Aldosterone and end-organ damage

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ABSTRACT

Aldosterone concentrations are inappropriately high in many patients with hypertension, as well as in an increasing number of individuals with metabolic syndrome and sleep apnoea. A growing body of evidence suggests that aldosterone and/or activation of the MR (mineralocorticoid receptor) contributes to cardiovascular remodelling and renal injury in these conditions. In addition to causing sodium retention and increased blood pressure, MR activation induces oxidative stress, endothelial dysfunction, inflammation and subsequent fibrosis. The MR may be activated by aldosterone and cortisol or via transactivation by the AT1 (angiotenin II type 1) receptor through a mechanism involving the EGFR (epidermal growth factor receptor) and MAPK (mitogen-activated protein kinase) pathway. In addition, aldosterone can generate rapid non-genomic effects in the heart and vasculature. MR antagonism reduces mortality in patients with CHF (congestive heart failure) and following myocardial infarction. MR antagonism improves endothelial function in patients with CHF, reduces circulating biomarkers of cardiac fibrosis in CHF or following myocardial infarction, reduces blood pressure in resistant hypertension and decreases albuminuria in hypertensive and diabetic patients. In contrast, whereas adrenalectomy improves glucose homoeostasis in hyperaldosteronism, MR antagonism may worsen glucose homoeostasis and impairs endothelial function in diabetes, suggesting a possible detrimental effect of aldosterone via non-genomic pathways.

INTRODUCTION

Activation of the RAAS [renin–Ang (angiotensin)–aldosterone system] is associated with increased morbidity and mortality among patients with hypertension and CHF (congestive heart failure). These effects have been attributed to the hypertrophic, proliferative, pro-inflammatory, prothrombotic and profibrotic effects of AngII. However, it is now appreciated that aldosterone or MR (mineralocorticoid receptor) activation contributes to many of these effects of AngII. In addition, although interruption of the RAAS with ACE (Ang-converting enzyme) inhibitors or ARBs [AT (AngII type 1) receptor blockers] significantly reduces morbidity and mortality...
in patients with CHF, at risk of coronary artery disease or with nephropathy, aldosterone concentrations 'escape' to baseline during chronic therapy.

In this article, we review results from studies in vitro and in whole-animal models indicating that aldosterone or MR activation causes oxidative stress and induces endothelial dysfunction, inflammation and fibrosis. We present these results in the context of recent clinical trials of MR antagonists in patients with CHF, hypertension and nephropathy. Understanding the mechanisms through which aldosterone contributes to end-organ damage during activation of the RAAS can lead to the development of new pharmacological strategies to decrease end-organ damage.

**ALDOSTERONE: A REGULATOR OF FLUID AND ELECTROLYTE HOMEOOSTASIS**

Classically, aldosterone regulates sodium excretion through MR-dependent and genomic effects in the distal nephron of the kidney [1–3]. AngII, potassium or corticotropin [ACTH (adrenocorticotropic hormone)] stimulates the adrenal zona glomerulosa to synthesize aldosterone. Circulating aldosterone then binds to the inactive cytosolic MR of target cells, resulting in dissociation of the ligand-activated MR from a multiprotein complex containing molecular chaperones and translocation of the ligand-activated MR into the nucleus, where it binds to hormone-response elements in the regulatory region of target gene promoters. In the distal nephron of the kidney, MR induction of sgk-1 (serum- and glucocorticoid-inducible kinase-1) gene expression triggers a cascade that leads to the absorption of sodium and water through the ENaC (epithelial sodium channel) and potassium excretion with subsequent volume expansion and hypertension (Figure 1).

The MR binds cortisol and aldosterone with equal affinity; however, in epithelial tissues, the enzyme 11β-HSD2 (type 2 isoform of 11β-hydroxysteroid dehydrogenase), converts cortisol (or in the case of rodents corticosterone) into its inactive 11-ketometabolite, such that MRs are occupied primarily by aldosterone [4]. MRs are also expressed in the hippocampus, heart
(cardiomyocyte), vasculature [endothelial cells and VSMCs (vascular smooth muscle cells)], renal cells in addition to the principal cell of the collecting duct (e.g. mesangial cells and podocytes) and monocytes [5–7]. The vasculature expresses 11β-HSD2 and here aldosterone acts as the primary ligand at the MR. In contrast, neither cardiomyocytes nor hippocampal cells express 11β-HSD2 [8]. Therefore cortisol, present in concentrations at least 10-fold in excess of aldosterone, acts as the primary MR ligand at these sites. Paradoxically, under normal redox conditions, cortisol acts as an antagonist in these tissues [9].

