Mineralocorticoid receptors and the heart, multiple cell types and multiple mechanisms: a focus on the cardiomyocyte

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Abstract
MR (mineralocorticoid receptor) activation in the heart plays a central role in the development of cardiovascular disease, including heart failure. The MR is present in many cell types within the myocardium, including cardiomyocytes, macrophages and the coronary vasculature. The specific role of the MR in each of these cell types in the initiation and progression of cardiac pathophysiology is not fully understood. Cardiomyocyte MRs are increasingly recognized to play a role in regulating cardiac function, electrical conduction and fibrosis, through direct signal mediation and through paracrine MR-dependent activity. Although MR blockade in the heart is an attractive therapeutic option for the treatment of heart failure and other forms of heart disease, current antagonists are limited by side effects owing to MR inactivation in other tissues (including renal targets). This has led to increased efforts to develop therapeutics that are more selective for cardiac MRs and which may have reduced the occurrence of side effects in non-cardiac tissues. A major clinical consideration in the treatment of cardiovascular disease is of the differences between males and females in the incidence and outcomes of cardiac events. There is clinical evidence that female sensitivity to endogenous MRs is more pronounced, and experimentally that MR-targeted interventions may be more efficacious in females. Given that sex differences have been described in MR signalling in a range of experimental settings and that the MR and oestrogen receptor pathways share some common signalling intermediates, it is becoming increasingly apparent that the mechanisms of MRs need to be evaluated in a sex-selective manner. Further research targeted to identify sex differences in cardiomyocyte MR activation and signalling processes has the potential to provide the basis for the development of cardiac-specific MR therapies that may also be sex-specific.

Key words: aldosterone, cardiac function, glucocorticoid, hypertension, inflammation, mineralocorticoid receptor

INTRODUCTION
The MR (mineralocorticoid receptor) is a ligand-activated steroid hormone receptor best known for its role in sodium and water homoeostasis in response to aldosterone. It is present not only in epithelial tissues, but also in many cell types in the heart where it plays a key role in cardiac inflammation and fibrosis. Cardiovascular disease is the leading cause of death and disability and the prevalence of heart failure and hypertension is increasing. Large scale clinical trials using low dose MR antagonists have demonstrated an important role for MR signalling in the development of heart failure [1,2]. One issue that arose in these studies was an increased incidence of hyperkalaemia in patients receiving MR antagonists owing to MR blockade in the kidney. Other side effects of the first generation MR antagonist spironolactone were also problematic (notably in men, gynacomastia and impotence) owing to cross-reactivity with other nuclear receptors. Although the cell-specific mechanisms involved in the benefits observed in these clinical studies are still being defined, emerging experimental evidence suggests that cardiomyocyte MR signalling plays a central role in the development of cardiac fibrosis, inflammation and hypertension [3–5]. The present review addresses the role of the cardiomyocyte MRs in cardiac pathophysiology and discusses the known mechanisms of cardiomyocyte MR action in influencing myocardial function and disease development in relation to oxidative stress, inflammation and fibrosis.
MR SIGNALLING MECHANISMS

The MR is expressed in epithelial cells in the kidney, colon and salivary glands and in non-epithelial tissues such as vascular smooth muscle cells, myeloid cells (i.e. monocytes and macrophages), neurons of the hippocampus and the placenta [6,7]. In the heart MRs are expressed in cardiomyocytes, macrophages, vascular smooth muscle and endothelial cells, but not at detectable levels in fibroblasts [8–11]. The MR is a member of the steroid hormone receptor family of ligand-activated transcription factors. Typically, as has been well studied in epithelial cell types, the MR is located within the cytosol and upon ligand binding forms dimers and translocates to the nucleus where, together with associated co-regulatory proteins, it binds to hormone-response elements in the promoter of target genes to regulate gene transcription (reviewed in [12]). Of note, cardiomyocyte MRs are mostly chromatin bound within the nucleus owing to hyperactive localization signals, rather than as a direct result of circulating corticosteroid levels [13,14]. MR signalling can also be non-genomic where MR signalling occurs rapidly within minutes of receptor activation and does not require nuclear translocation of the receptor–hormone complex or protein synthesis [15–17]. Numerous studies have been unable to identify a separate receptor for rapid MR effects, suggesting that rapid responses are mediated by the classical MR pathway in the cytoplasm that stimulates second messenger signalling [18–21]. The G-protein-coupled receptor GPR30 (G-protein-coupled receptor 30; also known as GPER [G-protein-coupled ER (oestrogen receptor) 1]), which has a well-described role in rapid oestrogen signalling, has recently been suggested to be involved in rapid MR signalling in the vasculature although the role of GPR30–aldosterone interaction in cardiomyocytes in currently unknown [22]. The convergence of ER and MR signalling pathways, perhaps via GPR30-related pathways, may be a mechanism whereby MR signalling is differentially regulated in males and females [22–26].

