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Contribution of aldosterone to cardiovascular and renal inflammation and fibrosis

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Abstract

The steroid hormone aldosterone regulates sodium and potassium homeostasis. Aldosterone and activation of the mineralocorticoid receptor also causes inflammation and fibrosis of the heart, fibrosis and remodelling of blood vessels and tubulointerstitial fibrosis and glomerular injury in the kidney. Aldosterone and mineralocorticoid-receptor activation initiate an inflammatory response by increasing the generation of reactive oxygen species by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and mitochondria. High salt intake potentiates these effects, in part by activating the Rho family member Rac1, a regulatory subunit of reduced NADPH oxidase that activates the mineralocorticoid receptor. Studies in mice in which the mineralocorticoid receptor has been deleted from specific cell types suggest a key role for macrophages in promoting inflammation and fibrosis. Aldosterone can exert mineralocorticoid-receptor-independent effects via the angiotensin II receptor and via G-protein-coupled receptor 30. Mineralocorticoid-receptor antagonists are associated with decreased mortality in patients with heart disease and show promise in patients with kidney injury, but can elevate serum potassium concentration. Studies in rodents genetically deficient in aldosterone synthase or treated with a pharmacological aldosterone-synthase inhibitor are providing insight into the relative contribution of aldosterone compared with the contribution of mineralocorticoid-receptor activation in inflammation, fibrosis, and injury. Aldosterone-synthase inhibitors are under development in humans.

Introduction

During volume depletion or hypoperfusion of the kidney, activation of the renin–angiotensin–aldosterone system leads to vasoconstriction and volume expansion. Aldosterone stimulates sodium reabsorption in the kidney via the sodium–chloride cotransporter (NCC) in the distal convoluted tubule and the epithelial sodium channel (ENaC) in the late distal convoluted tubule, the connecting tubule, and the collecting duct. In the principal cells of the collecting duct, aldosterone, acting at the mineralocorticoid receptor (MR), increases mRNA levels of serum/glucocorticoid-regulated kinase (SGK1).[1] SGK1 phosphorylates the ubiquitin-protein ligase neuronal precursor cell expressed developmentally down-regulated protein 4-2 (Nedd4-2), and prevents ubiquitylation and degradation of ENaC.[2] Aldosterone also induces the expression of glucocorticoid-induced leucine zipper (GILZ), which inhibits mitogen-activated protein-kinase (MAPK) regulation of ENaC.[3] In the distal convoluted tubule, SGK1 phosphorylates Nedd4-2 and WNK4 and

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attenuates their inhibitory effects on the NCC.[4,5] The net effect of aldosterone in the tubule is sodium retention and potassium excretion.

Over the past 20 years, investigators have come to appreciate that aldosterone exerts direct effects on the vasculature, heart and kidney beyond its effects on electrolyte handling in the distal tubule. MRs are expressed in non-epithelial cells such as those of the heart (cardiomyocytes[6]), vasculature (endothelial cells and vascular smooth muscle cells [VSMCs])[7], and kidney (mesangial cells[8] and podocytes[9]), adipocytes,[10] and monocytes.[11] Seminal studies by the groups of Weber, Hostetter, Safar and many others demonstrated that chronic administration of aldosterone in the setting of high salt intake causes both interstitial and perivascular fibrosis in the heart,[12] fibrosis of the aorta,[13] and glomerulosclerosis and interstitial fibrosis in the kidney.[14] Prior to the development of fibrosis, aldosterone causes monocyte and macrophage infiltration and increased expression of inflammatory markers such as cyclooxygenase-2, monocyte chemoattractant protein 1, and intercellular adhesion molecule 1 (ICAM1) in the heart, vasculature, and kidney.[15,16] In the heart, perivascular inflammation is followed by the proliferation of fibroblasts and myofibroblasts, collagen production, perivascular fibrosis, and lastly, interstitial fibrosis.[17] The proinflammatory and profibrotic effects of aldosterone are prevented by MR antagonism in most models.[13,16,18]

Studies in humans confirm that MR activation contributes to cardiovascular fibrosis and remodelling as well as to renal disease. In the Randomized Aldactone Evaluation Study (RALES), spironolactone reduced mortality in heart failure patients who were already being treated with standard therapy including an angiotensin-converting-enzyme (ACE) inhibitor.[19] The beneficial effect of spironolactone was associated with a reduction in circulating biomarkers of extracellular matrix turnover, such as procollagen type III N-terminal peptide.[20] In the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS), eplerenone treatment reduced mortality in patients with left ventricular dysfunction following myocardial infarction.[21] Eplerenone reduces the combined end point of death and hospitalization in patients with systolic dysfunction and mild symptoms.[22] Several small clinical trials have shown a beneficial effect of MR antagonism on proteinuric renal disease in patients already treated with an ACE inhibitor or angiotensin-receptor blocker;[23] however, no large outcomes trials have been conducted, in part because of concerns regarding the risk of hyperkalaemia during dual renin–angiotensin–aldosterone system blockade in patients with renal insufficiency.[23]

This Review discusses the proinflammatory and profibrotic effects of aldosterone and MR activation in the heart, vasculature and kidney. It focuses on recent studies attempting to address the following questions: how is the MR activated when endogenous aldosterone is suppressed, such as during high salt intake? Is activation of the MR in specific cell types required to induce inflammation and fibrosis in the heart, the vasculature, or the kidney? And does aldosterone promote inflammation and/or fibrosis through MR-independent mechanisms? In many cases the answers to these questions are not yet definitive, but the available evidence is discussed.

