

# Mineralocorticoid receptors in the heart: lessons from cell-selective transgenic animals

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## Abstract

The clinical impact of cardiovascular disease cannot be underestimated. Equally, the importance of cost-effective management of cardiac failure is a pressing issue in the face of an ageing population and the increasing incidence of metabolic disorders worldwide. Targeting the mineralocorticoid receptor (MR) offers one approach for the treatment of heart failure with current strategies for novel MR therapeutics focusing on harnessing their cardio-protective benefits, but limiting the side effects of existing agents. It is now well accepted that activation of the MR in the cardiovascular system promotes tissue inflammation and fibrosis and has negative consequences for cardiac function and patient outcomes following cardiac events. Indeed, blockade of the MR using one of the two available antagonists (spironolactone and eplerenone) provides significant cardio-protective effects in the clinical and experimental setting. Although the pathways downstream of MR that translate receptor activation into tissue inflammation, fibrosis and dysfunction are still being elucidated, a series of recent studies using cell-selective MR (NR3C2)-null or MR-overexpressing mice have offered many new insights into the role of MR in cardiovascular disease and the control of blood pressure. Dissecting the cell-specific roles of MR signalling in the heart and vasculature to identify those pathways that are critical for MR-dependent responses is an important step towards achieving cardiac-selective therapeutics. The goal of this review is to discuss recent advances in this area that have emerged from the study of tissue-selective MR-null mice, and other targeted transgenic models and their relevance to clinical disease.

## Key Words

- ▶ cardiovascular
- ▶ glucocorticoid
- ▶ mineralocorticoid receptor
- ▶ aldosterone
- ▶ knockout

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## Introduction

The primary physiological role of aldosterone is to stimulate ion transport in epithelial cells, in particular in the distal nephron and distal colon (Grundy *et al.* 1952). In epithelial cells, aldosterone stimulates sodium re-absorption and, by doing so, has direct effects on potassium and magnesium secretion and chloride re-absorption (Crabbe 1963, Wright & Giebisch 1978).

Aldosterone also has profound effects on the cardiovascular system, altering gene expression and influencing vascular tone, cardiac contractility and tissue composition. Moreover, it is becoming increasingly evident that the mineralocorticoid receptor (MR) exerts both cell- and ligand-specific actions in the cardiovascular system. The recent publication of a series of transgenic mouse models

targeting the MR in the cardiovascular system have highlighted novel and distinct roles for MR signalling in vascular smooth muscle cells (VSMCs), endothelial cells, cardiomyocytes and monocytes/macrophages in cardiac physiology and pathophysiology. These models represent important tools for understanding the specificity of MR action and have revealed many new mechanisms of action that were not possible in earlier pharmacological studies. The present review will discuss the importance of novel, cell-specific roles of MR signalling in the cardiovascular system.

### MR signalling in cardiac health and disease

Hans Selye first noted the pathological role of adrenal hormones in 1946; 50 years later, the significance of these findings was truly appreciated when inappropriate aldosterone-for-salt status in rodents was shown to produce hypertension, cardiac hypertrophy and fibrosis (Selye 1946, Brilla & Weber 1992). Large-scale clinical trials have subsequently validated the importance of MR signalling in cardiac pathology in patients with severe all-cause heart failures (RALES), heart failure post myocardial infarction (EPHESUS) or mild heart failure (EMPHASIS-HF; Pitt *et al.* 1999, 2001, Zannad *et al.* 2011). Addition of a low dose of spironolactone or the highly selective MR antagonist, eplerenone, to standard 'best practice' treatment with renin-angiotensin system inhibitors and  $\beta$ -blockers, reduces cardiovascular mortality and morbidity in patients with heart failure. Unfortunately, the clinical use of MR antagonists is limited by the side effect profile, which results from the blockade of renal tubule MR and the consequential elevation of serum potassium levels. Novel therapeutic options, which reduce MR activity in the heart while sparing renal function, would be beneficial. An increasing number of studies aim to elucidate the hormonal mechanisms and signalling pathways involved in MR-mediated cardiac pathology to facilitate the design of novel therapeutic approaches.

### Regulation of the MR epithelial vs non-epithelial mechanisms

The MR is a member of the steroid hormone receptor family of ligand-activated transcription factors that share structural homology with glucocorticoid (GR), progesterone, androgen and, to a lesser extent, oestrogen receptors respectively. The MR is, however, unique in that it can bind multiple classes of ligand including the mineralocorticoids, aldosterone and deoxycorticosterone

(DOC), and the GRs, cortisol or corticosterone. True mineralocorticoid responsiveness is achieved in the epithelial cells of the distal nephron, and blood vessel wall, via the action of  $11\beta$  hydroxysteroid dehydrogenase type 2 (HSD2). The absence of HSD2 in non-epithelial cells – heart, brain, adipose and inflammatory cells – results in cortisol (or corticosterone in rodents) being the predominant ligand for MR. Thus, in these tissues, MR serves to increase the dynamic range of cortisol/corticosterone signalling, given that they can signal via the high-abundance, low-affinity GR and the low-abundance, high-affinity MR.