An important consideration in the regulation of MR signalling is that the MR has a high affinity for both physiological glucocorticoids, cortisol in humans and corticosterone in rodents, as well as the mineralocorticoids [27]. Given that glucocorticoids are present at concentrations 10–100-fold higher than aldosterone, the specificity of the MR in epithelial tissues is conferred by the enzyme 11β-HSD2 (11β-hydroxysteroid dehydrogenase type 2) which converts glucocorticoids into their inactive metabolites (cortisone and 11-dehydrocorticosterone) [28–32]. In non-epithelial tissues, such as neural and myocardial tissue, the MR is not co-localized with 11β-HSD2 and thus the MR in these tissues is normally occupied by glucocorticoids [10,11,33,34]. Increasing evidence shows that, in contrast with MRs in the kidney, glucocorticoid-occupied MRs do not produce responses equivalent to aldosterone. However, in the presence of tissue damage or oxidative stress, glucocorticoids act as full agonists [35–37]. The aldosterone–MR complex is not only more stable than the glucocorticoid–MR complex, it is also more active at target gene promoters, which implies that the co-activator/repressor recruitment may be critical in MR activation by different ligands [38]. It is also of note that the ligand-mediated conformational change of the MR, i.e. the N–C interaction, only occurs with aldosterone and not with cortisol. Whether the presence of cellular oxidative stress modifies this interaction has not been addressed [39,40]. Regulation of MR signalling is therefore, in part, dependent upon the presence or absence of the 11β-HSD enzyme and the local context of different target cells. The contrasting role of glucocorticoid regulation of the MR in physiology and disease is discussed below.

MR ANTAGONISTS ARE CLINICALLY PROTECTIVE IN HEART FAILURE

MR antagonists, in combination with current best practice therapy including angiotensin receptor blockers and angiotensin-converting enzyme inhibitors, decrease morbidity and mortality in patients with primary heart failure and heart failure secondary to myocardial infarction [1,2]. Most patients in these trials did not have elevated plasma aldosterone levels and these clinical outcomes provide support for a critical role for inappropriate glucocorticoid activation of MR in heart failure progression. Serious hyperkalaemia was increased by MR blockade in ~1% of heart failure patients (serum potassium levels 0.6 mmol/l). Although serum potassium levels of 0.4 mmol/l have been correlated with increased mortality in heart failure patients, the incidence of adverse cardiac events was not increased [2,41]. Correcting plasma potassium levels may be beneficial. Patients receiving MR antagonists showed decreased risk of mortality from sudden cardiac death. This was associated with a reduction in arrhythmia consistent with a role for MR blockade in the regulation of excitation/contraction coupling. MR antagonism reduces the risk of death and hospitalization in patients with chronic heart failure and can improve left ventricular hypertrophy regression and systolic blood pressure control in patients with mild heart failure when used in conjunction with an angiotensin-converting enzyme inhibitor [42,43]. More recently, the incidence of atrial fibrillation in patients with mild heart failure was effectively decreased by the second generation MR antagonist eplerenone [44–46]. These clinical trials clearly indicate the importance of including MR antagonists with current combination therapy in the treatment of heart failure and hypertension.

The current clinical understanding of the use of MR antagonists in patients with heart failure, hypertension and atrial fibrillation has recently developed substantially [47]. In a randomized double blind placebo-controlled clinical trial involving heart failure patients with preserved ejection fraction, MR antagonism with eplerenone was associated with reduced collagen turnover and improved diastolic function [48]. Patients exhibiting heart failure with reduced ejection fraction treated with the MR antagonist Canrenone (the principle active metabolite in spironolactone) in addition to optimal therapy showed improved diastolic function, suggesting a synergistic effect of the combined therapies [49]. Evidence shows that hypertensive patients treated with MR antagonists have reduced left ventricular mass and hypertensive patients with diastolic dysfunction have improved left ventricular contractility and systolic function [50]. The inconsistencies between the clinical and experimental data...
are discussed below. More recently, a non-steroid MR antagonist, BAY 94-8862, has been developed and shows greater selectivity for MR compared with spironolactone and stronger binding to MR compared with eplerenone [51]. This compound is currently being utilized in a phase II clinical trial, with outcomes expected to identify biomarkers of cardiac and renal function or injury together with the safety and tolerability [52].