Aldosterone and MR activation increase ROS

Aldosterone and/or MR activation promote inflammation by stimulating the generation of reactive oxygen species (ROS) such as superoxide and hydrogen peroxide, which activate the proinflammatory transcription factors activator protein (AP)-1 and nuclear factor kappa B (NFκB) (Figure 1).[24] In the heart, the aldosterone-induced generation of ROS also activates Ca^{2+}/calmodulin (CaM)-dependent protein kinase II (CaMKII),[25] which contributes to left ventricular remodelling following myocardial infarction.

Aldosterone stimulates the formation of ROS through several mechanisms (Figure 1). Aldosterone increases nicotinamide adenine dinucleotide phosphate [NADP(H)] oxidase activity and oxidative stress in macrophages,[26] endothelial cells,[27] the heart,[28] aorta,[26,29] podocytes,[9] and mesangial cells.[30] MR activation also contributes to angiotensin-II-mediated activation of NADPH oxidase in the heart and aorta.[31–33] In the aorta, aldosterone stimulates expression of NOX2 (gp91phox) and p22phox through an MR-dependent mechanism and expression of p47phox through both angiotensin II type 1 (AT_1)-receptor-dependent and MR-dependent mechanisms.[29] Conversely, p47phox-deficient mice are protected against aldosterone-induced ROS production in the heart, as measured by dihydroethidium fluorescence following myocardial infarction and infusion of aldosterone by the osmotic minipump.[25] Aldosterone-stimulated activation of NFκB in the heart is prevented in NOX2-deficient mice.[32] In rodent models, antioxidants such as the super-oxide mimetic tempol, the NADPH oxidase inhibitor apocynin, and N-acetylcysteine have been shown to decrease inflammation and injury.[29,34–37]

In addition, aldosterone decreases the vascular expression of glucose-6-phosphate dehydrogenase, which reduces $NADP^+$ to NADPH.[38] The generation of ROS by aldosterone further leads to endothelial dysfunction via the formation of peroxynitrite and the oxidation of 5,6,7,8-tetrahydrobiopterin, an important co-factor for nitric oxide synthase (NOS).[39] Aldosterone causes dephosphorylation of Ser1177 of protein phosphatase 2A leading to an 'uncoupling' of NOS.[39] Aldosterone can contribute further to endothelial dysfunction by inducing swelling and stiffness of endothelial cells through the MR and activation of ENaC.[40,41] Increased ambient sodium exacerbates this effect.

Aldosterone also stimulates mitochondrial production of ROS (Figure 1), an effect that has been studied most extensively in renal cells. For example, aldosterone induces epithelial–mesenchymal transformation (EMT) of cultured human proximal tubular cells via MR-dependent phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK)1/2 and phosphorylation of p66Shc, an effector of mitochondrial dysfunction.[42] The mitochondrial respiratory chain complex I inhibitor, rotenone, but not the NADPH oxidase inhibitor apocynin, blocks EMT *in vitro* and *in vivo* in mice treated with deoxycorticosterone (DOCA) and salt (an animal model of steroid-induced, salt-sensitive hypertension).[43] Overexpression of genes encoding regulators of mitochondrial function such as peroxisome proliferator-activated receptor-γ coactivator-1α (*PGC1α*) or silent mating type information regulation 2 homolog 1 (*SIRT1*) prevents aldosterone- stimulated EMT.[42] Similarly, aldosterone-induced proliferation of mesangial cells is inhibited to a greater extent by the mitochondrial respiratory chain inhibitor rotenone than by apocynin.[44] Rotenone also reverses aldosterone-induced podocyte injury.[45] Rotenone and other inhibitors of mitochondrial ROS generation reduce blood pressure more effectively in DOCA–salt-treated mice than does genetic deletion of *NOX2* or *p47phox*.[46] The results of these studies suggest that mitochondrial dysfunction contributes to the aldosterone-dependent or MR-dependent formation of ROS to a greater extent than was previously appreciated.

Aldosterone can also stimulate NFκB activity and the inflammatory pathway directly by activating the MR and increasing the phosphorylation and activity of SGK1 in mesangial cells[47] and the cortical collecting duct.[48] Genetic deficiency of *Sgk1* in mice protects against angiotensin-II-induced macrophage infiltration and cardiac fibrosis,[49] as well as albuminuria and renal tubulointerstitial fibrosis.[50]

Inflammation begets fibrosis

Fibrosis occurs when collagen and matrix production exceeds their degradation by matrix metalloproteinases (MMPs). As noted earlier, aldosterone-induced mitochondrial

dysfunction leads to EMT of proximal tubule cells. Aldosterone stimulates the expression of profibrotic molecules, such as transforming growth factor-β1 (TGF-β1), plasminogen activator inhibitor 1 (PAI-1), endothelin 1 (ET-1), placental growth factor (PGF), connective tissue growth factor (CTGF), osteopontin, and galectin-3.

TGF-β1 promotes fibrosis by stimulating cellular transformation of many cells to fibroblasts, by increasing the synthesis of matrix proteins and integrins, and by decreasing the production of MMPs.[51] Aldosterone increases the expression of TGF-β1 in cultured cardiomyocytes and treatment with aldosterone plus salt increases cardiac TGF-β1 in the heart in most studies.[52] Aldosterone induces TGF-β1 expression via the ERK1/2 pathway in mesangial cells.[53] Treatment with aldosterone plus salt increases the renal expression of TGF-β1 in hypertensive rat models and increases the urinary excretion of TGF-β1 in normal rats through an MR-dependent post-transcriptional effect.[54]