Just as MRs have been identified in non-epithelial tissues, so have many of the downstream targets of MR activation. For example, sgk-1 is abundantly expressed in the heart [10]. Likewise, ENaC mRNA and protein are expressed in fibroblasts, VSMCs, endothelial cells and in cerebral vasculature [11].

**ALDOSTERONE EXERTS EFFECTS THROUGH GENOMIC AND NON-GENOMIC PATHWAYS**

In addition to its classical effects on gene expression, aldosterone can produce rapid (occurring within minutes) non-genomic (not blocked by inhibitors of transcription) effects [9,12]. In both VSMCs and endothelial cells, aldosterone causes a rapid increase in intracellular calcium through Ins(1,4,5)P3, diacylglycerol and PKC (protein kinase C) [13–15]. The consequence of these rapid effects of aldosterone in the vasculature depends on the bioavailability of endogenous NO [14–17]. For example, aldosterone causes vasoconstriction in micro-perfused afferent arterioles [15], whereas aldosterone decreases phenylephrine- [13] or potassium- [14] stimulated vasoconstriction in rat aortic rings and rabbit renal afferent arterioles via activation of NOS (NO synthase).

In the heart, aldosterone increases Na+–K+–2Cl− co-transporter activity and decreases Na+/K+ pump activity through a non-genomic PKCε-dependent mechanism [18]. In rabbit cardiomyocytes, these so-called ‘rapid’ effects of aldosterone persist for 7 days, suggesting an interaction with non-genomic effects [18]. Because PKCε activation also stimulates NF-κB (nuclear factor κB) activation of MAPK (mitogen-activated protein kinase), the genomic effects of aldosterone may influence the genomic effects [19]. Whether the non-genomic effects of aldosterone are also MR-independent is uncertain. In most studies, MR antagonism with spironolactone does not block the non-genomic effects of aldosterone; however, the open-ring water-soluble metabolite of spironolactone, canrenoate, or eplerenone have been reported to block some non-genomic effects [20].

**ALDOSTERONE AND ENDOTHELIAL FUNCTION**

Impaired endothelial function, defined by vasodilatation in response to shear stress or pharmacological agonists that stimulate NOS, is associated with increased mortality [21]. In animal models, aldosterone infusion causes endothelial dysfunction via the generation of ROS (reactive oxygen species) [22–24]. Aldosterone increases expression of NADPH oxidase subunits p22phox and gp91phox through an MR-dependent mechanism, whereas aldosterone stimulates expression of p47phox mRNA through both AT1 receptor and MR-dependent mechanisms (Figure 2) [25,26]. The resultant generation of ROS leads to the formation of peroxynitrite and oxidation of the NOS co-factor BH4 (5,6,7,8-tetrahydrobiopterin) [27]. In addition, aldosterone promotes the ‘uncoupling’ of NOS by inducing dephosphorylation of Ser1177 by PP2A (protein phosphatase 2A). Aldosterone also produces oxidative stress and endothelial dysfunction by decreasing the expression of G6PD (glucose-6-phosphate dehydrogenase), which reduces NADP+ to NADPH [28].

Increased aldosterone concentrations are associated with endothelial dysfunction in human hypertension [29,30], and acute aldosterone administration causes endothelial dysfunction in healthy individuals in some studies [31,32]. Administration of an MR antagonist improves endothelial dysfunction in patients with CHF [33]. A few investigators have reported that aldosterone can also cause vasodilatation via a rapid MR-independent mechanism involving PI3K (phosphoinositide 3-kinase)-dependent activation of NOS [16]. The clinical relevance of these observations is not yet known. As described below, MR antagonism worsens vascular function in patients with Type 2 diabetes [34].