MR antagonists are not only beneficial in patients with heart failure, but also in patients with atrial fibrillation. MR expression is higher in cardiac tissue samples from patients with atrial fibrillation compared with patients with normal sinus rhythm, suggesting that local aldosterone levels may not be as important as expression of the MR in the setting of atrial fibrillation [53]. Primary aldosteronism is accompanied by increased prevalence of atrial fibrillation and MR antagonists, along with surgery, reduced fibrillation incidence to a level comparable with essential hypertension [54,55]. The potential for MR antagonists to be of benefit in preventing atrial fibrillation was suggested by a trial that randomized patients with recurrent fibrillation into treatment groups with spironolactone in addition to a β-blocker and found that this combined treatment prevented episodes [56]. Further studies have validated the benefits of additive MR antagonist treatment for patients with coronary disease and/or heart failure in terms of a reduction in the risk of sudden cardiac death [57]. Collectively, these studies have highlighted a critical role for MR in the development and progression of cardiac injury.

Differences between males and females have been described for the prevalence, presentation and outcomes of cardiac events (reviewed in [58,59]). Whereas premenopausal women are less susceptible to ischaemic heart disease, post-myocardial infarction they experience increased mortality. After menopause, cardiovascular event risk is equivalent for males and females [60–62]. Both sex and age exert important influence on myocardial gene expression and signalling processes, yet few studies (clinical or experimental) have been conducted primarily on females [63]. Given that oestrogen can interact with many widely used cardiovascular drugs, including beta-blockers, calcium channels blockers and diuretics this may account for differences in drug activity between men and women [64]. Moreover, cytochrome P450 enzymes that are important in drug metabolism are more predominantly expressed in females compared with males, adding to the complexity of understanding the sex differences in drug action [65].

Clinical trials using MR antagonists have primarily comprised male participants. The RALES (Randomized Aldactone Evaluation Study) and EPHESUS (Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study) both consisted of ~30% females, and the 4E study of ~40% females. No significant sex differences were detected in these trials [1,2,42]. In contrast, the Framingham Heart Study, comprised ~72% females, demonstrated a correlation between plasma aldosterone levels and cardiac wall thickness in women, but not men. Other clinical studies have not demonstrated differences in cardiac morphology between males and females [66]. These discrepant findings between studies may reflect differences in the proportion of male and female patients studied and/or other confounding factors, such as age or different rates of co-morbidities, that may conceal sex dimorphism in heart failure.

CARDIAC OXIDATIVE STRESS, INFLAMMATION AND MR SIGNALLING

Experimental studies have been able to demonstrate that an important early event in the onset of cardiovascular fibrosis is oxidative stress and inflammation in the vessel wall (endothelial and vascular smooth muscle cells) and in the myocardium (cardiomyocytes and macrophages). MR-induced cardiac oxidative stress and inflammation occur within days of experimentally induced mineralocorticoid excess and precede the development of cardiac fibrosis and hypertrophy [37,67,68]. MR-induced inflammation and oxidative stress in vivo can be abrogated by either the MR antagonist spironolactone or the addition of an antioxidant, indicating that oxidative stress plays an important role in cardiac MR activation [69]. After coronary artery ligation, both aldosterone and AngII (angiotensin II) significantly increase NOX (NADPH oxidase) and mitochondrial superoxide generation. Blockade of MR and AngII signalling leads to a synergistic attenuation of oxidative stress in cardiomyocytes, demonstrating a key involvement of aldosterone in the cardiac oxidative stress response [70]. Studies have also suggested that the small GTPase Rac1 is required for oxidative stress-induced activation of cardiomyocyte MRs, potentially in a ligand-independent manner [71]. These data show that the redox state of a cardiomyocyte may modulate both MR activation and gene transcription. Therefore the MR can have both genomic and non-genomic effects on ROS (reactive oxygen species) production in the heart, and can in turn be regulated by oxidative stress itself.

Cardiomyocyte MR activation regulates the cardiac response to oxidative stress. Genetic loss of cardiomyocyte MR [myo-MRKO (MR-knockout)] decreases the generation of mitochondrial superoxide and reduces post-infarction up-regulation of the NADPH oxidase subunits NOX2 and NOX4 [72]. Myo-MRKO mice have lower basal levels of expression of the NADPH oxidase subunit p22phox which is not altered by mineralocorticoid excess over an 8 week treatment period [73]. In contrast, transverse aortic constriction increases cardiac NOX2 expression in both the wild-type and myo-MRKO mice [74]. These studies add further support to the hypothesis that the cellular response to disease is important in determining the effect of MR signalling on cardiac inflammation and oxidative stress.

Cardiomyocyte MRs can have direct effects on the coronary endothelium to elevate oxidative stress, decrease relaxation and to promote endothelial dysfunction [75]. Overexpression of human MR in mouse cardiomyocytes induces decreased nitric oxide-mediated coronary artery relaxation and increased cardiac ROS generation in endothelial cells. This raises the possibility of a paracrine signalling mechanism whereby activation of MR within cardiomyocytes induces expression of proteins/molecules that act on surrounding cells to incite an oxidative stress response. These studies highlight the involvement of multiple cell types, including cardiomyocytes, in the translation of MR signalling to produce cardiac inflammation and oxidative stress.