Aldosterone increases expression of PAI-1 in cultured endothelial cells, VSMCs, cardiomyocytes and monocytes.[11,55,56] In rat mesangial cells and renal fibroblasts, TGF-β1 and aldosterone act synergistically to increase PAI-1 expression and decrease extracellular degradation.[57] PAI-1, a member of the serpin (serine protease inhibitor) superfamily inhibits the activation of plasminogen to plasmin by tissue plasminogen activator. PAI-1 promotes fibrosis and remodelling by preventing plasmin-mediated MMP activation and extra-cellular matrix degradation, but PAI-1 can also exert anti-fibrotic effects by retarding cellular infiltration and impeding urokinase-type plasminogen activator (uPA) or plasmin- mediated activation and the release of latent growth factors.[58] PAI-1-deficient mice are protected against aldosterone-induced glomerular injury.[59] Aldosterone induces PAI-1 expression in the heart; however, PAI-1 deficiency in the heart enhances aldosterone-induced or angiotensin-II-induced fibrosis.[59–61] This finding is consistent with results of studies in senescent mice which have suggested that, in the heart, PAI-1 deficiency results in increased TGF-β activity[62] and increased infiltration of uPA-dependent macrophages.[63]

Increased expression of ET-1 can contribute to cardiovascular fibrosis and hypertrophy stimulated by aldosterone and salt. ET-1 increases collagen synthesis by cardiac fibroblasts, in part via a TGF-β1-dependent mechanism.[64] Mineralocorticoid plus salt treatment increases ET-1 expression in the heart and vasculature, whereas treatment with an endothelin-A-receptor blocker prevents aldosterone-induced cardiac and vascular fibrosis.[65,66] Endothelin-A-receptor blockade decreases renal injury in spontaneously hypertensive rats.[67] In the medullary collecting duct cells of the kidney, aldosterone modulates binding to a steroid-responsive element in the *Edn1* gene, which encodes ET-1.[68]

Jaffe *et al.* have implicated PGF, a member of the vascular endothelial growth factor (VEGF) family that promotes vascular cell proliferation, in aldosterone-mediated vascular injury.[69] Specifically, these investigators demonstrated that aldosterone simulates PGF expression in mouse aortas and atherosclerotic human vessels via an MR response element and found that PGF-deficient mice are protected against aldosterone-induced extracellular matrix deposition in injured carotid arteries.

Osteopontin is a negatively charged extracellular matrix protein that promotes adhesion of inflammatory cells.[70] In VSMCs, aldosterone stimulates osteopontin expression through an MR-dependent mechanism involving ERK and p38 MAPK.[71] Osteopontin-deficient mice are protected against aldosterone-induced fibrosis but they develop left ventricular dilatation.[72] Osteopontin-deficient mice are protected against oxidative stress, macrophage and fibroblast infiltration in the kidney as well as EMT, interstitial fibrosis, and podocyte injury.[73]

Galectin-3 is a lectin that causes collagen production in cultured VSMCs, cardiac fibroblast proliferation, fibrosis and left ventricular dysfunction.[74,75] Aldosterone increases the expression of galectin-3 in the heart.[76] Calvier *et al*. have reported that galectin-3-deficient mice are protected against aldosterone-induced aortic inflammation and fibrosis.[75]

Nitric oxide and the generation of cyclic GMP moderate many of the profibrotic effects of aldosterone. For example, aldosterone increases PGF expression and protein secretion in mouse aorta only after the endothelium has been denuded.[69] Administration of a NOS inhibitor exacerbates activation of the NFκB pathway, proteinuria and renal injury in DOCA–salt-treated rats.[77] Mice that lack the guanylyl cyclase-A receptor for natriuretic peptides and are treated with aldosterone and salt develop increased oxidative stress, increased proteinuria, mesangial expansion, and segmental sclerosis, compared with wild-type mice.[78]

Based on the findings from these studies, it is fair to say that aldosterone increases the expression of a number of profibrotic molecules and that the profile of these molecules is not unique to aldosterone-induced fibrosis.

Nongenomic and MR-independent effects

The classical effect of aldosterone on sodium transport in the renal tubule involves activation of the MR, which results in its dissociation from molecular chaperones, its translocation into the nucleus, and its binding to hormone response elements in the regulatory region of target gene promoters to enhance gene expression.[79] Aldosterone can also exert rapid nongenomic effects that are not blocked by inhibitors of transcription. These rapid, nongenomic effects of aldosterone have been most extensively described in vascular cells and tissues, where aldosterone causes a rapid increase in intra-cellular calcium through phosphatidylinositol 3-kinase (PI3 kinase), diacylglycerol and protein kinase C (PKC).[80] Aldosterone rapidly activates ERK1/2 in VSMCs, to promote a mitogenic and profibrotic phenotype.[71,81] Aldosterone enhances both rapid (10–15 min) and delayed (2 h) activation of ERK1/2 by angiotensin II. Potentiation of the early activation of ERK1/2 involves the transactivation of the epidermal growth factor receptor (EGFR), whereas the late phase involves increased expression of the fibrotic and proliferative small and monomeric GTP-binding protein Ki-ras2A and MAPK.[81] Angiotensin-receptor blockade prevents the rapid phosphorylation of ERK1/2 in VSMCs, but MR inhibition does not.[81,82] By contrast, spironolactone and inhibitors of transcription and protein synthesis prevent the late effect of aldosterone and angiotensin II.[82]

These findings highlight the contribution of the EGFR to the cardiovascular effects of aldosterone and to the crosstalk between aldosterone and angiotensin II in particular. In human aortic smooth muscle cells, aldosterone induces EGFR expression via an interaction between the ligand-bound MR and regions 316–163 bp and 163–1 bp of the EGFR promoter.[83] Interestingly, studies in mice with genetically decreased EGFR activity suggest that EGFR contributes to angiotensin-II-induced cardiac hypertrophy and aldosterone-induced vascular dysfunction, but not to aldosterone-salt-induced vascular or cardiac fibrosis and remodelling.[84,85] Systemic aldosterone administration induces phosphorylation of EGFR and ERK in the kidney within 30 min.[86] In cardiomyocytes, aldosterone phosphorylates and enhances activity of the Na^+/H^+ exchanger (NHE-1) via transactivation of the EGFR; this effect is blocked by MR antagonists but not by inhibitors of transcription (that is, it is MR-dependent and nongenomic).[87] In rabbit heart, aldosterone increases $Na^+/K^+/2Cl^-$ co-transporter activity and decreases Na^+/K^+ pump activity through a nongenomic effect on PKC epsilon type,[88] which stimulates NFκB activation via MAPKs.[89]