MR signalling can also be mediated by a number of other important cell signalling mechanisms including transrepression and second messenger pathways. Transrepression involves a protein-protein interaction with sequestration of other transcription factors of which the best characterised example is GR and the transcription factors AP1 and NF $\kappa$ B, which mediate an anti-inflammatory effect. Conversely, both transactivation and mutual transrepression of the MR by NF $\kappa$ B have been reported (Pearce & Yamamoto 1993, Kolla & Litwack 2000, Fiebele *et al.* 2001, Chantong *et al.* 2012). To date, signalling pathways characterised as mediating rapid MR/aldosterone responses include the protein kinase C family and the MAPK family (Gekle *et al.* 2001, McEneaney *et al.* 2008, McEneaney *et al.* 2010). Rapid MR signalling can also regulate intracellular levels of calcium, cAMP and nitric oxide (NO; Wehling 1994, Christ *et al.* 2005, Hashikabe *et al.* 2006). These responses can be mediated via the classical MR and may also occur via the MR being putatively membrane-bound (Grossmann *et al.* 2005). The MR can be localised to the membrane, for example via interactions with epidermal growth factor receptor (EGFR) and studies demonstrate that the genomic MR is required for rapid corticosterone signalling in the brain (Karst *et al.* 2005, Grossmann *et al.* 2010). By contrast, rapid aldosterone signalling is retained in MR (NR3C2)-null cardiac cells and recent studies have suggested that a G protein-coupled receptor, GPR30, may serve as a novel aldosterone receptor, as it does for oestrogen (Haserath *et al.* 1999, Gros *et al.* 2011).

In addition to regulation of pre-receptor ligand availability, canonical MR signalling is also regulated by the specific combination of coregulatory proteins that associate with the ligand-bound receptor as well as the DNA sequence in the promoter of the target gene to which the complex is bound. This has been recently reviewed elsewhere (Yang & Fuller 2012).

## MR signalling in the vessel wall: VSMCs and endothelial cells

VSMCs, the contractile units of vascular walls, exhibit remarkable plasticity (Owens 1995). This plasticity is exemplified by their response to vascular injury, whereby VSMCs dramatically increase cell proliferation, migration and synthetic capacity to facilitate vascular repair. However, the high degree of plasticity can also contribute to vascular disease states including atherosclerosis and hypertension (Owens *et al.* 2004).

### VSMC MR and vascular tone

In the kidney, the MR response to aldosterone is a key regulator of blood pressure via stimulation of sodium and water retention in the epithelial cells of the distal nephron. However, the extra-renal actions of MR can also have profound effects on the development of hypertension and the associated cardiovascular mortality. An important component of blood pressure control is arterial stiffness, which is also an important risk factor for cardiovascular disease. A number of recent studies have suggested that VSMC MR play a direct mechanistic role in vascular compliance and thus extra-renal blood pressure control. Mice in which VSMC MR genes were selectively deleted using an inducible Cre-recombinase approach identified an important role for extra-renal MR signalling in age-dependent blood pressure elevation (McCurley *et al.* 2012). Loss of VSMC MR resulted in a lower blood pressure as the mice aged, despite no change in their renal sodium handling or vascular structure. A reduction in vascular tone and contractile function of the vessel wall was identified as a key mediator of these effects, which may be dependent upon MR regulation of L-type calcium channel activity. Hypertensive responses to angiotensin II and other vasoactive substances, vascular oxidative stress and vascular contractility were also shown by the authors to be dependent upon VSMC MR. These data are also consistent with a close relationship between the MR and angiotensin II signalling in the control of vascular function.

### VSMC MR and vascular remodelling

VSMCs play a fundamental role in vascular remodelling and vascular reactivity. MR activity in VSMCs promotes vascular remodelling in response to endothelial cell injury, independently of blood pressure responses (Jaffe *et al.* 2010). Mechanisms of MR-dependent vascular remodelling include galectin 3 signalling, which serves

as an aldosterone-dependent regulator of vascular fibrosis and inflammation, and protein kinase B, which is required for aldosterone-induced vascular apoptosis and structural injury (Wei *et al.* 2009).

MR-dependent vascular remodelling has also been demonstrated in the venous system. The MR is upregulated in human venografts and MR antagonists can preserve graft patency (Ehsan *et al.* 2013). Vascular remodelling is a key feature of atherosclerosis and is dependent upon both turbulent flow in the aorta and activation of the MR (McGraw *et al.* 2013). Aldosterone significantly increases the development of early atherosclerotic changes in the aorta as well as increasing the degree of inflammation of plaques in *Apoe*-null mice. The authors identified placental growth factor (PIGF), a secreted growth factor and monocyte chemoattractant that binds the vascular endothelial growth factor receptor 1 (VEGFR1) on monocytes, as a critical mechanism downstream of the MR in the generation of plaque instability in the vessel wall. These detrimental processes are independent of haemodynamic changes, although the presence of endothelial cell injury has synergistic actions with VSMC MR signalling outcomes.

VSMC MR-null mice have a reduced vascular fibrotic response to both aldosterone treatment and a wire-induced carotid injury that is also associated with the loss of VEGFR1 signalling (Pruthi *et al.* 2014). A similar mouse model of MR-null VSMCs, this time using a constitutive Cre to drive recombinase activity, investigated MR signalling in vessel wall remodelling and compliance (Galmiche *et al.* 2014). In contrast to the previous model, these mice show lower blood pressure in the unchallenged state, which is consistent with the previously described reduced vasomotor tone. The elastic properties of the arterial wall of the aorta or carotids were not different between WT and VSMC MR-null mice at baseline but when challenged with aldosterone and salt, an increase in wall stiffness was detected in WT mice only. Although there was a similar induction of fibronectin and collagen between genotypes, the significant reduction in expression of a matrix attachment protein integrin  $\alpha 5$  in VSMC MR-null mice may result in reduced cell matrix attachments and account for reduced vascular stiffness (Galmiche *et al.* 2014; Table 1).

