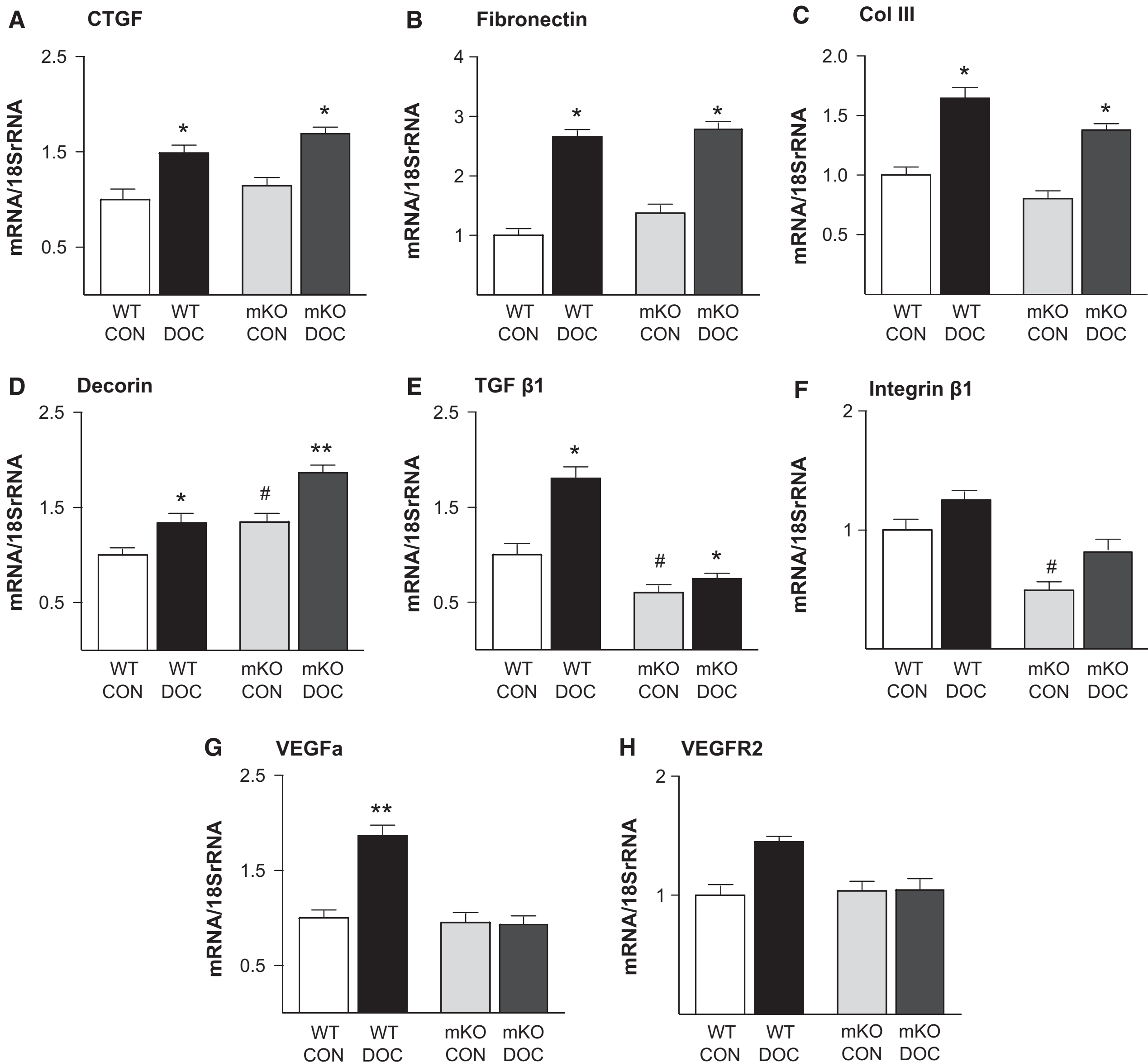

Figure 4. Cardiac tissue expression of genes associated with tissue remodeling at 8 weeks. Treatment groups are as for Figure 1. **A** through **D**, Deoxycorticosterone (DOC)/salt increased mRNA levels for connective tissue growth factor (CTGF), fibronectin, collagen III (Col III), and decorin in both genotypes. **D** through **F**, A significant genotype effect was detected for decorin, transforming growth factor-β (TGFβ1), and integrin-β1 mRNA levels. **G**, In wild-type (WT) mice only, DOC/salt increased vascular endothelial cell growth factor A (VEGFa) mRNA levels but not (**H**) VEGF receptor 2 (VEGFR2). *$P<0.05$ vs untreated mice; #$P<0.05$ WT vs mKO; **$P<0.05$ vs all other groups. Mean±SEM; n=8 to 10. WT CON indicates untreated wild-type mice; WT DOC, wild-type mice treated with deoxycorticosterone (DOC); mKO CON, untreated cardiomyocyte-specific MR-null mice; mKO DOC, cardiomyocyte-specific MR-null mice treated with DOC.

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DOC/salt treatment for 8 weeks increased SBP in WT mice (Figure S6A). Mean SBP values for untreated MyoMRKO mice were higher than for WT but not significant, with no difference in SBP found for MyoMRKO mice between CON and DOC/salt treatment. Plasma atrial naturietic peptide levels were equivalent in untreated WT and MyoMRKO mice at 8 weeks and were increased only in WT DOC mice (Figure S6B). Eight-week mRNA levels for brain natriuretic peptide were significantly lower in untreated MyoMRKO mice compared with WT; DOC/salt treatment significantly increased values in WT with similar relative change in MyoMRKO mice (Figure S6C). These limited changes in hemodynamic parameters are thus unlikely to account for the marked reductions in tissue fibrosis and inflammation observed in the MyoMRKO mice.

Discussion

The present study demonstrates a central role for cardiomyocyte MR in both the initiation and progression of DOC/salt-induced cardiac tissue inflammation and remodeling. Although the cardiomyocyte MR is not involved in the initial phase of DOC/salt-induced macrophage recruitment, we have identified a role for cardiomyocyte MR in the sustained inflammatory response at 8 weeks of DOC/salt treatment, in part via regulation of expression of chemoattractant proteins and chemokines. Upregulation of the CTGF-TGFβ1-Col III profibrotic pathway at the mRNA