**ANGII AND ALDOSTERONE INTERACT TO PRODUCE CARDIOVASCULAR INJURY**

A wealth of data supports the interaction of AngII and aldosterone in inducing inflammation, fibrosis and proliferation. For example, aldosterone increases ACE and AT1 receptor mRNA expression and AT1 receptor binding in the vasculature and heart, whereas MR antagonism decreases AT1 receptor mRNA and density in the heart [35,36]. AngII activates gene expression in VSMCs by transactivating the MR [37]. At low concentrations, AngII and aldosterone synergistically increase ERK (extracellular-signal-regulated kinase) activity in VSMCs through a biphasic mechanism; Horiuchi and co-workers have reported [38] that the early phase involves EGFR (epidermal growth factor receptor) transactivation through a rapid non-genomic mechanism, whereas the late phase involves MR-mediated effects on the fibrotic
Aldosterone stimulates the expression of NADPH oxidase subunits [129,130], and decreases the activity of G6PD, which reduces NADP$^+$ to NADPH [28]. Aldosterone promotes the ‘uncoupling’ of NOS by activating PP2A to dephosphorylate Ser$^{1177}$ [27]. AT1 receptor blockade abrogates many of the adverse effects of aldosterone in the heart and vasculature. Conversely, studies in vivo in rats in which the RAAS is up-regulated suggest that AngII induces cardiovascular injury in part through an aldosterone- and MR-dependent mechanism. For example, in rats doubly transgenic for the human renin and angiotensinogen genes, AngII stimulates AP-1 (activator protein-1) and NF-$\kappa$B expression and cardiac fibrosis via an MR-dependent mechanism [39]. Pharmacological inhibition of aldosterone synthase also decreases cardiac hypertrophy and renal injury in this model [40]. In humans, intravenous infusion of aldosterone or AngII increases circulating concentrations of inflammatory cytokines through an MR-dependent mechanism [41].

ALDOSTERONE PROMOTES OXIDATIVE STRESS, INFLAMMATION AND FIBROSIS

Chronic aldosterone infusion at doses that yield concentrations similar to those observed in CHF causes myocardial fibrosis in rat models in the setting of high-salt intake [42]. The development of fibrosis is preceded by coronary and myocardial inflammation characterized by monocyte and macrophage infiltration and increased expression of inflammatory markers, such as COX-2 (cyclo-oxygenase-2), osteopontin, MCP-1 (monocyte chemoattractant protein-1) and ICAM-1 (intracellular adhesion molecule-1) [43,44]. Both the inflammatory changes and subsequent fibrosis can be blocked by MR antagonism [42,45].

Aldosterone also causes aortic fibrosis and hypertrophy in hypertensive rats through an MR-dependent mechanism [46–48]. Similarly, aldosterone causes glomerular injury and tubulointerstitial fibrosis. In the rat remnant kidney model, aldosterone infusion reverses the protective effects of ACE inhibition and AT1 receptor antagonism [49]. MR antagonism decreases the development of glomerular damage (thrombosis, sclerosis and mesangiolysis) and arteriopathy in stroke-prone SHRs (spontaneously hypertensive rats) [50] in a renin-dependent radiation model of renal damage [51] and in diabetes [52], independent of effects on BP (blood pressure).

High-salt intake promotes cardiac fibrosis and vascular injury in aldosterone-treated animal models, but the...
pathophysiological basis for the effect of sodium has not been fully elucidated. Changes in potassium do not appear to contribute to aldosterone/salt-mediated injury [53]. On the other hand, during high-salt intake, activation of the MR causes Ca\(^{2+}\) loading and a fall in cytosolic-free ionized Mg\(^{2+}\) in monocytes and cardiomyocytes, through a mechanism involving Na\(^{+}\)/Mg\(^{2+}\) and Na\(^{+}\)/Ca\(^{2+}\) exchangers [54]. Intracellular Ca\(^{2+}\) loading, in turn, causes oxidative stress. Funder and Mihailidou [55] have proposed that changes in intracellular redox potential result in activation of cortisol (cortistosterone)--MR complexes in non-epithelial tissues, such as the heart.

In addition, it should be noted that, whereas high-salt intake suppresses the activity of the circulating RAAS, local cardiac [56], aortic [26] and renal [57] concentrations of AngII may be up-regulated in the setting of high-salt intake. Similarly, although high-salt intake is associated with decreased renal sympathetic nerve activity, lumbar sympathetic nerve activity is increased [58].