### Endothelial cells and MR

Endothelial cells are a highly heterogeneous cell population that differ in terms of morphology and function throughout the vascular tree (Fishman 1982). They are

**Table 1** Comparison of phenotypes in tissue-selective MR-null mice

	VSMC	Endothelial cell	Cardiomyocyte	Macrophage
Blood pressure	BP regulation in ageing	No direct role in DOC/salt BP regulation	No direct role in DOC/salt or L-NAME/salt BP regulation	DOC/salt BP regulation Delayed L-NAME/salt increase in BP
Tissue remodelling	Aldo/salt induction of VSMC $\alpha$ 5-sub-unit integrin	DOC/salt cardiac fibrosis	DOC/salt cardiac fibrosis	DOC/salt and L-NAME/salt cardiac fibrosis
Cardiac macrophage recruitment		DOC/salt macrophage recruitment	DOC/salt macrophage recruitment	No direct role in L-NAME/salt or DOC/salt macrophage recruitment Required for L-NAME/Ang II macrophage recruitment
Cardiac function			DOC/salt-positive inotropy	
Vascular tone and contraction	Vascular myogenic tone Ang II vascular contraction			
Oxidative stress	Ang II oxidative stress			
Vascular stiffness	Aldo/salt increase in vascular stiffness			
Endothelial dysfunction		Aldo-impaired endothelial NO function Aldo-induced endothelial dysfunction in obesity		

Aldo, aldosterone; Ang II, angiotensin II; BP, blood pressure; DOC, deoxycorticosterone; L-NAME, L-NG-nitroarginine methyl ester; NO, nitric oxide; empty cells indicate that the parameter was not tested.

actively involved in numerous pathological conditions including atherosclerosis, inflammation and tissue remodelling. The endothelium expresses MR, GR and HSD2 (HSD11B2) all of which drive numerous physiological and pathological functions in the vascular wall. Clinical and experimental studies depict the effect of aldosterone on the endothelium. Hyperaldosteronism, as a consequence of an aldosterone-producing adrenal adenoma, hypertension or congestive heart failure, is associated with impaired vascular reactivity (Blacher *et al.* 1997, Duprez *et al.* 1998).

### Endothelial cell MR and inflammation

Endothelial cells actively regulate both acute and chronic vascular inflammatory processes by the secretion of vasoactive agents and expression of adhesion molecules (Pober & Sessa 2007). In a mouse model of atherosclerosis (*Apoe*-deficient mice), which is characterised by an inflammatory response in which monocyte-derived macrophages and endothelial cells play a central role, antagonising the MR reduces oxidative stress and inflammation (Suzuki *et al.* 2006). *In vitro*, aldosterone activation of MR in endothelial cells specifically modulates expression of intercellular adhesion molecule 1 (ICAM1)

and promotes leukocyte adhesion, highlighting the importance of MR in macrophage recruitment (Caprio *et al.* 2008). This notion is further supported *in vivo*, with deletion of the MR from endothelial cells protecting against DOC/salt-induced macrophage recruitment and elevated vascular ICAM1 expression (Rickard *et al.* 2014).

It is important to note that to date, endothelial cell MR-null mice have been generated using the *Tie2* (*Tek*) promoter to drive Cre expression in endothelial cells. Given that *Tie2* is also expressed in myeloid cells, some degree of loss of MR expression in myeloid cells is expected, although this differs considerably between studies (Schafer *et al.* 2013, 2014). As discussed below, however, the phenotype observed in macrophage-specific MR-null mice (LysM Cre) in terms of cardiovascular protection and blood pressure regulation is significantly different from that observed in endothelial cell MR-null mice (*Tie2*) (Rickard *et al.* 2009, Rickard *et al.* 2014).

### Endothelial cell MR and endothelial dysfunction

Endothelial dysfunction is characterised by impaired endothelial cell NO production and bioavailability, increased permeability, altered endothelium-mediated vasodilation,

increased vascular reactivity, platelet activation and enhanced thrombogenicity (Endemann & Schiffrin 2004). A number of clinical and experimental animal studies have demonstrated that aldosterone impairs vascular reactivity, although the precise mechanisms responsible are largely unknown. Aldosterone increases endothelial cell stiffness, which, in turn, reduces NO release (Oberleithner 2005). Increased production of reactive oxygen species (ROS) by endothelial NO synthase (eNOS) uncoupling contributes to the pathology in angiotensin II and DOCA-salt-treated hypertensive rats (Mollnau *et al.* 2002, Landmesser *et al.* 2003).

We have recently shown that, in mice, aldosterone impairs endothelial NO function in small resistance-like mesenteric arteries in both WT and endothelial cell MR-null mice (Rickard *et al.* 2014). These findings suggest that endothelial cell MRs are unlikely to contribute to MR-dependent endothelial dysfunction in resistance arteries and are consistent with the equivalent blood pressure elevation in response to DOC/salt with both genotypes. By contrast, aldosterone impaired endothelial NO function in the aorta from WT mice only, supporting a role for endothelial cell MR in endothelial dysfunction in larger conduit arteries.

