Aldosterone and the mineralocorticoid receptor

Smail Messaoudi1,2 and Frédéric Jaisser1,2*

1INSERM, U872 team 1, Centre de Recherche des Cordeliers, 15 rue de l’Ecole de Médecine, 75006 Paris Cedex 06, France
2Pierre et Marie Curie University, 75006 Paris, France

For decades, the role of aldosterone was thought to be limited to salt and water homeostasis control. This traditional view of the action of aldosterone restricted to sodium reabsorption in epithelial tissues must be revisited.

Clinical trials have demonstrated the therapeutic benefit of MR blockade in heart failure of various origins. The beneficial effects of MR inhibition in patients with heart diseases emphasize the importance of this receptor in cardiovascular tissue. Significant progress have been made in understanding the pathological situations in which MR blockade is beneficial as well as the mechanisms underlying the deleterious effects of MR activation. Experimental studies indicated that chronic activation of the MR in target tissues induces structural and functional changes in the heart, kidneys and blood vessels. These deleterious effects include cardiac and renal fibrosis, inflammation and vascular remodeling. Aldosterone is generally considered as the main ligand of MR. However, MRs bind mineralocorticoids and glucocorticoids with equal affinity. Since the selectivity enzyme 11 beta hydroxysteroid dehydrogenase type II (11 beta HSD2) is not active in cardiomyocytes, the question of which ligand activates MR in these cells is thus the subject of intense debate. The interaction of MR activation with other important players in cardiac pathophysiology, such as salt, oxidative stress, and Ang II, suggests that the combined use of MR antagonists and other drugs may have synergic beneficial effects. Experimental data also suggest that MR antagonists could have wider use than they do today and perhaps extended to various systemic diseases that affect cardiac function.

KEYWORDS
Aldosterone; Mineralocorticoid receptors; Cardiovascular disease; Angiotensin II; Oxidative stress

Introduction

Since the discovery of aldosterone by Simpson et al.1 in 1953 until the mid-1990s, the role of this mineralocorticoid hormone was thought to be limited to salt and water homeostasis. The pioneering work of Brilla and Weber,2 showing a profibrotic effect of aldosterone in the myocardium, gave new dimensions to this hormone and its receptor, the mineralocorticoid receptor (MR). Since then, knowledge of the pathophysiological role of aldosterone has continually been growing. Significant progress has been made in understanding the pathological situations in which MR antagonism is beneficial as well as the mechanisms underlying the deleterious effects of MR activation in the heart. Clinical trials have demonstrated the therapeutic benefit of MR blockade in heart failure of various origins. Thus, the deleterious effects of aldosterone and the beneficial effects of mineralocorticoid receptor antagonists in hypertension, heart failure, the metabolic syndrome, atherosclerosis, and vascular diseases place aldosterone and its receptor at the heart of the continuum from hypertension to heart failure.

The aim of this article is to review recent advances in this field and discuss some points that are subject to debate: (i) the importance of MR antagonists in heart diseases of various origins; (ii) the effects of non-cardiac MR blockade on cardiac function; (iii) the nature of the
ligand activating the MR in the heart; and (iv) the model of co-incidence (angiotensin II [Ang II] interaction, role of salt, and oxidative stress).

**MR antagonism and heart diseases**

Chronic co-administration of aldosterone and sodium chloride (NaCl) stimulates perivascular and interstitial cardiac fibrosis in both left and right ventricles independently of blood pressure.\(^2^\)\(^-^\)\(^4^\) MR blockade at sub-hypertensive doses prevents these effects. The development of cardiac fibrosis seems to start with a perivascular fibrosis (associated with coronary and myocardial inflammation) that extends to the interstitium.\(^5^\) Aldosterone alone (ex vivo) or in association with NaCl (in vivo) stimulates the expression of several pro-inflammatory molecules that may contribute to the pathogenesis of cardiac remodelling. For example, aldosterone increases endothelin type 1, transforming growth factor \(\beta\), and plasminogen activator inhibitor through MR-dependent mechanisms as well as collagen and metalloproteinases.\(^6^\)

Oxidative stress is also induced, mainly through activation of NADPH oxidases.\(^7^\) Concomitant administration of NaCl is a prerequisite to induce cardiac fibrosis; aldosterone administration alone (or chronic aldosteronemia in models of hyperaldosteronism) is not sufficient to induce cardiac fibrosis in rats or mice.\(^8^\)\(^,^\)\(^9^\) Likewise, cardiomyocyte-specific MR over-expression or cardiac synthesis of aldosterone does not induce fibrosis.\(^10^\)\(^,^\)\(^11^\) This suggests that one or more co-factors are required to induce the profibrotic effects of aldosterone. This point will be discussed later.