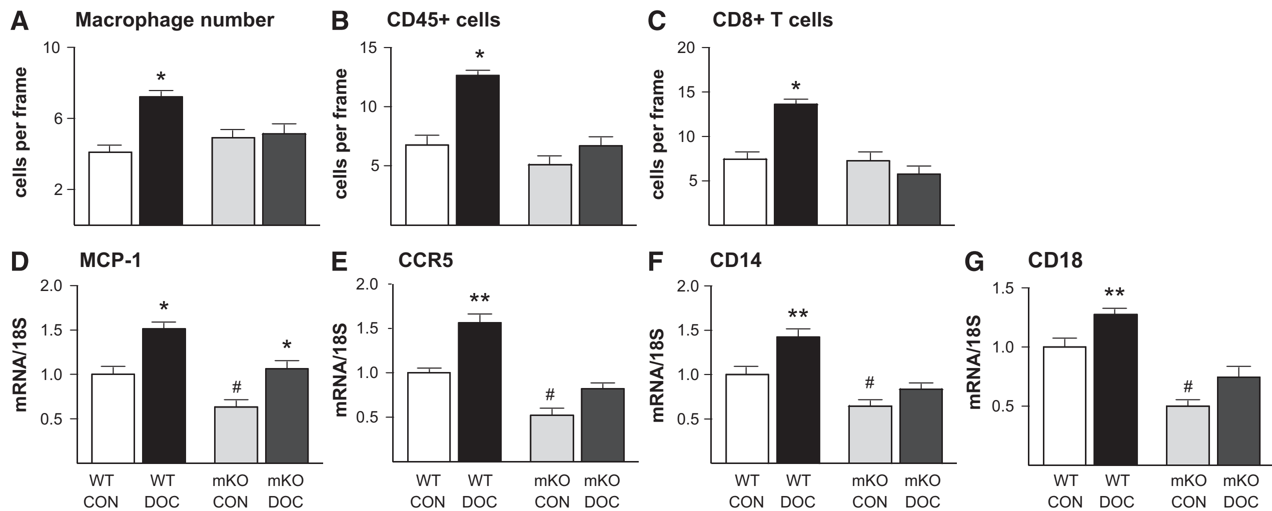

Figure 5. Markers of inflammation at 8 weeks. Treatment groups as for Figure 1. DOC/salt increased (**A**) CD11b+ monocytes/macrophages, (**B**) CD45+ leukocytes, and (**C**) CD8+ T cells in wild-type (WT) mice but not mKO mice and mRNA levels for (**D**) membrane cofactor protein (MCP)-1, (**E**) C-C chemokine receptor type 5 (CCR5), (**F**) CD14, and (**G**) CD81 in WT but not MyoMRKO mice. For all mRNA values, a significant genotype effect was detected. *$P<0.05$ vs untreated mice; **$P<0.05$ vs WT CON; #$P<0.05$ WT vs mKO. Mean±SEM; n=9 to 10. WT CON indicates untreated wild-type mice; WT DOC, wild-type mice treated with deoxycorticosterone (DOC); mKO CON, untreated cardiomyocyte-specific MR-null mice; mKO DOC, cardiomyocyte-specific MR-null mice treated with DOC.

level was not mirrored by protein expression in MyoMRKO mice. This differential response may reflect increased expression of the CTGF/TGFβ1 inhibitor decorin in MyoMRKO mice. MyoMRKO mice also showed higher cardiac MMP2/MMP9 activity compared with WT mice. Protection from DOC/salt-induced fibrosis in MyoMRKO mice may also involve reduced tissue inflammation because of loss of chemokine expression and reduced recruitment of monocytes/macrophages and lymphocytes. Collectively, these data demonstrate an important and independent role for cardiomyocyte MR in the progression of DOC/salt-induced tissue remodeling.

Cardiomyocyte MR and Collagen Deposition

A significant finding of the current study is that DOC/salt-mediated cardiac fibrosis is prevented in MyoMRKO mice. Our data show a parallel change in gene expression in MyoMRKO mice that favors reduced tissue fibrosis at 8 days (reduced plasminogen activation inhibitor 1) and 8 weeks (reduced TGFβ1).[12,13] Together with a higher MMP2/MMP9 activity in MyoMRKO mice versus WT, these tissue responses suggest a mechanism for the absence of a fibrotic response to DOC/salt treatment in MyoMRKO mice.

Our finding that profibrotic gene expression (CTGF, Col III, and fibronectin) was not altered by loss of cardiomyocyte MR may reflect high vascular expression of these markers (shown by immunostaining) or a limited role for cardiomyocyte MR in their regulation. A novel finding in the present study is that the CTGF/TGFβ-inhibitory protein decorin was upregulated in MyoMRKO mice. Given that decorin is a negative regulator of CTGF and TGFβ1[14] and that loss of decorin is associated with significant tissue fibrosis,[15] it is possible that elevated levels of this protein in the MyoMRKO mouse heart may serve to limit the profibrotic effects of CTGF and TGFβ1.