There is evidence that macrophage recruitment following tissue damage is a central feature of cardiac inflammation and fibrosis [76]. Mice in which the MR is selectively deleted from
monocytes and macrophages (mac-MRKO) display normal inflammatory cell recruitment in response to pharmacological activation of the MR receptor by DOC (deoxycorticosterone; a precursor to aldosterone that has potent mineralocorticoid activity), with reduced cardiac inflammation, oxidative stress, fibrosis and hypertension [77]. Similarly, the hearts of mac-MRKO mice also exhibit reduced L-NAME (N\textsuperscript{\O}-nitro-L-arginine methyl ester)/salt-induced cardiac fibrosis even though macrophage recruitment, inflammation and oxidative stress occurrence is robust [78]. These findings contrast with other studies using a different mac-MRKO model, reporting that loss of macrophage MRs was associated with reduced AngII/L-NAME-induced hypertrophy and fibrosis in a setting where macrophage infiltration was concomitantly reduced [79].

In addition to cardiomyocyte MRs facilitating the recruitment of macrophages, cardiomyocyte MRs also play a key role in the activation of macrophages [80]. In vivo, cardiac MRKO macrophages have reduced inducible nitric oxide synthase and tumour necrosis factor α expression and thus loss of the M1 macrophage pro-inflammatory phenotype [79]. Isolated peritoneal MRKO macrophages have been suggested to be polarized towards alternative activated or M2 macrophages; however, these cells were stimulated with thioglycolate prior to collection and similar results were not found in non-stimulated cells [78,79]. Macrophage MRs are thus necessary for the translation of inflammation and oxidative stress into interstitial and perivascular fibrosis after nitric oxide deficiency, even when plasma aldosterone is not elevated, providing further evidence for redox alteration of MR signalling multiple cell types.

Cortisol is normally anti-inflammatory acting via the GR (glucocorticoid receptor), but it also has a role in MR induced-inflammation [81,82]. As noted above, glucocorticoids can bind to both the GR and, when 11β-HSD2 is not present, to the MR. The differential role of cortisol compared with aldosterone at the MR in the heart is illustrated by both experimental overexpression of 11β-HSD2, which allows aldosterone to access the receptor, and tissue damage (e.g. induced-oxidative stress), which enables cortisol activation of MR resulting in equivalent cardiac inflammation to aldosterone excess [83,84]. Cortisol has been shown to mimic the effect of aldosterone on the sodium/potassium pump in isolated cardiomyocytes when the redox state is altered by the addition of GSSG [84]. Similarly, corticosterone exerts a more pronounced chronotropic effect on spontaneously contracting cardiomyocytes in culture when cellular oxidative stress is promoted pharmacologically [85]. Interestingly, cortisol signalling through the MR and GR regulates distinct pathways as determined by transcriptome analysis of mice overexpressing the MR or GR [86]. Further evidence from in vivo MR overexpression studies indicates different transcriptional networks operating for MR and GR signalling when activated by glucocorticoids [86]. Despite high levels of circulating glucocorticoids, aldosterone can activate MR and regulate transcription of specific genes, suggesting a mechanism other then 11β-HSD2 could be responsible for regulating MR activation by mineralocorticoids and glucocorticoids in cardiomyocytes [87]. The precise action and response of aldosterone versus endogenous glucocorticoids at the MR remains unclear.

**THE MR IS A KEY DETERMINANT OF CARDIAC FIBROSIS**

MR signalling is well recognized as a major contributor to the development of cardiac fibrosis and heart failure [88–90]. Long-term clinical and experimental studies have demonstrated a direct relationship between cardiac fibrosis and circulating mineralocorticoid levels [91–94]. Tissue fibrosis and collagen synthesis are characterized by the early presence of markers of inflammation, including cytokines and chemokines (monocyte chemoattractant protein 1, intercellular adhesion molecule 1 and vascular cell adhesion molecule) that stimulate extracellular matrix accumulation and tissue scarring [3]. Mineralocorticoid excess in the presence of high salt increases the accumulation of collagen type I and type III fibres in rat hearts, leading to myocardial stiffness [88,95–97].