The involvement of MR activation in the development of several heart diseases has been demonstrated through the use of specific MR antagonists (spironolactone, eplerenone) in experimental models. Pharmacological blockade of MRs ameliorates the transition to heart failure in models of systolic left ventricular dysfunction\(^12^\) and myocardial infarction\(^13^\) as well as in models of diastolic dysfunction in rats\(^14^\) and mice.\(^15^\)

Recently, the deleterious role of aldosterone and the beneficial role of MR antagonists have emerged in the study of atrial fibrillation (AF). Milliez et al.\(^16^\) have shown an increased rate of AF in patients with hyperaldosteronism. Aldosteronemia increases in patients with chronic AF and decreases rapidly after electrical cardioversion.\(^17^\) Atrial MR expression is increased in patients with AF.\(^18^\) Spironolactone prevents atrial remodelling (fibrosis and dilation) as well as apoptosis in an experimental model of AF and prevents the increased inducibility and duration of tachypacing-induced AF\(^19^\).

MR activation can also modulate cardiac electrical activity and thus lead to ventricular arrhythmias. Aldosterone increases T-type calcium channel expression and beating frequency in neonatal rat cardiomyocytes ex vivo.\(^20^\) In vivo, aldosterone infusion or over-expression of MRs in cardiomyocytes induces ionic remodelling. The potassium current \(I_{to}\) is decreased while the \(I_{cal}\) channel activity is increased; the activity of the ryanodine receptor is also impaired.\(^21^\)\(^,^\)\(^22^\) This has important consequences in the control of calcium homeostasis, modulation of calcium transients, sarcoplasmic reticulum diastolic leaks, and promotion of rhythm disorders. Conditional over-expression of MRs in cardiomyocytes is associated with ventricular extrasystoles and increased sensitivity to the triggering of ventricular arrhythmia.\(^11^\) Furthermore, in cardiomyopathic hamsters (TO-2), eplerenone reduces cardiac remodelling and decreases the rate of spontaneous ventricular tachycardia and the spatial dispersion of the QT interval, while the ST-segment depression is considerably improved. The propagation and conduction velocity are also improved.\(^22^\)\(^,^\)\(^23^\) Similarly, spironolactone prevents gap junction remodelling and restores the decreased transverse conduction velocity in the thoracic aortic constriction model.\(^23^\) This may be the basis of the strong beneficial effect of MR antagonism in the Randomized Aldactone Evaluation Study (RALES) and Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS).\(^24^\)\(^,^\)\(^25^\)

**Cardiac effects of non-cardiomyocyte MR**

The beneficial effects of MR antagonists on the heart are often considered to result solely from blockade of MR expressed in the heart or more specifically in cardiomyocytes. Although MRs are expressed in cardiomyocytes,\(^26^\) these receptors are also expressed in other cardiac cell types: coronary endothelial and vascular smooth muscle cells,\(^27^\) inflammatory cells such as macrophages, as well as fibroblasts.\(^28^\) Cardiomyocyte-specific MR deficiency, resulting from conditional MR gene inactivation, prevented adverse cardiac remodelling in heart failure following ischaemia or pressure overload.\(^29^\)\(^,^\)\(^30^\) It has recently been demonstrated that specific knock-out of MRs in macrophages prevents the development of cardiac interstitial fibrosis in the DOCA (deoxycorticosterone acetate)-Salt model, although the cardiac recruitment of these cells was not prevented.\(^31^\) These observations indicate that MR signalling in macrophages is required to elicit a full fibrotic response in the heart in some pathophysiological conditions.

The importance of coronary circulation in cardiac pathophysiology is well known, although its role in the harmful effects of mineralocorticoids has been poorly studied. Two recent studies suggest a significant effect. In a model of cardiomyocyte-specific over-expression of aldosterone synthase, the aldosterone concentration in cardiac tissue is moderately increased without alteration of cardiac structure and function. In contrast, coronary arteries develop a major NO-independent vascular dysfunction that is due to altered potassium channel BKCa expression and activity in vascular smooth muscle cells (VSMCs).\(^32^\) Cardiomyocyte-specific over-expression of MRs is associated with a local oxidative stress, leading to severe coronary endothelial dysfunction that is prevented by MR antagonist, vitamin E/vitamin C, or a NADPH oxidase inhibitor.\(^33^\) The consequences of such MR-dependent, aldosterone-induced alterations of coronary reactivity are still to be evaluated, but they could increase the sensitivity of the heart to ischaemia or predispose cardiac tissue to heart dysfunction.
Heart failure is associated not only with peripheral endothelial dysfunction, often with renal dysfunction (which in turn will affect cardiac function), but also with an increase of sympathetic tone. It is difficult to distinguish the role of each element. However, in a series of elegant experiments using intracerebroventricular (ICV) injection of an aldosterone synthesis inhibitor or an MR antagonist, the role of activation of MRs expressed in the central nervous system and its effects on sympathetic tone have been elucidated.44 Leenen’s group demonstrated the beneficial effects of ICV MR blockade, using a low dose of spironolactone, on cardiac remodelling after myocardial infarction. More surprising, the effects were equivalent between oral and ICV administration of spironolactone.34 Similar results were obtained with the ICV injection of an inhibitor of the aldosterone synthase (FAD286).35 These studies indicate that locally synthesized aldosterone and MR activation in the brain influences peripheral mechanisms involved in cardiac remodelling and contributes substantially to progressive heart failure post-MI.