Obesity is a major risk factor for hypertension and cardiovascular disease. The importance of endothelial cell MR in obesity-induced endothelial dysfunction has recently been described (Schafer *et al.* 2013). In diet-induced obese mice, eplerenone treatment prevented the impaired endothelium-dependent relaxation in response to acetylcholine, reduced the expression of pro-oxidative NADPH oxidase and increased the expression of anti-oxidant genes. Specifically, exploring the role of endothelial cell MR signalling in diet-induced obesity, Schäfer *et al.* (2013) demonstrated that specific deletion of MR from endothelial cells prevents obesity and aldosterone-induced endothelial dysfunction without affecting glucose tolerance and pro-inflammatory activity in white adipose tissue. Moreover, endothelial cell MR activity plays an important role in endothelial dysfunction by increasing endothelial p22phox expression.

Experimental animal studies suggest that aldosterone exerts prothrombotic actions by enhancing platelet activation and reducing fibrinolysis, and that MR antagonism can block these effects (Schafer *et al.* 2003, 2010, Bodary *et al.* 2006, Stankiewicz *et al.* 2007, Gromotowicz *et al.* 2011). Interestingly, MR signalling specifically in endothelial cells has recently been shown to reduce atrial thrombus formation in mice overexpressing MR conditionally in endothelial cells (Lagrange *et al.* 2014). The authors propose

a hypothesis that aldosterone-activated endothelial cell MR possesses antithrombotic properties in healthy vessels, while aldosterone acts synergistically with endothelial injury to exert prothrombotic effects (Newfell *et al.* 2011).

### Endothelial cell MR and blood pressure regulation

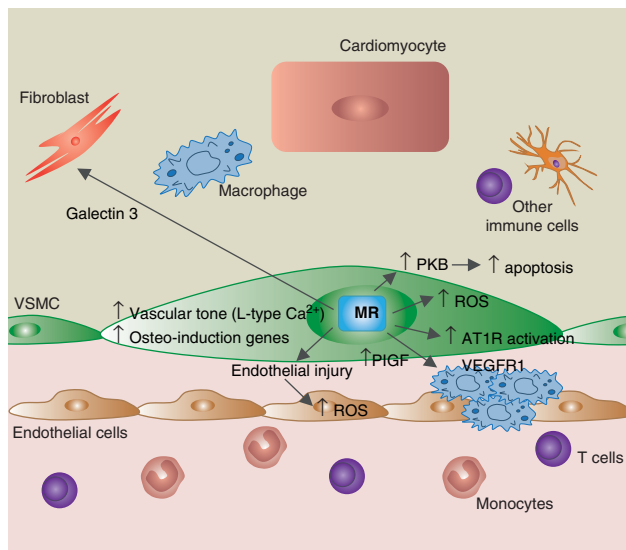
Endothelial dysfunction is associated with the pathophysiology of hypertension. A significant proportion of patients with stage I essential hypertension exhibit impaired small artery vasodilation (Park & Schiffrin 2001). Increased production of ROS by eNOS uncoupling has been demonstrated in a number of models of hypertension, including rats treated with peroxynitrite, which degrades the eNOS cofactor BH4, spontaneously hypertensive rats and angiotensin II or DOCA-salt-treated hypertensive rats. In DOCA-salt-induced hypertensive rats, it has been proposed that activation of NADPH oxidases provides the initial source of superoxide, which then reacts with BH4 and reduces BH4 levels. The loss of BH4 alters the function of eNOS, leading to reduced NO and elevated ROS production. In these rats, treatment with BH4 prevents eNOS uncoupling and reduces the blood pressure increase, suggesting that eNOS uncoupling contributes to the pathology of MR-induced hypertension (Fig. 1).

Interestingly, endothelial cell MR-null mice are not protected against DOC/salt-induced blood pressure elevation, suggesting that MR in other cell types of the myocardium regulates blood pressure in this model (Rickard *et al.* 2014). These data, together with the reduced fibrosis in endothelial cell MR-null mice, provide further support for the well-defined concept that MR-mediated fibrosis and hypertrophy are independent of changes in blood pressure (Rickard *et al.* 2014).

### Endothelial cell MR and tissue remodelling

Endothelial cells appear to be involved in the early pathological events of cardiac tissue remodelling. Activated endothelial cells release profibrotic mediators, inducing fibroblast activation and increased collagen deposition. Moreover, endothelial-mesenchymal transition, a recently recognised type of cellular transition, suggests that endothelial cells may play an important role in the pathogenesis of cardiac tissue fibrosis (Piera-Velazquez *et al.* 2011). Endothelial-mesenchymal transition is a process in which endothelial cells lose their specific markers and gain a mesenchymal or myofibroblastic phenotype. To date, the importance of the MR in endothelial-mesenchymal transition has not been defined. Interestingly, endothelial cell-specific MR knockout mice



**Figure 1**

Mineralocorticoid receptor activity in vascular smooth muscle cells. Mineralocorticoid receptor signalling in VSMCs alters vascular function via several mechanisms including PIGF signalling, VEGFR1 signalling, Mac2 activity, increasing ROS and activation of the AT1R. MR in VSMCs increases vascular tone via regulation of L-type calcium channels and contributes to endothelial cell injury. VSMCs, vascular smooth muscle cells; MR, mineralocorticoid receptor; Mac2, galectin 3; VEGFR1, vascular endothelial growth factor receptor 1; PKB, protein kinase B; PIGF, placental growth factor; ROS, reactive oxygen species; AT1R, angiotensin II receptor type 1.