Lemarie *et al.* used small interfering RNA to explore the specific contributions of the AT_{1a} and AT_{1b} receptors and MR to crosstalk between aldosterone and angiotensin II in mouse VSMCs.[90] Their data suggest that activation of NFκB by either angiotensin II or aldosterone requires both the AT_1 receptor and the MR. Aldosterone can stimulate activation of ERK1/2 through an AT_1-dependent, MR-independent mechanism, but requires AT_1 and MR to activate c-Jun NH2-terminal kinase (JNK).[90] VSMC-specific knockout of the MR protects mice from angiotensin-II-induced vascular superoxide production, contraction of resistance blood vessels and hypertension, without affecting vascular structure.[91]

Identification of the receptor mediating the rapid, nongenomic effects of aldosterone has proven difficult. Some rapid effects of aldosterone cannot be blocked by the MR antagonist spironolactone, but may be blocked by its open-ring water-soluble metabolite, canrenoate, or by eplerenone.[92] Studies on fibroblasts derived from MR-deficient mice and on MR-transfected human embryonic kidney (HEK) cells suggest that the MR contributes to the nongenomic effects of aldosterone on the ERK1/2 and JNK pathway, but not to its rapid effects on calcium.[93,94] In addition, the nongenomic effects of aldosterone may facilitate classical MR-mediated effects.[88]

Recent studies suggest a new candidate receptor for the nongenomic effects of aldosterone. G-protein-coupled receptor (GPR) 30, an 'orphan' G-protein-coupled receptor expressed on the cell surface, acts via members of the G-protein family of GTP-binding proteins (Figure 1).[95] Gros *et al.* found that, in VSMCs, aldosterone activates both GPR30 and the MR to activate PI3 kinase, ERK and myosin light chain phosphorylation.[95] The contribution of GPR30 to the effects of aldosterone depends on the relative expression of GPR30 versus MR in cells; importantly, the authors reported that GPR30 expression declines in VSMCs when they are cultured, suggesting that studies in cultured VSMCs may underestimate the rapid, MR-independent effects of aldosterone *in vivo*. The authors further observed that spironolactone and eplerenone partially blocked the effects of a GPR30 agonist in cells that lacked the MR, consistent with prior studies suggesting that these drugs could prevent the rapid effects of aldosterone.[95] In a later paper, Batenburg *et al.* reported that either an EGFR antagonist or a GPR30 antagonist prevented potentiation of angiotensin II-induced vasoconstriction by aldosterone.[96]

Key role of salt and MR activation

The observation that MR antagonism decreases inflammation and fibrosis during conditions of high salt intake, which is associated with suppression of circulating aldosterone concentrations, suggests that ligands other than aldosterone might activate the MR. As noted earlier, angiotensin II stimulates the MR via transactivation of the EGFR. Cortisol may activate the MR in cells that lack the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD2). Cortisol circulates at concentrations 100-fold to 1,000-fold higher than aldosterone and binds to the MR with equal affinity. In epithelial cells, expression of 11β-HSD2 converts cortisol (or corticosterone in rodents) to an 11-ketometabolite that is inactive at the MR.[97] Some non-epithelial cells, such as endothelial cells,[98] also express 11β-HSD2, but cardiomyocytes and macrophages do not.[99,100] In the glomerulus, both mesangial cells and podocytes express 11β-HSD2.[101,102]

Because cardiomyocytes lack 11β-HSD2, the MR is typically occupied by glucocorticoids. Funder and co-workers have proposed that glucocorticoids activate the MR under conditions of oxidative stress. In a Langendorff model of myocardial infarction, researchers found that cortisol increased cardiac infarct size and that this effect was blocked by spironolactone or tempol, but not by the glucocorticoid-receptor (GR) antagonist, RU486.[103] The GR agonist dexamethasone also increased infarct size in this model and, paradoxically, so did the GR

antagonist RU486.[103] In a study investigating rats treated with the mineralocorticoid deoxycorticosterone plus salt, RU486 did not reverse cardiac inflammation and fibrosis; however, interpretation of this study is confounded by the fact that RU486 increased blood pressure.[104] Administration of corticosterone and salt to adrenalectomized rats did not increase mRNA expression of osteopontin, NOX4 or p22phox in the heart unless 11β-HSD2 was inhibited, suggesting that activation of MR by corticosterone does not cause cardiac inflammation under physiological conditions.[105] In summary, the case that activation of local cardiac MRs by glucocorticoids leads to inflammation has not been made beyond a reasonable doubt.

Compelling evidence suggests that the Rho family member Rac1, a regulatory subunit of reduced NADPH oxidase, activates the MR. Fujita and co-workers have reported that mice in which Rac1 activity is increased due to the loss of a regulatory factor, RhoGDIa, have increased MR activity and MR-dependent gene expression in the kidney, and develop focal sclerosing glomerulosclerosis.[106] Administration of either a Rac1 inhibitor or an MR antagonist prevents kidney injury in this model, even though aldosterone concentration is not increased. Likewise, overexpression of constitutively active Rac1 potentiates MR-dependent transcription *in vitro* in HEK293 cells, even in the absence of aldosterone.