**Aldosterone, cortisol, MR: all guilty**

The involvement of MRs in the deleterious effects described above is demonstrated in transgenic models with targeted over-expression of MRs and the beneficial effects of MR antagonists. Of course, the specificity of MR blockers can be discussed, especially for spironolactone, which can also antagonize the androgen receptor; in contrast, eplerenone specifically antagonizes the MR.

The question of which ligand activates MR in cardiomyocytes is the subject of intense debate. MRs bind mineralocorticoids and glucocorticoids with equal affinity. In classic target tissues such as the distal nephron, sweat glands, and distal colon, MR selectivity is achieved by the co-expression of 11β-HSD2 activity.26,43 Thus, there must be another selectivity mechanism(s) to allow MR activation by aldosterone. These mechanisms may be diverse. Sub-cellular compartmentalization or post-translational modifications of MRs could also be mechanisms to vary MR affinity for aldosterone or glucocorticoids.

Another important point, often underestimated, is that aldosterone/MR or glucocorticoid/MR, with their co-factors is different depending on the nature of the complex, aldosterone/MR or glucocorticoid/MR, with each modulating a specific set of genes. The use of transgenic models with targeted over-expression of MRs and demonstrated that aldosterone exhibited a significant response on beating frequency in isolated cardiomyocytes. However, this response was not affected by the presence of either corticosterone or dexamethasone (even in the presence of a glucocorticoid receptor antagonist), in spite of a glucocorticoid to mineralocorticoid ratio (100:1) favouring glucocorticoid binding to MRs.38 These results argue against a competition between glucocorticoids and mineralocorticoids for MR occupancy but do not exclude an effect of glucocorticoids in the basal state or in certain pathophysiological situations.

**Ex vivo**, corticosterone has effects equivalent to those of aldosterone. In isolated cardiomyocytes, aldosterone and cortisol induce a similar increase in the expression of various genes.39 In an isolated perfused heart model, aldosterone, like cortisol, increases the size of infarction.40 These effects are prevented by concomitant administration of MR antagonists. However, there is no evidence that this is also the case in the more complex *in vivo* environment.

According to Funder and Young, MRs expressed in cardiomyocytes are constantly occupied by glucocorticoids, which act as antagonists.41 Reduced nicotinamide adenine dinucleotide (NAD) has been shown to reduce gene expression by activating co-repressors.42 A change of the NAD/NADH ratio, by oxidative stress, for example, will activate MRs by converting glucocorticoids from antagonists to agonists.43 A recent study demonstrated that modification of the redox potential of isolated cardiomyocytes leads to activation of MR by cortisol.38 but how in this case can the agonist effects of aldosterone described above be explained? It is possible that both hypotheses are compatible if another mechanism of selectivity exists that does not rely on the activity of the 11β-HSD2. In the distal nephron, 11β-HSD2 activity cannot convert all of the glucocorticoids present in the cell into compounds that bind to MRs with low affinity. Funder estimated the intracellular residual level of glucocorticoids to be 10-fold higher than that of aldosterone, and therefore at a level sufficient to compete with aldosterone for MR binding, assuming that his model is correct *in vivo*.43 Thus, there must be another selectivity mechanism(s) to allow MR activation by aldosterone. These mechanisms may be diverse.

Perrier et al.37 showed an excellent correlation between aldosteronaemia and calcium channel activity (iCal) in cardiomyocytes isolated from experimental models of pseudo-hypaldosteronism (knock-out mutation of the epithelial sodium channel [ENaC]), hypoaldosteronism (activating mutation of ENaC), or chronic administration of aldosterone.41 It was recently shown that administration of aldosterone in mice increases the release of calcium from the sarcoplasmic reticulum because of an abnormally prolonged opening of ryanodine receptors.21 A modest increase of MR expression in cardiomyocytes produces a similar effect. Furthermore, Rossier et al.38
administration of glucocorticoids or aldosterone could help to address this issue.