Gene expression of growth factors and markers of remodeling were significantly lower in MR-null cardiomyocytes. Of these, VEGFa and integrin-β1 remained low at 8 weeks. In particular, DOC/salt stimulation of VEGFa in WT mice was completely absent in MyoMRKO mice. Although a role for VEGFa regulation of capillary density has been described in ischemic remodeling, it is less well described in reactive fibrosis models; these data are consistent, however, with a role for VEGFa in tissue remodeling. Interestingly, a decorin-derived peptide has been shown to inhibit VEGF-stimulated NO release and endothelial cell migration in other model systems; decorin may thus play a broader role in the modulation of DOC/salt fibrosis.[16]

Per2 was selected for analysis, given that it is regulated by aldosterone in cardiomyocytes and that mice null for Per2 are protected from cardiac remodeling.[17] Per2 was upregulated at 8 days of DOC/salt in WT but not MyoMRKO mice, suggesting a potential role for cardiomyocyte MR signaling in the modulation of clock genes, although detailed time-course studies are required to fully characterize this response. Given that the cardiomyocyte circadian clock influences myocardial gene expression, heart rate, function, and tolerance to reperfusion injury, Per2 may represent a novel contributor to DOC/salt-mediated cardiac pathology.[18]

Cardiomyocyte MRs and Inflammation

Cardiomyocyte MRs are important for the recruitment of inflammatory cells at 8 weeks but not 8 days. The parallel increase in macrophage number in both WT and MyoMRKO mice at 8 days is consistent with the role of vascular MR in the initial inflammatory infiltrate.[5] Our data suggest that cardiomyocyte MR signaling may also regulate cell adhesion factor intercellular adhesion molecule-1 at baseline, but not in response to DOC/salt treatment as suggested previously.[11,19] Although macrophage recruitment is maintained in MyoMRKO mice, oxidative stress (NOX2 and p22phox) and inflammatory markers (CCR5) are substantially lower than for

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WT mice, suggesting that the tissue response to the DOC/salt stress is already limited at this early stage.

At 8 weeks, the MyoMRKO hearts do not show the characteristic DOC/salt inflammatory cell infiltrate observed in WT mice. Our present data are thus consistent with our previous studies showing the importance of macrophage MR in cardiac remodeling.[4] The mechanisms by which cardiomyocytes promote tissue fibrosis may therefore be macrophage dependent. Although decreased macrophage infiltration is associated with reduced tissue remodeling, our current data also show that a range of leukocytes play a role in DOC/salt cardiac pathology, that is, $CD45^+$ leukocytes and $CD8^+$ T cells that have previously been linked with tissue remodeling.[20]

Several proinflammatory genes not previously explored in the DOC/salt model, upregulation of CCR5 (chemokine for T-cell recruitment),[21] CD14 (myeloid cell surface receptor),[22] and CD81 (key regulatory protein on B cells and T cells),[23,24] were lost in MyoMRKO mice. Although differential expression of these markers may reflect differences in immune cell infiltrate, these data offer further insights into the specific subsets of immune cells in the tissue remodeling process.

Cardiomyocyte MRs, Blood Pressure Regulation, and Cardiac Function

DOC/salt was associated with a positive inotropic effect in WT hearts only indicated by an increased developed pressure. A positive chronotropic effect of DOC/salt was also found for WT but not MyoMRKO mice (+Dp/dt and −Dp/dt; 2-tailed *t* test). These data are consistent with previous studies showing aldosterone-dependent increased factional shortening of cardiomyocytes and increased chronotropic responses in isolated cardiomyocytes.[25–27] Although plasma volume contributes to developed pressure, the hypervolemia induced by DOC/salt is equivalent in WT and MyoMRKO mice, given that renal and vascular MR are intact. A lack of effect of fibrosis on cardiac relaxation is consistent with this stage of the model, which is has been selected to assess mediators of tissue remodeling. That cardiac function is normal in unchallenged mice shows that cardiac development is unaffected by the absence of MRs and is thus not compromised before the onset of the disease.[9]

DOC/salt treatment produced treatment effects on SBPs that were more marked in WT mice than MyoMRKO. However, SBP for MyoMRKO mice was not different from WT in either control or DOC/salt-treated mice, indicating that any effect on blood pressure by cardiomyocyte MR is modest. However, reduced atrial naturietic peptide (serum) and brain natriuretic peptide (mRNA) responses to DOC/salt suggest that the heart may be subject to less wall stress in MR-null mice.

