The renin–angiotensin–aldosterone system (RAAS) is a powerful hormone regulatory mechanism serving multiple functions as a key determinant of tissue perfusion pressure and cellular homeostasis. Since the original demonstration of renin in renal extracts, characterization of the biochemical physiology and mechanisms of action of this system have become one of the major accomplishments of the biomedical research enterprise. It is also a fruitful example of translational medicine, with major impact on the therapy of cardiovascular disease as a consequence of the development of drugs that inhibit the pathological actions of angiotensin II (Ang II) and its interplay with the neurohormonal mechanisms regulating salt and water intake. The translational lessons derived from the original reports of Ang II and aldosterone biosynthesis embody an extensive medical literature, the highlights of which resulted in the development of angiotensin-converting enzyme (ACE) inhibitors, Ang II receptor antagonists, direct renin inhibitors, and mineralocorticoid receptor (MR) antagonists. It would be a futile effort to detail accurately the complete history of these accomplishments, as they constitute a body of literature of several hundred papers and more than one hundred years of exemplary discoveries. This review provides a succinct summary of the knowledge that exists on aldosterone actions in the cardiovascular system and, in providing the translational impact of this knowledge to the clinical arena, illustrates how much more needs to be achieved in exploring the use of mineralocorticoid receptor blockers in less advanced stages of heart, renal, and vascular disease. (Circ Res. 2015;116:206-213. DOI: 10.1161/CIRCRESAHA.116.302706.)

Key Words: heart failure ■ hypertension ■ kidney failure, chronic

Role of Mineralocorticoid Receptor Antagonists in Cardiovascular Disease

Carlos M. Ferrario, Ernesto L. Schiffrin

Abstract: Aldosterone exerts its best known sodium homeostasis actions by controlling sodium excretion at the level of the distal tubules via activation of the apical epithelial sodium channel and the basolateral Na+/K+ATPase pump. Recently, this mineralocorticoid hormone has been demonstrated to act on the heart and blood vessels. Excess release of aldosterone in relation to the salt status induces both genomic and nongenomic effects that by promoting endothelial dysfunction, and vascular and cardiorenal adverse remodeling, contribute to the target organ damage found in hypertension, heart failure, myocardial infarction, and chronic renal failure. Mineralocorticoid receptor blockers have been shown to be highly effective in resistant hypertension and to slow down heart failure progression, and in experimental animals, the development of atherosclerosis. Blockade of the action of aldosterone and potentially other mineralocorticoid steroids has been increasingly demonstrated to be an extremely beneficial therapy in different forms of cardiovascular disease. This review provides a summary of the knowledge that exists on aldosterone actions in the cardiovascular system and, in providing the translational impact of this knowledge to the clinical arena, illustrates how much more needs to be achieved in exploring the use of mineralocorticoid receptor blockers in less advanced stages of heart, renal, and vascular disease.
**Nonstandard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>11-β-OHSD</td>
<td>11-β-hydroxysteroid dehydrogenase enzyme</td>
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<tr>
<td>ACE</td>
<td>angiotensin-converting enzyme</td>
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<tr>
<td>Ang II</td>
<td>angiotensin II</td>
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<tr>
<td>HF</td>
<td>heart failure</td>
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<tr>
<td>MR</td>
<td>mineralocorticoid receptor</td>
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<tr>
<td>RAAS</td>
<td>renin-angiotensin-aldosterone system</td>
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<tr>
<td>VSMC</td>
<td>vascular smooth muscle cell</td>
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A narrative synthesis of accomplishments and the inclusion of the timeline of discoveries documented in Table 1.

**Aldosterone Biosynthesis and Functions**

The isolation of aldosterone can be traced back to the early 1930s when researchers succeeded in obtaining biologically active extracts free of contaminating compounds from the adrenal medulla. As narrated by Tai et al., this work was accomplished by several teams of investigators working at Columbia University, the Mayo Clinic, and the Ciba group in Basel. Aldosterone crystals were obtained first from human urine collected from 2 patients with congestive heart failure (HF). Table 1 documents the evolution of the research leading to the final synthesis of aldosterone by the Ciba group of investigators in 1955. Whereas the clinical role of aldosterone in disease processes continues to expand, the classification provided by Genest et al. (Table 2) summarizes the involvement of this adrenal hormone in human hypertension.