In RALES, the starting levels of plasma aldosterone were in the low/normal range, which raises the question of what spironolactone was antagonizing. Cardiac expression of MRs is increased in hypertension, myocardial infarction, and diastolic heart failure. Increased MR activation could occur in the presence of normal aldosterone and glucocorticoid levels because of increased local expression of MRs. Another possibility, currently not explored in the heart but in the kidney, may be ligand-independent activation of MRs. Fujita and his collaborators recently provided evidence that MRs may be activated by the small protein Rac1, even in the absence of aldosterone. This is reminiscent of an earlier observation in which MRs were activated in cultured smooth muscle cells by Ang II (probably not directly but through angiotensin II receptor [AT1R] transactivation) in the absence of aldosterone in the culture medium. This aspect clearly needs further analysis in cardiomyocytes and in cardiac pathophysiology in vivo.

The co-incidence model (interaction with salt, Ang II, and oxidative stress)

It is difficult to distinguish the molecular effectors directly related to the activation of cardiac MRs by aldosterone or glucocorticoids from those associated with other effectors such as salt, Ang II, and oxidative stress. Infusion of aldosterone or glucocorticoids in the presence or absence of MR antagonists or manipulation of MR expression (up or down) in the absence of pathological stress can help to address this issue.

The effect of salt is a crucial element in the pathological activation of cardiac MRs and its consequences. The deleterious effects of aldosterone in the aldosterone–salt model are observed only when aldosterone is abnormally high in comparison with sodium loading. Thus, if sodium intake is normal, administration of aldosterone does not induce cardiac remodelling, oxidative stress, or inflammation. Similarly, activation of MRs is required to observe the deleterious effects of salt on diastolic function. The underlying mechanisms are unclear but may involve a synergism between activation of MRs and oxidative stress. While aldosterone alone does not lead to an increase in collagen deposit but to a small, significant increase in fibronectin synthesis in human aortic smooth muscle cells, co-incubation with aldosterone and hydrogen peroxide leads to a much more pronounced increase in collagen I and III secretion than with hydrogen peroxide alone. This synergism also involves the interaction between MRs and AT1Rs. Ang II in vivo significantly increases NADPH oxidase activity, interstitial fibrosis, expression of fibronectin, procollagen I and connective tissue growth factor mRNA, MMP-2 activity, and NF-kB activation. Spironolactone inhibits the Ang II-induced increase in NADPH oxidase activity and the increase in interstitial fibrosis. Similarly, losartan, an AT1R antagonist, prevents the cardiac effects of aldosterone in the aldosterone–salt model. The synergism between MRs and AT1Rs induces diastolic dysfunction, cardiac remodelling, and an increase in inflammatory markers in the heart. All these effects are prevented by administration of an MR antagonist. This synergy seems to be based, at least in part, on increased expression of AT1Rs (not always observed, depending on the models studied) as well as on the transactivation of this receptor by MRs. This synergism has been well documented in VSMCs but has not been specifically studied in cardiomyocytes. Gekle has recently proposed a model of co-incidence integrating these different aspects.

Cardiopathies from diverse origins

The beneficial effects of MR blockade could be extended to other cardiac conditions such as cardiac hypertrophy, chronic adrenergic activation, and arrhythmias. MR activation is probably a risk factor to be considered in other diseases that impact the heart, including genetic diseases such as myodystrophy or Fabry disease (personal data), and systemic diseases such as the metabolic syndrome or diabetes. For example, in a model of type 1 diabetes, spironolactone decreases oxidative stress and cardiac ionic remodelling (potassium currents). In db/db mice, a model of type 2 diabetes, eplerenone normalizes the cardiac expression of several adipokines.

On the other hand, two polymorphisms have been reported in the MR gene (G215→C215, rs2070951) and (A754→G754, rs5522). Studies in vitro have demonstrated greater activation of MRs with the 215G and 754A alleles. These polymorphisms may be linked to increased sensitivity to spironolactone (as reported recently for the K sparing effect) and thus reflect altered MR activity. Whether these polymorphisms (or others) may explain the involvement of MRs in cardiac diseases, especially with normal aldosterone levels, remains to be explored.

Conclusions

MR activation plays a deleterious role in the development of heart failure in experimental models of myocardial overload and infarction. Significant deleterious effects on diastolic function are also reported. The interaction of MR activation with other important players in cardiac pathophysiology, such as salt, oxidative stress, and Ang II, suggests that the combined use of MR and AT1R antagonists may have synergistically beneficial effects. Experimental data also suggest that MR antagonists could have wider use than they do today, and perhaps their use could even be extended to various systemic diseases that affect cardiac function.

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