Because animal models of aldosterone-stimulated cardiovascular and renal injury involve the systemic administration of aldosterone, it has been difficult to dissect local tissue effects of aldosterone from systemic effects of sodium retention. This point is highlighted by the observation that MR-deficient mice die from sodium wasting and dehydration [59]. In addition, studies in genetically modified mice provide conflicting data as to whether aldosterone or MR activation is the critical step in initiating fibrosis and whether aldosterone acts exclusively through MR-dependent pathways. On the one hand, local aldosterone expression causes coronary endothelial dysfunction, not fibrosis, suggesting that MR activation is critical [60]. On the other hand, cardiac-specific overexpression of the MR does not cause fibrosis [61]. On the contrary, conditional knockdown of the cardiac MR using antisense mRNA results in the development of cardiac fibrosis and heart failure [62]. Although this has been attributed to an artifact related to overexpression of a foreign protein in myocytes, the finding that spironolactone, which increases circulating aldosterone concentrations, enhanced fibrosis in this model raises the possibility that knockdown of the cardiac MR unmasked an MR-independent effect of aldosterone [62].

The availability of pharmacological aldosterone synthase inhibitors [40] and aldosterone-synthase-deficient mice [63] should contribute to our understanding of the relative importance of aldosterone compared with MR activation, if not to our understanding of local compared with systemic effects.

**POSSIBLE MECHANISMS OF ALDOSTERONE-INDUCED FIBROSIS**

Although many studies have focused on the role of oxidative stress and inflammation in the initiation of aldosterone-mediated end-organ damage, much remains to be learned about the pathways involved in progression from inflammation to remodelling and fibrosis. Aldosterone stimulates the expression of several profibrotic molecules that may contribute to the pathogenesis of cardiac remodelling. For example, aldosterone increases the activity of TGF-\(\beta_1\) (transforming growth factor-\(\beta_1\)) in cultured cardiomyocytes [64]. Aldosterone/salt treatment increases and MR antagonism decreases myocardial TGF-\(\beta_1\) expression in some studies [44,65], although not all [66]. Aldosterone increases renal TGF-\(\beta_1\) mRNA expression and signalling [67,68], and causes a rapid increase in urinary excretion of TGF-\(\beta_1\) through an MR-dependent post-transcriptional mechanism [69]. TGF-\(\beta_1\) promotes fibrosis and tissue remodelling by stimulating cellular transformation to fibroblasts [70], increasing the synthesis of matrix proteins and integrins and decreasing the production of MMPs (matrix metalloproteinases) [71].

In addition, TGF-\(\beta_1\) increases the expression of PAI-1 (plasminogen activator inhibitor-1) (Figure 3) [72]. Aldosterone also increases PAI-1 expression in endothelial cells, VSMCs, cardiomyocytes and monocytes [25,73–75]. PAI-1, a member of the serpin (serine protease inhibitor) superfamily and the major physiological inhibitor of t-PA (tissue-type plasminogen activator) and uPA (urokinase plasminogen activator) in vivo, can, in turn, accelerate fibrosis by decreasing both direct and indirect effects of plasmin on ECM (extracellular matrix) [76]. Treatment of mice or rats with aldosterone increases cardiac PAI-1 expression, whereas MR antagonism reduces cardiac PAI-1 expression [44,77]. Similarly, MR antagonism decreases renal injury and PAI-1 expression in a radiation model of glomerular injury and in streptozotocin-induced diabetes [51,67]. Importantly, treatment with spironolactone can reverse pre-existing glomerular injury and associated PAI-1 expression [78].

On the basis of these findings, one might predict that PAI-1 deficiency would decrease aldosterone-induced cardiac fibrosis and renal injury. Indeed, genetic PAI-1 deficiency protects against AngII-stimulated aortic remodelling and aldosterone-mediated glomerular injury [79,80]. However, paradoxically, genetic PAI-1 deficiency increases cardiac fibrosis, as well as the expression of pro-inflammatory genes, in AngII- and aldosterone-treated rodents [79,80]. These findings highlight the complex role of the plasmin/protease system in the regulation of ECM degradation and cell migration. PAI-1 promotes fibrosis and remodelling by inhibiting plasmin-mediated MMP activation and ECM degradation, but PAI-1 may also prevent fibrosis by retarding cellular infiltration and inflammation or by impeding uPA- or plasmin-mediated activation or release of latent growth factors, such as FGF (fibroblast growth factor) and VEGF (vascular endothelial growth factor) [81,82]. In support of a role for PAI-1 in modulating uPA-dependent cell infiltration,
Figure 3 Effect of aldosterone on the expression of profibrotic factors

AngII and aldosterone increase ET-1, TGF-β and PAI-1 mRNA expression through MR-dependent mechanisms. Aldosterone also stimulates PAI-1 expression in cardiomyocytes through a non-genomic pathway. TGF-β stimulates collagen expression, as well as PAI-1 expression. PAI-1 inhibits the formation of plasmin from plasminogen. This decreases ECM degradation by decreasing plasmin-mediated activation of MMPs; however, increased PAI-1 can also prevent plasmin-mediated activation of latent TGF-β and affect cell migration through complex mechanisms involving the interaction of u-PA with urokinase receptor (uPAR), lipoprotein-receptor-related peptide (LRP) and cell-surface integrins. HRE, hormone-responsive element.