Baseline Cardiac Responses to Deletion of MR Signaling

An important finding in the present study is the difference in gene expression profiles of untreated MyoMRKO mice compared with WT mice. Loss of MR signaling in cardiomyocytes resulted in significantly lower baseline levels of oxidative stress markers (p22phox), inflammatory markers (membrane cofactor protein-1, CCR5, CD14, and CD81), and profibrotic factors (plasminogen activation inhibitor 1, VEGFa, p22phox, TGFβ, and integrin β1). These data suggest an important role for the MR in cardiomyocyte signaling pathways responsible for these processes. Given that cardiomyocytes do not express 11βHSD2, expression of these genes may reflect glucocorticoid-bound MR.[28] We recently described a similar pattern of expression for a set of proinflammatory and profibrotic genes in untreated monocyte/macrophage MR-null mice, further supporting a distinct role for glucocorticoid-occupied MRs in cardiac gene regulation.[4]

Perspectives

Clinical studies have demonstrated cardiac protection with MR antagonists in heart failure, although the cellular mechanisms are not fully understood. Previously, the MR has been shown to regulate leukocyte adhesion on endothelial cells[5] and to promote a proinflammatory macrophage phenotype[29]; our data show that the cardiomyocyte MR regulates multiple stages of the DOC/salt inflammatory and fibrotic cardiac responses. The early responses to DOC/salt are suppressed in MR-null cardiomyocytes, which may be translated into a loss of the expanded tissue inflammatory response at 8 weeks and abrogation of tissue fibrosis. Our data highlight a specific role for cardiomyocyte MRs in the regulation of decorin levels and the CTGF-TGFβ pathway, and increase MMP2/MMP9 activity, together resulting in a net reduction in tissue fibrosis. Although a unique role for the cardiomyocyte MR in cardiac remodeling is consistent with observations of higher MR expression in failing heart tissue,[30] it should be noted that although clinical studies show significantly greater cardiac mass in primary aldosteronism versus essential hypertension, left ventricular function has not been found to differ between these 2 patient groups.[31] However, given that the majority of patients in the Randomized Aldactone Evaluation Study who benefited from MR blockade did not have high aldosterone levels, MR activation in the setting of left ventricular dysfunction may not be aldosterone dependent.

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Disclosures

None.

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Novelty and Significance

What Is New?

- Cardiomyocyte MR activation plays a direct role in deoxycorticosterone/salt cardiac remodeling, independent of cardiac hypertrophy and systolic blood pressure.
- Lack of cardiomyocyte MR limits early oxidative and inflammatory events, which limits expansion of the tissue inflammatory response and fibrosis.
- Increased decorin, a transforming growth factor-β-inhibitory protein, in cardiomyocyte MR-null mice is a novel mechanism, whereby profibrotic signaling pathways may be regulated by a cell-specific MR.

What Is Relevant?

- Cardiac histology and function in untreated cardiomyocyte MR-null mice is equivalent to wild-type mice showing that MR in these cells is not critical for cardiac development but is important for responses to disease stimuli.
- Identification of the specific cellular mechanisms regulated by cardiomyocyte MRs may allow for the development of targeted therapies, such as tissue-selective MR blockers, that will provide cardiovascular protection but spare renal function.

Summary

- Cardiomyocyte MRs play a critical role in initiation and developing tissue inflammation and collagen deposition via regulation of proinflammatory and profibrotic pathways in the heart.
- Cardiomyocyte MR-null mice show normal tissue structure and function when unchallenged, highlighting the importance of the MR in promoting cardiovascular disease but not in maintaining normal cardiac development and function.