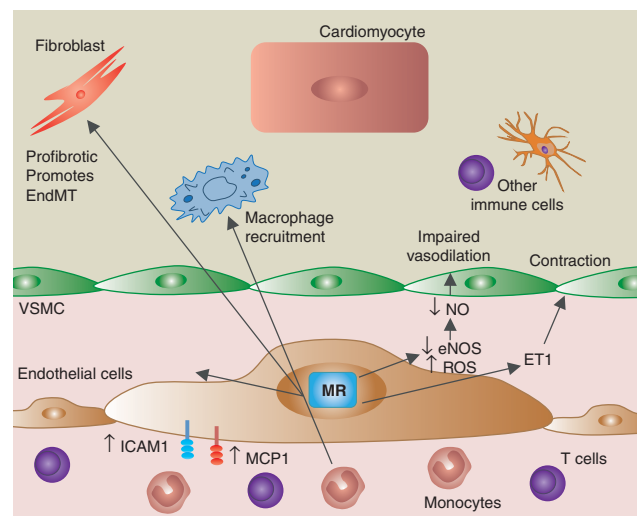
are protected against DOC/salt-induced fibrosis (Rickard *et al.* 2014). As noted earlier, DOC-induced inflammatory cell infiltrate is also attenuated in these mice, suggesting that the cardiac protection observed in this model may be primarily due to a reduced inflammatory response (Rickard *et al.* 2009). Further studies to explore the role of the MR and the intracellular interactions within the myocardium are required to understand the mechanisms responsible for DOC/salt-induced cardiac fibrosis. MR-dependent signalling pathways in the endothelial cells are summarised in Fig. 2.

## The MR in cardiomyocytes

The MR was identified in cardiomyocytes over two decades ago and, as noted earlier, acts as a high-affinity cortisol receptor, given that HSD2 expression is absent (Pearce & Funder 1988, Iqbal *et al.* 2014). Although cortisol is the predominant ligand for cardiac MR, aldosterone can be considered to play a physiological role in the maintenance of cardiac output as part of the response to volume depletion. The cardiac MR, however, is best known for its role in cardiac tissue remodelling processes, the mechanisms of which are discussed in this section.

## Cardiomyocyte MR, tissue remodelling and heart failure

The recent generation of cardiomyocyte MR-null mice, using either the myosin light chain 2a (*Mlc2a* (*Myl7*)) or the *Mlc2v* (*Myl2*) promoter, has facilitated the direct investigation of MR in cardiomyocytes *in vivo*. Fraccarollo *et al.* (2011) demonstrated that cardiomyocyte-specific MR deficiency improved infarct healing and prevented progressive adverse cardiac remodelling, cardiac hypertrophy of the surviving tissue, contractile dysfunction and gene expression profiles in ischaemic heart failure (Fraccarollo *et al.* 2011). Specifically, early tissue changes after infarct were significantly improved in cardiomyocyte MR-null mice, including increased scar thickness in the infarct zone and increased capillary density in the viable tissue. Tissue inflammation and markers of apoptosis were similarly improved in the first 7 days after infarct, compared with WT mice. These early improvements were associated with marked improvements in left ventricular filling pressures, left ventricular end diastolic and end systolic volumes and left ventricular ejection fraction in cardiomyocyte MR-null mice after infarct when compared with WT mice. These data underscore the importance of cardiomyocyte MR for heart failure onset

**Figure 2**

Mineralocorticoid receptor activity in endothelial cells. Mineralocorticoid receptor signalling in endothelial cells modulates ICAM1 and MCP1 expression, exerts profibrotic effects, stimulates macrophage recruitment to the myocardium and promotes EndMT. Endothelial cell MR alters vascular tone by increasing VSMC contraction via ET1 and impairing vasodilation by decreasing NO production. VSMCs, vascular smooth muscle cells; MR, mineralocorticoid receptor; EndMT, endothelial-to-mesenchymal transition; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; ROS, reactive oxygen species; ET1, endothelin 1; MCP1, monocyte chemoattractant protein 1; ICAM1, intracellular adhesion molecule 1.

and development and, taken together with previous pharmacological studies, provide strong evidence that cardiac dysfunction and failure can be limited by early initiation of MR blockade after infarct. Notably, immediate MR blockade improves myocardial infarct healing by modulation of the inflammatory response (Fraccarollo *et al.* 2008). Loss of the cardiomyocyte MR was also protective in the transaortic constriction model of pressure overload (Lothar *et al.* 2011). Cardiomyocyte MR-null mice were protected from cardiac dilatation and failure, demonstrating a significantly higher left ventricular ejection fraction compared with control mice. The authors suggested that a mild increase in cardiac wall thickness at baseline in the mutant mice (which was not detected in the previous study) may have contributed to the reduction in left ventricular wall tension after pressure overload. However, loss of cardiomyocyte MR did not prevent the development of cardiac hypertrophy, fibrosis, apoptosis index or inflammatory response after pressure overload, suggesting that the cardiomyocyte MR does not play a universal role in the onset of cardiac remodelling.