Dichek and co-workers [83] have reported that PAI-1-deficient mice and transgenic mice overexpressing macrophage uPA develop cardiac fibrosis through a TGF-β-independent mechanism.

Increased expression of (pro) ET (endothelin)-1 also contributes to aldosterone-mediated cardiac and vascular fibrosis and hypertrophy. Aldosterone/salt treatment increases ET expression in the heart, vasculature and kidney [84,85]. ET stimulates cardiomyocyte hypertrophy and increases collagen synthesis by cardiac fibroblasts, in part, through effects of TGF-β1 [86,87]. Importantly, ET,A (ET type A receptor) antagonism prevents small artery remodelling and cardiac and aortic fibrosis and hypertrophy in aldosterone- and aldosterone/salt-treated rats respectively [84,88].

CLINICAL TRIALS OF MR ANTAGONISTS IN CHF

Increased plasma aldosterone concentrations portend a poor prognosis in CHF. The observation that aldosterone concentrations ‘escape’ during chronic ACE inhibition spurred clinical trials examining the effect of combined MR antagonism and ACE inhibition in patients with CHF. In RALES (Randomized Aldactone Evaluation Study), the addition of 25 mg of spironolactone to patients with NYHA (New York Heart Association) Class III or IV heart failure, who were already treated with an ACE inhibitor, diuretics and digoxin, reduced mortality by 30% [89]. The EPHESUS (Eplerenone Neurohormonal Efficacy and Survival) trial studied the effect of the addition of 25–50 mg of eplerenone/day to standard therapy with ACE inhibitors, AT1 receptor antagonists, β-blockers, digoxin and diuretics in patients with LV (left ventricular) dysfunction following a recent myocardial infarction [90]. The addition of eplerenone reduced all-cause and cardiovascular mortality.

MR antagonism reduces the incidence of fatal arrhythmias in patients with CHF [91]. This could result from classical effects of MR antagonism on sodium and potassium homeostasis and decreased relative hypokalaemia or from alterations in noradrenaline (norepinephrine) re-uptake. CHF is characterized by activation of the sympathetic nervous system; impaired cardiac re-uptake of noradrenaline is associated with a poor
prognosis in CHF patients [92]. Studies in the Dahl salt-sensitive rat indicate that aldosterone also inhibits NET (noradrenaline transporter) via an ET_{A}-dependent mechanism, whereas MR antagonism restores NET function [93]. Alternatively, MR antagonism could also reduce mortality by decreasing fibrosis and remodelling and, in turn, affecting the substrate for the generation of arrhythmias. In this regard, it is notable that the reduction in markers of ECM turnover, such as PIINP (procollagen type III N-terminal peptide), predicted the reduction of mortality in RALES [94].

RALES and the EPHESUS trial were conducted in patients with systolic dysfunction. Heart failure due to LV hypertrophy and diastolic dysfunction causes considerable morbidity and mortality in patients with long-standing hypertension. The 4E-LV Hypertrophy Study compared the effects of the ACE inhibitor enalapril (40 mg/day), eplerenone (200 mg/day) or a combination of enalapril (10 mg/day) and eplerenone (200 mg/day) [95]. Both enalapril and eplerenone decreased LV mass as measured by MRI (magnetic resonance imaging), but the combination of enalapril + eplerenone decreased LV mass to a greater degree than the MR antagonist alone. Unfortunately, interpretation of this study is confounded by the fact that systolic BP was also reduced to a greater degree in the combination arm compared with the MR-antagonist-treatment arm.