Increased Rac1 activity may contribute to MR activation during high salt intake. Shibata *et al.* have reported that high salt intake induces Rac1 overexpression in the salt-sensitive Dahl rat but not in salt-resistant Dahl rats.[107] Furthermore, they showed that Tiam1, a Rac guanine nucleotide exchange factor that promotes the transition of inactive GDP-bound Rac to active GTP-bound Rac, mediates salt-induced Rac1 activation. Although aldosterone concentrations are suppressed in both salt-sensitive and salt-resistant Dahl rats, eplerenone or adrenalectomy prevented Rac1 activity being induced by a high salt intake in the Dahl salt-sensitive rat, suggesting some dependence of Rac1 activity on aldosterone or MR activation. Rac1-dependent activation also contributes to kidney injury in mice treated with angiotensin II and salt.[108]

More recently, Nagase *et al.* have reported that Rac1 and ROS contribute to activation of the MR in rat cardiomyocytes.[109] Treatment of cardiomyocytes with an inhibitor of glutathione synthesis increased ROS and increased translocation of MR into the nucleus, even when the ligand binding site was mutated. The anti-oxidant N-acetylcysteine blocked activation of the MR in cultured myocytes.

In short, the Rac1 pathway may account for MR activation under conditions such as high salt intake, when endogenous aldosterone is decreased.

Effects of cell-specific MR activation

MR antagonism prevents cardiovascular and renal injury in rodent models and in clinical trials. Because systemic administration of an MR antagonist increases sodium excretion and reduces blood pressure in rodent models, it can be difficult to distinguish the effects of systemic MR activation, sodium retention and hypertension on inflammation and fibrosis from effects of local MR activation.

In order to dissect the effects of aldosterone from the effects of systemic hypertension on the expression of profibrotic genes in the heart, Azibani *et al.* crossed hypertensive global renin (Ren) transgenic mice with normotensive cardiac-specific aldosterone synthase (AS) transgenic mice.[76] Hypertension was associated with increased cardiac expression of genes encoding fibronectin, CTGF and TGF-β1 and there was no difference in the cardiac expression of these profibrotic genes between Ren and Ren-AS mice. By contrast, local overexpression of aldosterone synthase increased cardiac expression of genes encoding

monocyte chemoattractant protein-1, osteopontin, galectin-3, and the macrophage marker CD68, independent of blood pressure (that is, expression of these genes was increased in AS and Ren-AS mice but not in Ren mice). Interestingly, expression of brain natriuretic peptide and bone morphogenic protein (BMP)-4, known to antagonize the effect of TGF-β1, was decreased by expression of aldosterone synthase in the heart.

Studies in mice in which the MR has been selectively deleted from specific cell types highlight the important contribution of inflammatory cell MR activation to inflammation and fibrosis, and will be discussed below.

As noted earlier, McCurley *et al.* have reported that VSMC-specific knockout of the MR protects mice from ageing-induced and angiotensin-II-induced hypertension, as well as from angiotensin-II-induced oxidative stress.[91] These mice are not protected against hypertension induced by aldosterone plus salt, suggesting that renal sodium retention is the primary driver of aldosterone-induced hypertension in this model. The central effects of MR activation on salt appetite, vasopressin release and the sympathetic nervous system[110] could also contribute to the development of hypertension in these VSMC-specific knockout mice.

Role of cardiomyocyte MR

Cardiomyocyte-specific knockout of the gene encoding the MR protects mice against ventricular dilatation and dysfunction following myocardial infarction.[111] Infiltration by inflammatory cells is increased in cardiomyocyte-MR-deficient mice following infarction (Figure 2). At 8 weeks, cardiac expression of NADPH subunits NOX2 and NOX4 and of profibrotic genes such as CTGF, fibronectin, and collagen, is decreased, as is the formation of ROS. Lother *et al.* reported that cardiomyocyte-specific knockout of the gene encoding the MR protected mice against left ventricular dilation and dysfunction during pressure overload caused by aortic banding, whereas fibroblast-specific knockout of the gene encoding the MR did not have a beneficial effect.[112] In addition, neither cardiomyocyte-specific nor fibroblast-specific knockout of the gene encoding the MR prevented macrophage infiltration, inflammatory gene expression in the heart or cardiac hypertrophy or fibrosis in this model. The data from these two studies indicate that the activation of MR on cells other than cardiomyocytes contribute importantly to inflammation in the heart.

Role of macrophage MR

The macrophage plays a key role in cardiovascular inflammation, fibrosis and remodelling induced by aldosterone and high salt intake. Aldosterone at physiological concentrations increases the expression of pro-inflammatory genes in cultured macrophages, an effect that is prevented by MR antagonism.[99] MR antagonism decreases macrophage expression of the profibrotic genes *TGF-β1* and *PAI-1* and increases expression of anti-fibrotic genes such as *HTRA1*, which encodes a member of the trypsin family of serine proteases (Figure 2).[99] Because macrophages do not express 11β-HSD2, physiological concentrations of corticosterone may also be expected to activate the MR; however, although a low concentration (10 nmol/l) of corticosterone induces the expression of the gene encoding tumour necrosis factor in cultured macrophages, this effect is prevented by blocking the GR, not by blocking the MR.[99] Moreover, a higher concentration of cortisol (1 μmol/l) induces alternative activation of macrophages.

Two groups have reported that macrophage-specific knockout of the gene encoding the MR protects mice against interstitial and perivascular cardiac fibrosis induced by DOCA plus salt[113] or by chronic NOS inhibition and high salt intake (L-NAME plus salt).[99,114] Young and co-workers reported that mice with macrophage-specific knockout of the gene encoding the MR have lower blood pressure but are not protected against cardiac hypertrophy induced

by DOCA plus salt or by L-NAME plus salt, whereas Usher *et al.* reported that mice with macrophage-specific knockout of the gene encoding the MR have increased blood pressure during treatment with L-NAME plus salt and are protected against cardiac hypertrophy.[99,114] The two groups report differing effects of macrophage-specific knockout of the gene encoding the MR on the recruitment of macrophages to the heart. The reason for this difference is not readily apparent, but may reflect differences in the models used (for example, differences in duration of L-NAME treatment) or differences in the derivation of the macrophage-specific knockouts.