Studies from our laboratory investigated cardiovascular responses to the DOC/salt model of cardiac fibrosis and hypertension in mice null for cardiomyocyte MR (Rickard *et al.* 2012). We showed that activation of the cardiomyocyte MR was required from the primary inflammatory response and recruitment of inflammatory cells to the heart. As described in detail below, blocking the recruitment of macrophages to the myocardium is protective in itself (Shen *et al.* 2014). However, this model allowed for the identification of important signalling intermediates that were selectively regulated by the cardiomyocyte MR and point to a direct role for cardiomyocyte MR in DOC/salt-induced cardiac tissue remodelling. Selective regulation of matrix metalloproteinase 2/matrix metalloproteinase 9 activity and the transforming growth factor beta (TGF $\beta$ )–connective tissue growth factor (CTGF) profibrotic pathway by the MR in these cells suggest potential mechanisms for the cardioprotective effects of the loss of MR signalling. The TGF $\beta$ –CTGF inhibitor decorin was also significantly upregulated in cardiomyocyte MR-null mice, thus it is possible that lower collagen levels may reflect an increase in inhibitors of the fibrotic process in the MR-null cardiomyocytes.

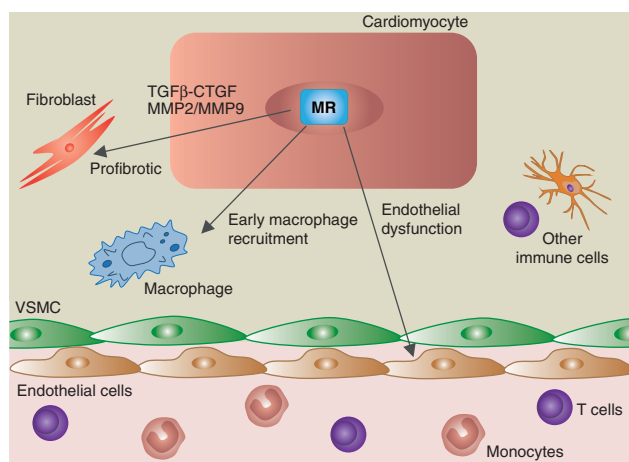
Further insights into the role of the cardiomyocyte MR in cardiac function and pathology have been identified *in vivo* in an earlier work on transgenic MR-overexpressing mice. First, genome-wide analyses of the MR-overexpressing heart, untreated and treated with aldosterone for 7 days, offer some insight into selective aldosterone–MR-regulated

genes in cardiomyocytes (Messaoudi *et al.* 2013). CTGF was one example of an MR target gene linked to cardiac remodelling that showed aldosterone-dependent regulation despite higher levels of intracellular GRs. Neutrophil gelatinase-associated lipocalin is another novel MR target in the cardiovascular system that may serve as a useful biomarker for MR-dependent cardiac dysfunction (Latouche *et al.* 2012). Notably, these mice did not show increased fibrosis, inflammation or apoptosis in the myocardium, supporting the hypothesis that an elevated sodium status is required for MR-mediated cardiac pathology.

### Cardiomyocyte MR and contractile function

An important observation in these studies is that the MR does not appear critical for normal cardiac development nor cardiac function in the unchallenged state. Baseline data from all three studies in cardiomyocyte MR-null mice showed normal systolic and diastolic functions and chamber diameters. Deletion of cardiac MR signalling did, however, result in the loss of the expected chronotropic and inotropic responses to DOC/salt (Rickard *et al.* 2012). These data are consistent with previous studies showing aldosterone-dependent increased fractional shortening of cardiomyocytes and chronotropic responses in isolated cardiomyocytes (Bravo *et al.* 1977, Barbato *et al.* 2004, Rossier *et al.* 2010). Rossier *et al.* (2008) have also shown the MR and GR to have additive chronotropic effects on the cardiomyocytes and that these can be modulated by the oxidative status of the cell. Notably, in cells expressing the MR alone, cortisol requires the presence of an oxidant to have an effect. To produce these responses, the MR and GR act to regulate T-type and L-type calcium channel expression (current amplitude) and activity (frequency) and may therefore also enhance the pro-arrhythmogenic potential of the myocardium (Rossier *et al.* 2008).

Conditional cardiac-specific overexpression of the human MR in mice also produced a high rate of sudden cardiac death (Ouvrard-Pascaud *et al.* 2005). Downregulation of the potassium transient outward current (I<sub>to</sub>), an increase in the L-type calcium current (I<sub>Ca</sub>) and the resultant prolonged ventricular repolarisation and severe ventricular arrhythmias are very likely to account for this phenotype. NOX-dependent, ROS-mediated coronary endothelial dysfunction are also evident in cardiomyocyte-specific MR overexpression indicating the importance of cell–cell signalling and the broader implications for cardiac MR activation in one cell type within the heart (Favre *et al.* 2011). As noted by the authors, the pro-arrhythmic effects

**Figure 3**

Mineralocorticoid receptor activity in cardiomyocytes. Mineralocorticoid receptor signalling in cardiomyocytes stimulates increase in collagen deposition via the selective regulation of MMP2/MMP9 activity and the TGFβ–CTGF pathway. MR in cardiomyocytes also contributes to early macrophage recruitment and endothelial dysfunction. VSMCs, vascular smooth muscle cells; MR, mineralocorticoid receptor; TGFβ, transforming growth factor beta; CTGF, connective tissue growth factor; MMP2, matrix metalloproteinase 2; MMP9, matrix metalloproteinase 9.

of the MR may reflect an important component of the protective effects of MR antagonists, which include decreased sudden death (Pitt *et al.* 1999, 2001).