Activation of MR directly in the coronary vasculature and in cardiomyocytes is an important regulatory step in the development of cardiac fibrosis [75,87,97]. Mineralocorticoid-induced cardiac fibrosis occurs independently of hypertension and hypertrophy, demonstrating that increased collagen accumulation is cardiac MR-dependent [4,98]. Treatment with aldosterone or the MR agonist DOC combined with elevated sodium intake induces left ventricular hypertrophy and increased collagen accumulation over a period of 8 weeks. This response is not seen when aldosterone or DOC are administered alone, highlighting the importance of moderate salt loading in this pathology [5,88,89,91,93]. Clinical evidence similarly indicates that high dietary sodium is required for aldosterone’s detrimental cardiac effects [99]. Chronic blockade of nitric oxide synthesis induced by L-NAME causes hypertension and vascular damage through increased oxidative stress on the vessel lumen, without direct and immediate up-regulation of the renin–angiotensin–aldosterone system [100–103]. That MR antagonism with eplerenone does not alter systolic blood pressure, but can decrease cardiac fibrosis and inflammation, suggests that cardiovascular responses to the inhibition of nitric oxide production are only partially MR-dependent [104]. Aldosterone increases the activity of MMP (matrix metalloproteinase) 2 and MMP9 in adult rat ventricular myocyte cultures in a ROS-dependent manner that involves the MEK1/2 [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) 1/2, ERK1/2, and MEK1/2 signalling cascade [105]. Thus cardiac MR can alter the breakdown and turnover of the extracellular matrix. In a cardiomyocyte MR-deficient model, suppressed expression of pro-fibrotic markers, as well as enhanced MMP2/9 activity following mineralocorticoid excess, has been observed [73]. These findings suggest a mechanism by which MR signalling in the cardiomyocyte increases fibrosis and inhibits extracellular matrix breakdown.

As discussed above, increased collagen gene expression is apparently an effect of MR activation in non-fibroblast cell types, such as macrophages and cardiomyocytes. Whether mineralocorticoids directly increase collagen gene expression in cardiac fibroblasts remains controversial, with direct evidence of cardiac fibroblast MR expression not consistently reported in the literature [8,88,106]. That MRs do not play a direct role in fibroblast function is supported by a recent study in which transgenic mice...
null for fibroblast MR showed no reduction in cardiac fibrosis or improvement of cardiac function when subjected to pressure overload [74]. It is thus unlikely that MRs have a direct effect on cardiac fibroblasts, and other cell types may be responsible for fibroblast stimulation through paracrine mechanisms (Figure 1).

As previously noted, myo-MRKO mice showed no detectable baseline differences in cardiac morphology following infarction induced in vivo compared with the wild-type; however, pulmonary oedema, cardiac hypertrophy and accumulation of extracellular matrix proteins were attenuated and capillary density was increased after infarction. At 7 days post infarct, myo-MRKO mice showed enhanced neovascularization and improved collagen structural organization. Interestingly, while these studies were conducted on male and female mice, sex differences were not assessed [72]. Of note, two studies which used the same strain of myo-MRKO mouse described different basal hypertrophy phenotypes: one exhibiting mild hypertrophy at the baseline and the other finding no differences between the wild-type and myo-MRKO mice [74]. Other studies using a different strain of myo-MRKO mice (i.e. myosin light chain-2V compared with atrial myosin light chain driven deletion) showed reduced cardiac fibrosis and increased extracellular matrix turnover compared with the wild-type in response to DOC/salt treatment [73]. Although some aspects of these studies/models are discrepant, overall the indication is that cardiomyocyte MR signalling is involved in both cardiac remodelling and ischaemic heart failure and is directly involved in infarct healing, hypertrophy and the fibrotic process (Table 1).

There is evidence of interaction between MR and oestrogen signalling in the development of cardiac fibrosis and in blood pressure control. Oestrogen acts in the cardiovascular system via two receptors, ERα and ERβ [107]. These receptors belong to the same nuclear receptor hormone family as the MR and can have important regulatory effects in MR-induced cardio-vascular changes [108,109]. In females, loss of ERβ is known to induce cardiac hypertrophy, fibrosis and maladaptive calcinurin signalling which is not observed in males. Specific sex differences in DOC/salt-induced cardiomyopathy are mediated by ER signalling [109]. mTOR (mammalian target of rapamycin) is involved in the ERβ regulation of cardiac fibrosis and hypertrophy in normotensive mice during mineralocorticoid excess, supporting a differential role for MR activation in males relative to females [110]. DOC/salt treatment exacerbates blood pressure-independent cardiac hypertrophy in male mice only [111]. Blood pressure increases in response to DOC/salt treatment are milder in female rats compared with the males. Adrenal medullectomy only reduces blood pressure in male mice, suggesting that sex differences in DOC/salt hypertension may be attributed to differences in the adrenal medulla and catecholamine levels [112,113]. In rats, a very high salt diet (4% NaCl) significantly increases systolic blood pressure in both males and females. This effect can be blocked in males, but not females, by the MR antagonist spironolactone independently of gonadal steroids [114]. Females may thus also have different sensitivity to aldosterone, although how responses in the adrenal medulla differ is unclear [115–117]. The relative contribution of MR and ER to cardiac fibrosis and

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**Figure 1** *Indirect effect of MR signalling on induction of cardiac fibrosis*