Role of endothelial cell MR

The endothelial cell promotes macrophage adhesion and infiltration via an MR-dependent mechanism. Endothelial cells express functional MRs as well as 11βHSD-2.[115] Aldosterone stimulates expression of ICAM1 by endothelial cells (Figure 2) and promotes adhesion of leukocytes to endothelial cells.[115] Jeong *et al.* found that physiological concentrations of aldosterone stimulate the release of von Willebrand factor and interleukin (IL)-18 from human aortic endothelial cells, without stimulating tissue plasminogen activator (t-PA) release.[116] In this study, aldosterone enhanced the adherence of leukocytes to endothelial cells by increasing P-selectin expression. The authors reported that aldosterone stimulates endothelial exocytosis through a nongenomic, MR-dependent mechanism. Aldosterone stimulated endothelial exocytosis within 10 min and exocytosis was not blocked by actinomycin, but was blocked by MR inhibition.

Other immune-cell-specific MR effects

T-cell activation contributes to the inflammatory response to MR stimulation. Adoptive transfer of T cells into mice lacking T cells and B cells increases the inflammatory and hypertensive response to DOCA plus salt.[117,118] In addition, aldosterone modulates interactions between antigen-presenting cells (such as dendritic cells) and T cells (Figure 2). Dendritic cells express MRs, and aldosterone increases activation of the MAPK pathway and secretion of IL-6 and TGF-β1 from dendritic cells.[119] Aldosterone-treated dendritic cells activate $CD8^+$ T cells and enhance IL-17 excretion by $CD4^+$ T cells, through an MR-dependent pathway.[119] On the other hand, regulatory T cells decrease vascular injury induced by aldosterone plus salt. Specifically, Kasal *et al.* found that adoptive transfer of $CD24^+CD25^+$ cells prevented oxidative stress, inflammation, endothelial dysfunction, and remodelling in mesenteric arteries from mice with high salt intake treated with aldosterone.[120]

Endogenous aldosterone vs MR activation

Because endogenous aldosterone concentrations are suppressed during high salt intake and as ligands other than aldosterone can activate the MR, the contribution of endogenous aldosterone to MR-mediated inflammation and fibrosis has been the subject of interest.

Messaoudi *et al.* sought to clarify the role of aldosterone-specific activation of the MR in the heart by identifying genes that were upregulated in mice overexpressing the cardiomyocyte MR, and further upregulated by 7 days of treatment with low-dose aldosterone, but not by treatment with corticosterone.[121] They identified 43 genes regulated by aldosterone-mediated activation of cardiac MR (23 that were upregulated and 20 that were downregulated), including the genes encoding the growth factors CTGF and hepatocyte growth factor, several potassium and calcium channel genes, and genes encoding cell adhesion molecules such as cadherin 4 and integrin β6.

The availability of mice lacking active aldosterone synthase, as well as pharmacological aldosterone-synthase inhibitors, is enabling investigators to study the contribution of endogenous aldosterone to inflammation and fibrosis. Lee *et al.* disrupted the mouse gene encoding aldosterone synthase (*Cyp11b2*) by replacing its first two exons with sequences coding for enhanced green fluorescent protein.[122] These mice exhibit mild hypotension, as well as hyperkalaemia and increases in renin that are both corrected by high salt intake.[123]

Using aldosterone-deficient ($AS^{-/-}$) mice, Luther *et al.* determined that the induction of *Pai-1* and *Et-1* expression in the heart by acute angiotensin II administration required endogenous aldosterone, whereas acute angiotensin-II-induced expression of these profibrotic genes in the aorta was aldosterone-independent. The investigators later compared the effect of pharmacological blockade of the MR with spironolactone with the effect of genetic aldosterone-synthase deficiency on chronic end-organ damage induced by angiotensin II and salt in mice.[124] They found that blockade of the MR with spironolactone and genetic aldosterone synthase deficiency were associated with similar reductions in cardiac and aortic fibrosis in mice treated with angiotensin II plus salt, but MR antagonism prevented glomerular injury whereas aldosterone deficiency did not. Therefore, in the kidney, angiotensin II caused injury via aldosterone-independent activation of the MR. Rafiq *et al.* have reported that hydrocortisone also causes renal injury via activation of the MR.[125]

Trials of aldosterone-synthase inhibition

FAD286, the D-enantiomer of the aromatase inhibitor fadrozole, was developed as an aldosterone-synthase inhibitor after it was recognized that fadrozole reduced aldosterone concentrations without affecting cortisol concentrations in patients with breast cancer. Fiebeler and co-workers demonstrated that either treatment with FAD286 or adrenalectomy significantly reduced mortality, cardiac hypertrophy, albuminuria, and cardiac and renal inflammation in rats doubly transgenic for the human genes encoding renin and angiotensinogen.[126,127] FAD286 also reduces inflammation and atherosclerosis in apolipoprotein-E-deficient mice.[128]

LCI699 is the first orally available aldosterone-synthase inhibitor to be developed for use in humans. In a proof-of-concept study, LCI699 decreased plasma aldosterone concentrations by approximately 70% and increased concentrations of the aldosterone precursor 11-DOC by more than 700% in patients with primary hyperaldosteronism.[129] LCI699 had no effect on basal concentrations of cortisol. LCI699 reduced office and ambulatory systolic blood pressure and increased serum potassium concentration. In a subsequent study, LCI699 at doses of 0.5 mg or 1.0 mg twice daily was less effective in reducing blood pressure in patients with primary hyperaldosteronism than was eplerenone 50–100 mg twice daily, even though LCI699 decreased aldosterone concentrations by 70–80%.[130]