The cellular mechanisms involved in MR regulation of cardiomyocyte function are illustrated in Fig. 3.

### MR signalling in inflammatory cells

Research over the past two decades has illustrated the remarkable impact of the immune response to the pathogenesis of cardiovascular disease (Mann 2011, Schiffrin 2014). The immune system comprises two components: the innate and the adaptive immune responses. The innate immune response employs a defined set of germline-encoded pattern-recognition receptors, such as toll-like receptors, NOD-like receptors, RIG1-like receptors and C-type lectin, which recognise pathogen-associated molecular patterns or damage-associated molecular patterns. The adaptive immune response relies on the generation of antigen receptors on T- and B-lymphocytes and the subsequent activation and clonal expansion of cells carrying the appropriate antigen-specific receptors.

### Macrophage MR and inflammation

Macrophages are key regulators of the initiation and progression of many pathologies including hypertension and cardiovascular disease (Rickard & Young 2009). They

exhibit a high level of functional plasticity, which is predominantly controlled by signals from the microenvironment in which they reside. Signals from the tissue microenvironment dictate the specific phenotype they acquire, which lies along a spectrum broadly defined as classically (M1) and alternatively (M2) activated macrophages (Goerdts *et al.* 1999, Gordon 2003, Martinez *et al.* 2008). Classical activation refers to stimulation by interferon  $\gamma$  and lipopolysaccharide, whereas alternative activation requires interleukins (IL10, IL4 and IL13), TGF $\beta$  or GRs to promote an M2-like phenotype. M1-like macrophages produce pro-inflammatory cytokines, chemokines and oxidative intermediates. By contrast, an M2-like phenotype is typically associated with anti-inflammatory signals, tissue remodelling and angiogenesis.

Macrophages express MR and GR but not HSD2; hence, under normal circumstances, the MR in macrophages is occupied by GRs (Lim *et al.* 2007). Corticosteroid receptor signalling in macrophages and other immune cells (microglia) appear to be mediated in a concentration-dependent manner. In microglia, low-dose corticosteroids (1 nM) promote inflammatory gene expression via MR, whereas the immunosuppressive effects of higher dose corticosteroid (100 nM) are conferred through GR (Tanaka *et al.* 1997).

It is well accepted that cardiac tissue inflammation plays an integral role in the initiation and progression of MR-mediated cardiac pathology. Early studies demonstrated elevated myocardial and/or vascular expression of inflammatory mediators after just one week of mineralocorticoid treatment (Fujisawa *et al.* 2001, Rocha *et al.* 2002, Young *et al.* 2003). Moreover, the critical role of inflammation in hypertension is also becoming increasingly apparent (Schiffrin 2014).

With the use of macrophage-specific MR-null mice, we identified a critical role for macrophage MR signalling in both mineralocorticoid-dependent and -independent models of cardiac pathology (Rickard *et al.* 2009, Bienvenu *et al.* 2012). Deletion of MR from macrophages altered the baseline expression of a number of pro-inflammatory genes, suggesting that, under normal circumstances, GR-occupied MR maintain normal macrophage function. Interestingly, macrophage recruitment was not altered by the loss of macrophage MR signalling in these models, suggesting that the MR in other cardiovascular cell types is responsible for macrophage recruitment in DOC/salt-induced cardiac pathology. As noted earlier, we have recently demonstrated a critical role for endothelial cell MR signalling in DOC/salt-induced macrophage recruitment (Rickard *et al.* 2014). Usher *et al.* (2010) expanded



upon these findings, demonstrating that macrophage MR is necessary for classical macrophage activation, given that macrophage MR-null mice displayed a transcriptional profile of alternative macrophage activation (Usher *et al.* 2010). In contrast to what has been observed in response to DOC/salt treatment for 8 weeks, macrophage MR-null mice were protected against NG-nitro-L-arginine methyl ester (L-NAME)/angiotensin II-induced macrophage recruitment (Usher *et al.* 2010).