INAPPROPRIATELY HIGH ALDOSTERONE CONCENTRATIONS IN RESISTANT HYPERTENSION

In 1955, Dr Jerome Conn [96] first described a patient with severe hypertension, hypokalaemia and an aldosterone-secreting adenoma. Although aldosterone-secreting adenomas are relatively uncommon among patients with hypertension, the work of Calhoun et al. [97,98] and others suggests that aldosterone concentrations are inappropriately high relative to salt intake in a substantial proportion of individuals with hypertension, particularly among those who are overweight or have sleep apnoea. Whether the apparent increase in the prevalence of primary hyperaldosteronism results from the increased use of the ARR (aldosterone/renin ratio) in screening or due to a true increase in the prevalence, perhaps related to the epidemic of obesity (see below), is a topic of debate. Although elevated plasma aldosterone concentrations provide a useful screening tool, increased plasma renin activity due to antihypertensive treatment can falsely decrease the ARR; therefore the saline-suppression test and measurement of urinary aldosterone excretion during sodium loading remain the screening tests of choice for primary hyperaldosteronism [99].

Nevertheless, studies demonstrating the effectiveness of MR antagonism in patients with resistant hypertension support a high prevalence of hyperaldosteronism among this population. For example, among patients requiring treatment with adequate doses of three or more antihypertensive medication, 20% have elevated urinary aldosterone concentrations and suppressed plasma renin activity. Treatment with an MR antagonist dramatically lowered BP in these patients [97]. In the ASCOT (Anglo-Scandinavian Cardiac Outcomes Trial)-Blood-Pressure-Lowering Arm, the non-randomized addition of spironolactone as a fourth-line antihypertensive agent reduced BP by 21.9/9.5 mmHg in participants who were already taking an average of 2.9 antihypertensive medications [100]. As in animal models, MR antagonism reduces circulating biomarkers of inflammation, such as PAI-1, in individuals with hypertension [101].

CLINICAL STUDIES OF THE EFFECT OF MR ANTAGONISM IN RENAL DISEASE

Although ACE inhibitors and AT_{1} receptor antagonists slow the progression of diabetic and non-diabetic nephropathy, albuminuria can return to baseline levels with chronic therapy [102]. Escape from the renoprotective effects of ACE inhibitors has also been associated with aldosterone escape in patients with Type 2 diabetes mellitus [103].

Several studies have examined the effect of MR antagonism on microalbuminuria in individuals with essential hypertension. In addition to predicting progression to renal insufficiency, urinary albumin excretion has been associated with an increased risk of cardiovascular events [104]. In the 4E-LV Hypertrophy Study, combination treatment with the ACE inhibitor enalapril and eplerenone reduced the urine albumin/creatinine ratio to a greater degree than did either eplerenone or enalapril alone [95]. Again, it is not possible to conclude whether this renoprotective effect of a combination of ACE inhibition/MR antagonism resulted from interruption of the RAAS or superior BP reduction in this study. However, in a study of patients with mild-to-moderate hypertension, 50–200 mg of eplerenone/day reduced urine albumin excretion to a significantly greater extent than did 10–40 mg of enalapril, despite equivalent effects of the two drugs on BP [105]. Similarly, in older patients with systolic hypertension, eplerenone reduced urine albumin to a greater extent than did amlodipine at comparable hypotensive doses [106].

MR antagonism also reduces microalbuminuria in patients with mild diabetic nephropathy. For example, addition of 25 mg of spironolactone/day to patients with Type 2 diabetes with aldosterone escape significantly reduced urinary albumin excretion without affecting BP [103]. Rachmani et al. [107] reported the effect of randomized treatment with either spironolactone (100 mg/day) or the ACE inhibitor cilazapril (5 mg/day) on a
background of atenolol and hydrochlorothiazide in a group of hypertensive post-menopausal female diabetic patients. Despite equivalent effects on BP and 
HbA1c (glycated haemoglobin), spironolactone reduced urinary albumin excretion to a greater extent than did cilazapril. Addition of spironolactone enhanced the effect of cilazapril on urine albumin excretion when both groups were crossed over to combination therapy with spironolactone and cilazapril at the end of the study. In patients with Type 2 diabetes, 50–200 mg of eplerenone/day reduced urinary albumin/creatinine ratio to a greater extent than treatment with 10–40 mg of enalapril/day and the combination of eplerenone + enalapril was more effective than either agent alone [108].