Multiple cell types within the myocardium are responsible for the induction of cardiac fibrosis. MR signalling in the cardiomyocyte can induce the development of cardiac fibrosis, potentially by indirectly altering the activation state of macrophages. Alteration of macrophage activation state (M1 to M2 activation, signalling mechanisms unidentified) can stimulate myofibroblast differentiation and the expression of pro-fibrotic proteins, such as connective tissue growth factor (CTGF) and transforming growth factor-β (TGF-β), and the subsequent production of collagen fibres including collagen I and III (COL I and COL III respectively).
stress increased T-type Ca$^{2+}$ currents and cardiac arrhythmia [118, 119]. Interestingly, oxidative stress increased T-type Ca$^{2+}$ channel (Cav3.2/$\alpha_1$C subunit) to promote electrical alterations in cardiomyocytes and cardiomyocyte cell lines. MR activation can increase the expression of multiple ion channels including the potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4 (a non-selective pacemaker channel), T-type Ca$^{2+}$ channel (Cav3.2/$\alpha_1$H subunit) and L-type Ca$^{2+}$ channel ($\alpha_1$C subunit) to promote electrical alterations in cardiomyocytes and cardiac arrhythmia [118, 119]. Interestingly, oxidative stress increased T-type Ca$^{2+}$ current amplitude, but decreased L-type channel activity indicating that the redox state of a cardiomyocytes, as well as MR activation, contribute to cardiac Ca$^{2+}$ signalling and arrhythmia [119].

Some links have been made at the mechanistic level relating MR action with the cellular substrates of arrhythmia. Overexpression of human MR in mouse cardiomyocytes alters the behaviour of the ryanodine receptor, which regulates the release of activator Ca$^{2+}$ from internal stores in these cells [120]. Abnormal operation of the ryanodine receptor and subsequent Ca$^{2+}$ release is associated with increased occurrence of delayed afterdepolarizations, diastolic dysfunction and arrhythmogenesis. Overexpression of human MR also decreases heart rate variability and increases the risk of arrhythmia in vivo [121]. In contrast, MR antagonism in the clinical setting decreases heart rate variability [50]. These data highlight the inconsistencies between the clinical and experimental settings and suggest that both low and high MR activation can lead to a pro-arrhythmic state, the mechanisms of which are unclear. MR blockade was also observed to prevent infarction-associated prolongation of cardiomyocyte action potential, suggesting that MR plays a role in early electrical changes prior to hypertrophy [122]. Although MR activation by itself is enough to significantly alter cardiac function and the incidence of arrhythmia, interstitial fibrosis can also have functional arrhythmogenic consequences related to conduction disruption. Aged mice treated with MR and/or AngII receptor antagonists have significantly decreased incidence of arrhythmia that correlates with reduced interstitial fibrosis [123]. To understand the mechanisms involved in MR-induced oxidative stress, inflammation and cardiac fibrosis and how this affects cardiac function and induction of arrhythmia, further research is required.

**A ROLE FOR THE MR IN CARDIAC ARRHYTHMIA**

As mentioned above, in the clinical setting, MR antagonists are beneficial in patients with atrial fibrillation, the most common form of cardiac arrhythmia. Involvement of MR in cardiac arrhythmia has now been identified in both isolated ventricular cardiomyocytes and cardiomyocyte cell lines. MR activation can increase the expression of multiple ion channels including the potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4 (a non-selective pacemaker channel), T-type Ca$^{2+}$ channel (Cav3.2/$\alpha_1$H subunit) and L-type Ca$^{2+}$ channel ($\alpha_1$C subunit) to promote electrical alterations in cardiomyocytes and cardiac arrhythmia [124,125]. In contrast, rats fed a low-salt diet show increased plasma aldosterone levels in vivo, but this aldosterone elevation was not associated with an increased cardiomyocyte L-type Ca$^{2+}$ current, demonstrating that increased plasma aldosterone alone is not sufficient to modulate L-type Ca$^{2+}$ channel flux [126]. Physiological concentrations of corticosterone were found to induce L-type Ca$^{2+}$ currents in an MR-dependent manner, but aldosterone in combination with corticosterone did not result in a further increase in the Ca$^{2+}$ current. These observations provide evidence that aldosterone does not affect cardiomyocyte Ca$^{2+}$ signalling when physiological glucocorticoid is present [126]. Cultured rat neonatal cardiomyocytes exhibit increased Ca$^{2+}$ current in response to aldosterone treatment, an effect reflecting increased L-type Ca$^{2+}$ current amplitude and associated with