Calhoun *et al.* tested the effect of 8 weeks of therapy with LCI699 (0.25 mg once daily, 0.5 mg once daily, 1.0 mg once daily and 0.5 mg twice daily) on blood pressure in a double-blind, placebo-controlled and eplerenone-controlled trial in patients with essential hypertension.[131] LCI699 induced a dose-dependent reduction in systolic and diastolic blood pressure, with the 1.0 mg per day dose having a similar effect on blood pressure as a 50 mg twice daily dose of eplerenone. LCI699 and eplerenone both increased plasma renin activity. At the highest dose (0.5 mg twice daily), LCI699 significantly decreased plasma aldosterone concentration, whereas eplerenone (50 mg twice daily) increased plasma aldosterone concentration. Paradoxically, the 0.5 mg twice-daily dose of LCI699 had less effect on blood pressure than did the 1.0 mg per day dose, even though the 0.5 mg twice-daily dose had a greater inhibitory effect on aldosterone synthesis. This finding that LCI699 0.5 mg twice daily suppressed aldosterone but did not decrease blood pressure has been attributed to increases in precursor mineralocorticoids.[132] In Calhoun *et al.*'s study, one individual in

each LCI699 treatment group had a recorded potassium concentration of >6 mmol/l, which resolved on repeat testing without discontinuation of study medication.[131] Overall, the effect of LCI699 on serum potassium concentration was similar to the effect of eplerenone. Although LCI699 had no effect on basal cortisol concentrations, the aldosterone-synthase inhibitor significantly blunted the cortisol response to adrenocorticotropic hormone. To date, the effects of aldosterone-synthase inhibition on inflammation or on cardiovascular or renal injury have not been reported in humans.

Conclusions

In conclusion, aldosterone stimulates the production of ROS, inflammation and fibrosis of the heart, vasculature, and kidney through both MR-dependent and MR-independent mechanisms. GPR30 may mediate MR-independent effects of aldosterone in vascular cells. In addition, during conditions of high salt intake, Rac1 activates the MR to produce oxidative stress. Findings in mice with cell-specific deficiency of the MR suggest that activation of local MRs (for example, on macrophages) induces fibrosis. The MR may be activated or transactivated by ligands other than aldosterone. Studies in mice treated with aldosterone synthase or aldosterone synthase inhibitors suggest that aldosterone is the primary ligand involved in cardiac and vascular fibrosis, but that MR antagonism may be necessary to prevent renal injury. Human studies suggesting that aldosterone-synthase inhibitors may be less effective in reducing blood pressure than are MR antagonists also suggest that ligands other than aldosterone activate the MR in the kidney.

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Key points

- Aldosterone or mineralocorticoid-receptor activation trigger the formation of reactive oxygen species by NADPH oxidase and mitochondria that, in turn, induce a proinflammatory and profibrotic phenotype
- Under conditions of high salt intake, Rac1 activates the mineralocorticoid receptor and increases the formation of reactive oxygen species
- Aldosterone exerts rapid, transcription-independent effects (nongenomic effects) that may be mediated by G-protein-coupled receptor 30 and transactivation of the epithelial growth factor receptor
- Studies in mice in which the mineralocorticoid receptor has been selectively deleted on specific cells indicate that systemic mineralocorticoid-receptor activation is not necessary to induce local inflammation and fibrosis
- Aldosterone-synthase inhibition or deficiency prevents inflammation and fibrosis in many rodent models of cardiovascular or renal injury

Review criteria

PubMed was searched for articles published between 1960 and the present using the terms “aldosterone”, “mineralocorticoid”, and/or “mineralocorticoid receptor” together with the terms “oxidative stress”, “inflammation” and/or “fibrosis”. Full-text English-language papers were reviewed and the bibliographies of these papers were searched for additional leads. Focused searches were also conducted regarding topic areas identified in the initial search. Emphasis was placed on papers published from 2009 onwards.

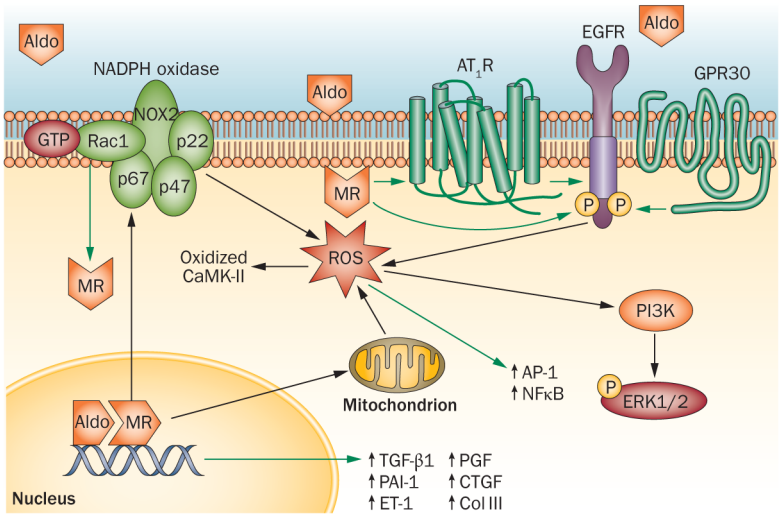

Figure 1.