Studies have also examined the effect of adjuvant MR antagonism on renal function in patients with overt proteinuria or renal insufficiency. For example, in patients with residual proteinuria despite ACE inhibition and BP control, the addition of 25 mg of spironolactone/day significantly reduced proteinuria and urinary excretion of Type IV collagen, a potential marker of renal collagen turnover, without affecting BP [109]. In patients with depressed glomerular function treated with ACE inhibitors and/or AT1 receptor antagonists, the addition of 25 mg of spironolactone/day significantly reduced urinary protein excretion through a BP-independent mechanism [110].

These clinical studies suggest that MR antagonism reduces urine albumin excretion in hypertension, mild diabetic nephropathy and mild-to-moderate chronic renal disease; however, whereas ACE inhibition and AT1 receptor antagonism have been demonstrated to improve outcomes in patients with renal disease, the effect of MR antagonism on progression to end-stage renal disease and mortality has yet to be determined.

**ALDOSTERONE, THE METABOLIC SYNDROME AND DIABETES**

Elevated aldosterone concentrations are associated not only with difficult-to-control hypertension, but also with obesity and the metabolic syndrome [111,112]. Aldosterone concentrations correlate with BMI (body mass index), particularly in women [113,114]. The stimulation of aldosterone synthesis by oxidized fatty acids such as 12,13-epoxy-9-keto-15(trans)-octadecenoic acid may contribute to this association [115]. However, results from clinical trials indicating that interruption of the RAAS by ACE inhibition or AT1 receptor antagonism decreases the incidence of Type 2 diabetes mellitus [116] suggests the alternative possibility that aldosterone concentrations influence the development of the metabolic syndrome and diabetes.

Aldosterone decreases glucose-stimulated insulin release [117] and may affect insulin release indirectly by lowering potassium [118]. Aldosterone can also decrease insulin receptor expression in adipocytes and monocytes via an MR-dependent mechanism [119]. MR activation attenuates the effect of insulin on hepatic gluconeogenesis [120]. These effects do not appear to be mediated by expression of sgk-1, as sgk-1 up-regulates GLUT-1 (glucose transporter-1) activity and expression, and sgk-1-deficient mice exhibit decreased glucose uptake and increased circulating glucose concentrations after a glucose load [121].

Most studies on the effect of aldosterone on insulin sensitivity and glucose homeostasis in humans have focused on patients with primary hyperaldosteronism. Conn [122] reported the association of hypokalaemia with impaired carbohydrate tolerance over 40 years ago. Surgical resection of aldosterone-secreting adenomas improves glucose concentrations and has been reported to improve or to have no effect on HOMA-IR (homeostasis model assessment-insulin resistance), an indirect measure of insulin sensitivity [123,124]. Hyperinsulinaemic euglycaemic clamp studies indicate that surgical resection of aldosterone-secreting adenomas improves insulin sensitivity, whereas MR antagonism in patients with idiopathic hyperaldosteronism does not [125]. On the contrary, spironolactone has been reported to increase HbA1c concentrations in patients with Type 2 diabetes [126,127]. Given that circulating aldosterone concentrations are often increased during MR antagonism, this suggests that aldosterone affects glucose metabolism and/or insulin sensitivity through an MR-independent effect, a possibility that merits further investigation.

**CONCLUSIONS**

Aldosterone contributes to cardiac and vascular hypertrophy by acting on MR-dependent pathways in the kidney to foster systemic sodium retention and hypertension. During high-salt intake, inappropriately high aldosterone concentrations also promote cardiovascular remodelling and renal injury by stimulating oxidative stress, endothelial dysfunction, inflammation and fibrosis. Induction of oxidative stress, inflammation and fibrosis involves complex interactions between the MR and the AT1 receptor, as well as EGFR, and may involve both genomic and non-genomic mechanisms. Continuing research *in vitro* and *in vivo* in animal models is required to discern the ligand specificity of MR activation, the role for MR-dependent compared with -independent effects, and the importance of genomic compared with non-genomic pathways. Likewise, further studies are needed to elucidate the mechanism through which salt facilitates the profibrotic effects of aldosterone or MR activation, and to identify tissue-specific pathways involved in the progression from inflammation to fibrosis.
At the same time, clinical studies provide unequivocal evidence of a beneficial effect of MR antagonism in patients with CHF, who are screened and monitored to reduce the risk of hyperkalaemia. MR antagonism shows promise in the treatment of resistant hypertension and diabetic and hypertensive nephropathy, but large trials with morbidity and mortality outcomes are needed.

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