### T lymphocytes, MR and cardiac pathology

In addition to macrophages, a number of other immune cells are increasingly recognised as playing an important role in the pathophysiology of cardiovascular disease and hypertension. T lymphocytes are adaptive immune cells that play a central role in orchestrating the immune response. The expression of MR in mouse splenic T lymphocytes was first described over 25 years ago (Armanini *et al.* 1985). T lymphocytes lack HSD2, thus MRs in T lymphocytes are hypothesised receptors for cortisol. A number of studies have implicated T lymphocytes in MR-mediated pathology. Mice lacking T and B lymphocytes (*Rag1*<sup>-/-</sup>) demonstrate a blunted blood pressure increase to both angiotensin II and DOCA-salt-induced hypertension (Guzik *et al.* 2007). Adoptive transfer of T lymphocytes, but not B-lymphocytes, restores the hypertensive response to angiotensin II and DOCA-salt in *Rag1*<sup>-/-</sup> mice, suggesting a role for T lymphocytes in the pathogenesis of hypertension. Furthermore, *in vivo* studies show that aldosterone can enhance the severity of Th17-mediated autoimmune disease responses, suggesting that MR-mediated inflammation may in part be mediated by amplification of the Th17 immune response (Herrada *et al.* 2010). This notion is further supported by a recent study in DOCA-salt-treated rats, where MR activation promoted Th17 polarisation and suppressed Treg function (Amador *et al.* 2014). Despite these recent advances, however, it remains unclear whether the effects of MR signalling on T lymphocyte polarisation are mediated by MR activation in T lymphocytes or via MR signalling in other inflammatory cells, i.e. macrophages or dendritic cells.

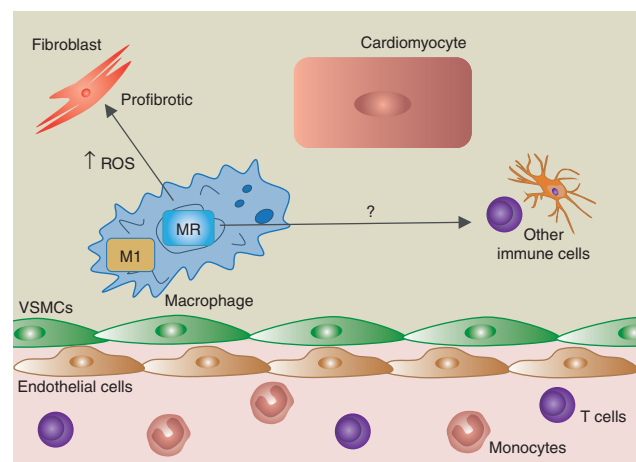
### Macrophage MR and cardiac fibrosis

Macrophages play a key role in fibrotic disease by altering the balance between matrix synthesis and degradation, via the secretion of various proteases and cytokines (Lupher & Gallatin 2006, Wynn 2008). With the use of macrophage-specific MR-null mice, we identified a critical role for

macrophage MR signalling in both mineralocorticoid-dependent and -independent models of cardiac fibrosis and profibrotic gene expression (Rickard *et al.* 2009, Bienvenu *et al.* 2012). The importance of macrophage MR in L-NAME/angiotensin II-induced cardiac hypertrophy, fibrosis and vascular damage has also been demonstrated (Usher *et al.* 2010).

### Macrophage MR and stroke

Immune cells play a central role in ischaemic brain injury. In experimental models of ischaemic stroke, MR antagonists reduce the infarct size and neurological deficit, without altering blood pressure (Dorrance *et al.* 2001, Iwanami *et al.* 2007, Oyamada *et al.* 2008). In a mouse model of transient middle cerebral artery occlusion, deletion of MR from macrophages reduced infarct volume and inflammation and improved neurological function (Frierler *et al.* 2012). Interestingly, in contrast to MR antagonists, which were protective against both transient and permanent occlusions in WT mice, specific deletion of MR from macrophages does not protect against permanent middle cerebral artery occlusion (Frierler *et al.* 2012). While the mechanism responsible for this difference in protection is unknown, it supports the notion that macrophages, and the MR in macrophages, play an important role in the initial response to reperfusion injury. These and the aforementioned pathways regulated by the MR in macrophages are illustrated in Fig. 4.



**Figure 4**

Mineralocorticoid receptor activity in macrophages. Mineralocorticoid receptor signalling in macrophages contributes to fibrosis by increasing ROS. Macrophage MRs are necessary for classical macrophage activation. VSMCs, vascular smooth muscle cells; MR, mineralocorticoid receptor; ROS, reactive oxygen species; M1, classically activated macrophages.

## Conclusion and perspectives

Pharmacological studies over the 20+ years following Brilla & Weber's (1992) publication describing the profibrotic effects of aldosterone plus salt, have defined the important role of MR in cardiac fibrosis, inflammation and vascular dysfunction. Collectively, these studies paved the way for a series of large-scale clinical trials of MR antagonists for heart failure management (Pitt *et al.* 1999, 2001, Zannad *et al.* 2011). The outcomes of these trials in turn have validated MR activity as an independent risk factor for cardiovascular disease and led to the development of eplerenone, which has a significantly greater specificity for the MR.

The recent series of studies using transgenic MR overexpressing or cell-selective MR-null mice described in this review have represented a new direction in understanding the role of MR in cardiovascular disease and provide a significant advantage over earlier pharmacological studies by allowing the dissection of unique cellular actions of MR. The overall cardiovascular 'outcome' of enhanced MR signalling is a composite of both rapid second messenger signalling (EGFR, Na/K/2Cl cotransporter, etc.) and gene/protein expression (calcium channels, growth factors, cytokines, etc.), which represent both compensatory physiological responses and defined pathological pathways. Taken together, these studies serve to illustrate the complex and coordinated role of MR in both the regulation of plasma volume homeostasis and the development of cardiovascular disease.

Whether or not therapeutically silencing MR in one cell type alone will be sufficient to protect against cardiovascular diseases remains unclear. Future studies are required for exploring MR-dependent cell–cell interactions in the initiation and progression of cardiovascular disease, which is an important step towards realising this goal.

### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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