**MR SIGNALLING REGULATES CARDIOMYOCYTE CONTRACTILITY AND RHYTHMICITY**

*In vitro* experiments have shown that aldosterone can modulate cardiomyocyte contractility via increased density of L-type Ca$^{2+}$ currents (the primary trigger for contraction) and decreased transient outward potassium current (involved in action potential plateau shaping) [124,125]. In contrast, rats fed a low-salt diet show increased plasma aldosterone levels in vivo, but this aldosterone elevation was not associated with an increased cardiomyocyte L-type Ca$^{2+}$ current, demonstrating that increased plasma aldosterone alone is not sufficient to modulate L-type Ca$^{2+}$ channel flux [126]. Physiological concentrations of corticosterone were found to induce L-type Ca$^{2+}$ currents in an MR-dependent manner, but aldosterone in combination with corticosterone did not result in a further increase in the Ca$^{2+}$ current. These observations provide evidence that aldosterone does not affect cardiomyocyte Ca$^{2+}$ signalling when physiological glucocorticoid is present [126].

**Table 1** Comparison of the cardiac features described in studies of cardiomyocyte MR-null mice exposed to different cardiovascular insults

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elevated expression of L-type channel subunits (α1c and β2). The addition of aldosterone to isolated rat cardiomyocytes increases the L-type Ca\(^{2+}\) current that can be blocked by an antioxidant, providing further evidence for redox regulation of MR signalling. In neonatal mouse cardiomyocytes aldosterone up-regulates peak Ca\(^{2+}\) current levels as a direct effect of MR activation. The use of MRKO and GR-knockout cardiomyocytes isolated from global MRKO or GR-knockout mice directly demonstrates the importance of the MR in the regulation of cardiac Ca\(^{2+}\) current [127]. Collectively, these experimental studies identify MR involvement in influencing cardiomyocyte contractile responses through direct regulation of the Ca\(^{2+}\) fluxes involved in activating excitation/contraction coupling.

There is also a growing body of experimental evidence which identifies a role for MR in the regulation of cardiomyocyte rhythmicity. In cardiomyocytes the T-type Ca\(^{2+}\) channel is involved in determining pacemaker depolarization activity. Mineralocorticoids and glucocorticoids promote T-type Ca\(^{2+}\) channel protein expression (Ca\(_{a1c2}\)/α1H subunit) linked with increased Ca\(^{2+}\) current and cardiomyocyte contraction frequency. Suppression of the expression of T-type channel subunits reduced cardiomyocyte chronotropic responses to corticosteroids. Changes in redox state were found to increase chronotropic responses by increasing T-type channel activity and decreasing L-type channel activity, but not gene expression. Glucocorticoids and mineralocorticoids increase cardiac contraction via a T-type channel-dependent mechanism [119]. Both MR and GR induce chronotropic responses in adult and neonatal rat ventricular cardiomyocytes; aldosterone and corticosterone induce increased occurrence of spontaneous contraction and expression of T-type Ca\(^{2+}\) channels in isolated rat ventricular cardiomyocytes [118,128].

Aldosterone has a dose-dependent positive chronotropic effect in isolated ventricular cardiomyocytes, an effect blocked by MR antagonism and reduced by GR antagonism, establishing a clear role for MR in cardiac Ca\(^{2+}\) signalling and regulation of cardiac rhythm in a mixed mineralocorticoid/glucocorticoid manner [85,118,128]. MR overexpression in embryonic stem cell-derived cardiomyocytes increased the frequency of contractions coincident with increased expression of the hyperpolarization-activated cyclic nucleotide-gated potassium channel 1 pacemaker channel [129]. Conversely, a reduction in heart rate has cardioprotective effects and MR blockade by spironolactone decreases both the heart rate and heart rate variability, demonstrating parasympathetic nerve activity is, in part, MR-dependent [130–132], although MR-mediated heart rate change is not inversely observed [133]. These extensive data demonstrate an important involvement of MR activation by corticosteroids and mineralocorticoids in the regulation of myocardial rhythmicity at the level of the myocyte and intact heart.

Some progress has been made in identifying the cell signalling events which mediate the action of MRs and GRs on cardiomyocyte contractility and rhythmicity. The direct positive inotropic effect of aldosterone on basal cardiomyocyte shortening in vitro occurs coincidentally with increased generation of oxygen radicals and elevated diastolic and systolic Ca\(^{2+}\) levels [134]. In contrast, other reports suggest that aldosterone does not directly alter cardiac contractility, rather it has been found to induce an increase intracellular sodium concentration via sodium/hydrogen exchanger up-regulation, which would directly modulate pH (and hypertrophic growth response) and indirectly modulate Ca\(^{2+}\) [135]. An interesting finding is that the MR antagonist spironolactone can act as a weak MR agonist in certain situations [136]. Both aldosterone and spironolactone can exert rapid effects on cardiac contractility when administered in the physiological range for aldosterone and the therapeutic range for spironolactone. Co-infusion of both ligands in ex vivo rat hearts has an additive effect on cardiac contractility suggesting that parallel pathways and/or signalling cascades may be involved [137]. Subsequent studies demonstrated that increased cytosolic pH promotes aldosterone-induced inotropic actions, whereas increased diastolic Ca\(^{2+}\) and increased myosin ATPase Ca\(^{2+}\) sensitivity are responsible for spironolactone-induced inotropic effects [138].