Mechanisms by which aldosterone and/or MR activation induce oxidative stress, inflammation, and fibrosis. Activation of the MR by aldosterone results in dissociation of chaperone proteins, translocation of the MR to the nucleus and activation of transcription. MR activation induces oxidative stress by both NADPH oxidase and mitochondria. ROS formed by NADPH oxidase oxidize CaMK-II. Oxidative stress triggers the activation of proinflammatory transcription factors such as AP-1 and NFκB. Rac1 can also activate the mineralocorticoid receptor. Aldosterone can induce rapid, nongenomic effects via GPR30. Aldosterone and angiotensin II interact to cause rapid, nongenomic effects via transactivation of the EGFR, resulting in the generation of ROS and phosphorylation of ERK1/2. The green arrows indicate an activating or increasing effect; the black arrows indicate a downstream consequence. See text for references. Abbreviations: Aldo, aldosterone; AP-1, activator protein 1; AT_1R, angiotensin II type 1 receptor; CAMK-II, calcium/calmodulin-dependent protein kinase type II subunit gamma; Coll III, collagen III; CTGF, connective tissue growth factor; EGFR, epidermal growth factor receptor; ERK1/2, extracellular signal-regulated kinases 1 and 2 (ERK)1/2; ET-1, endothelin 1; GPR30, G-protein-coupled receptor 30; MR, mineralocorticoid receptor; NADPH, nicotinamide adenine dinucleotide phosphate; NFκB, nuclear factor kappa B; P, phosphate; PAI-1, plasminogen activator inhibitor 1; PGF, placental growth factor; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species; TGF-β1, transforming growth factor-β1.

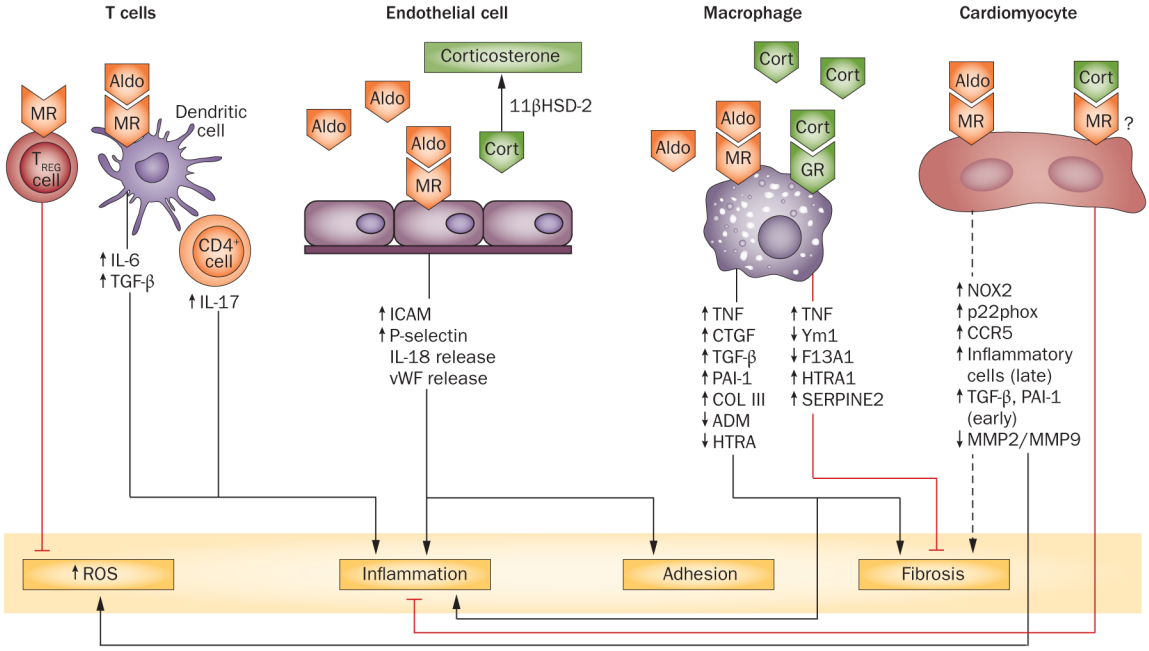

Figure 2.

Effects of cell-specific activation of the MR. Aldosterone activates the MR on macrophages to produce inflammation, hypertrophy, and fibrosis in the heart. At low concentrations, cortisol can also cause an inflammatory response via activation of glucocorticoid receptors. At higher concentrations, cortisol induces alternative activation of macrophages. Activation of MRs in cardiomyocytes increases the formation of ROS and might promote fibrosis. Studies in which *11βHSD-2* is overexpressed in cardiomyocytes suggest that cortisol opposes these effects. In endothelial cells, MR activation by aldosterone promotes inflammation and adhesion of leukocytes; endothelial cells produce 11βHSD-2, rendering cortisol inactive at the mineralocorticoid receptor. Stimulation of the MR on dendritic cells increases production of IL-6 and TGF-β1, primes $CD8^+$ T cells, and induces T_H17 production by $CD4^+$ cells. The dotted line indicates that the positive effect is not consistent across studies (see text). The red lines indicate a decreasing effect. See text for references. Abbreviations: 11βHSD-2, 11β-hydroxysteroid dehydrogenase type II; ADM, adrenomedullin; Aldo, aldosterone; CCR5, C-C chemokine receptor type 5; Cort, cortisol; COL III, collagen III; CTGF, connective tissue growth factor; F13A1, coagulation factor XIII, A1 polypeptide; GR, glucocorticoid receptor; HTRA, high temperature requirement factor A1; ICAM1, intercellular adhesion molecule 1; IL, interleukin; MMP, matrix metalloproteinase; MR, mineralocorticoid receptor; PAI-1, plasminogen activator inhibitor 1; ROS, reactive oxygen species; TGF-β, transforming growth factor-β; TNF, tumour necrosis factor; T_{REG}, regulatory T cell; vWF, von Willebrand factor; Ym1, a secretory protein produced by activated macrophages.