Further studies have identified a feedback loop where AngII and aldosterone cross-talk can activate MEK1/2 signalling leading to a time-dependent increase in T-type Ca\(^{2+}\) channel activity and subsequent MEK1/2 inactivation which was associated with PP2A (protein phosphatase 2A)-induced CREB inactivation [139,140]. MR antagonism with eplerenone was shown to inhibit the AngII-induced increase in Ca\(^{2+}\) current density in cardiomyocytes derived from a myopathic model [41]. The same affect was observed acutely in vitro and was partially reversed by aldosterone treatment. Expression of the angiotensin receptor was significantly reduced by eplerenone treatment, indicating that MR signalling is essential in the intracrine angiotensin regulation of Ca\(^{2+}\) current [141].

In overview, there are numerous lines of evidence which support the conclusion that MR signalling in the cardiomyocyte has a direct role in modulating myocardial contractility and rhythmicity by altering the expression and activity of proteins involved in excitation/contraction coupling, including Ca\(^{2+}\) fluxes in particular (Figure 2). On-going work will determine how these cellular and tissue influences contribute to in vivo cardiac regulation, acutely and chronically, in a variety of myocardial disease states.

**CARDIOMYOCYTE ISCHAEMIC INJURY AND DEATH RESPONSES ARE REGULATED BY MR SIGNALLING**

In isolated rat heart preparations acute cortisol and aldosterone infusion increase infarct size at low doses [136]. Eplerenone decreased infarct size and increased the recovery of left-ventricular-developed pressure [142]. These data support the hypothesis that in disease states, oxidative stress may allow the glucocorticoid activation of MR. Pre-treatment with MR antagonists decreased infarct size and phosphorylation of Akt and ERK1/2, signalling intermediates known to mediate ischaemic cardioprotection [143]. Moreover the beneficial effects of an MR antagonist at reperfusion were similar to the effects of pre-conditioning [144]. After myocardial infarction, eplerenone can suppress the extent of ventricular dilatation, the reduction in ejection fraction and the magnitude of the transcriptional response. Interestingly, these attenuation effects were more pronounced in females than...
Genomic MR activation involves the binding of aldosterone in the cytosol, leading to conformational changes and dimerization of the receptors. This process allows the receptors to translocate into the nucleus, bind to the MR-response element (MRE), and induce gene transcription. This results in the increased expression of inflammatory, pro-fibrotic, and oxidative stress proteins, such as NOX subunits, which can also up-regulate the expression of T- and L-type Ca\(^{2+}\) channel subunits, increasing Ca\(^{2+}\) signalling, beating frequency, and arrhythmia.

Non-genomic MR activation is characterized by rapid, ligand-independent signalling pathways. It involves the activation of MEK1/2 and ERK1/2 pathways and increases ROS generation and Ca\(^{2+}\) signalling through T- and L-type Ca\(^{2+}\) channels. These processes rapidly increase cell beating and contraction, as well as altering cell death responses in both isolated cells and intact hearts.

Cell death occurs both as a normal part of cardiac development and in disease conditions such as ischaemia and heart failure. Apoptotic cell loss plays a critical role in many forms of cardiac injury including post-infarction. The outcomes of cardiac injury are known to differ significantly between males and females and are likely to be due, at least in part, to oestrogen- and testosterone-induced alterations of the cell death processes.

Cardiac MRs directly affect cell death and survival under stress conditions. Cardiomyocytes contain an aldosterone-responsive plasma membrane-associated receptor that is critical for aldosterone-induced apoptosis through calcineurin/Bad (Bcl-2/Bcl-xL-antagonist, causing cell death) pathway. Low dose MR antagonists suppress infarction-induced apoptosis by attenuating the degradation of anti-apoptotic proteins and by reducing processing of pro-apoptotic proteins.

Cardiac MRs in males suggest greater MR sensitivity of females in the post-infarct environment.

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Although MR blockade in the heart is an attractive therapeutic option for the treatment of heart failure and other forms of heart disease, current antagonists are limited by side effects owing to MR inactivation in other tissues (including renal targets). This has led to increased efforts to develop therapeutics that are more selective for the cardiac MR and which may have reduced occurrence of side effects in non-cardiac tissues. Further research targeted to identify sex differences in cardiomyocyte MR activation and signalling processes has the potential to provide the basis for the development of cardiac specific MR therapies that may also be sex-specific.

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MR signalling in the heart


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