Normal adrenal steroidogenesis leads to the production of glucocorticoids, mineralocorticoids, and sex hormones. Although glucocorticoid secretion is regulated primarily by pituitary ACTH (adrenocorticotropic hormone), Ang II acts as the primary stimulus for aldosterone secretion. The mechanisms by which aldosterone interplays with the regulation of blood pressure and the control of renal salt excretion (Figure) are mediated by the MR, a ligand-activated transcription factor that is a member of the nuclear receptor superfamily. The relatively slow genomic aldosterone actions influence renal gene expression of proteins that regulate Na+/K+-ATPase activity and the renal epithelial sodium and outer medullary potassium channels. Of importance, aldosterone has a significant influence in the activation of genes participating in the genesis of adverse cardiac and vascular remodeling. Aldosterone can exert direct vasoconstrictor actions and in normal healthy volunteers the effects of aldosterone in forearm blood flow were dose dependent.

In vascular smooth muscle cells (VSMCs), aldosterone induces a proliferative response through increased expression of p53-binding protein. Wehling and colleagues and Rautureau et al. provide a comprehensive discussion of aldosterone nongenomic effects, which are in the order of minutes rather than hours. In addition, aldosterone can upregulate vascular angiotensin receptors, a finding that is critically important in understanding vasoconstrictor and inflammatory mechanisms of the interplay among Ang II, aldosterone, and salt intake. Collagen deposition in the vasculature leading to vascular stiffness was prevented in VSMCs from mice with conditional inactivation of the MR. The authors concluded that the MR influences vascular elasticity by modulation of cell-matrix attachment proteins independent of major vascular structural changes. The interactions between MRs and angiotensin receptors seem to explain many of the effects found when blocking either of these receptors. These signaling pathways are important in explaining the mechanisms by which MRs induce cardiac and vascular collagen deposition, activation of proinflammatory cytokines, and increases in oxidative stress. Recently, evidence of a role of placental growth factor has been produced in the mediation of aldosterone action. Furthermore, aldosterone proatherogenic effects may in part be mediated by stimulation of placental growth factor. The effects on atherosclerosis are mediated via MRs as demonstrated in mice with deletion of smooth muscle MRs or administration of MR antagonists. Furthermore, the aldosterone contribution to atherosclerosis progression may include facilitation of aortic aneurysm formation.

Atherogenic effects of aldosterone may relate to its role in inflammation, stimulation of oxidative stress, endothelial dysfunction, and prothrombotic activity. Aldosterone, in association with inflammatory mediators such as interferon-γ and tumor necrosis factor-α, stimulates growth and proliferation of VSMCs, leading to adverse vascular remodeling. Moreover, new fascinating data show prevention of vascular and renal inflammatory responses after administration of T-regulatory lymphocytes both to Ang II–infused and to aldosterone-infused mice. The finding that Ang II functions as an obligatory mediator pathway for the formation of aortic fatty streaks in the presence of hypercholesterolemia and no changes in arterial pressure was first demonstrated in monkeys. These findings led to the hypothesis that the RAAS is a critical contributor to the pathogenesis of atherosclerosis through stimulation of oxidative stress, inflammatory cytokines, and recruitment of monocytes into the subendothelial vascular spaces. Proatherogenic aldosterone mechanism occurs via induction of oxidative stress, endothelial dysfunction, activation of adhesion molecules, and upregulation of ACE and AT1 receptors. Atherogenic actions of aldosterone can be prevented by epelone administration. In primary aldosteronism, plasma osteopontin levels are higher than those in essential hypertensive subjects, and in osteopontin-knockout mice reduced renal interstitial fibrosis is induced by aldosterone infusions. An intriguing study showed that aldosterone can stimulate the release of Willebrand factor and interleukin-8 via exocytosis of Weibel–Palade bodies. This nongenomic effect is a potentially powerful mechanism for the activation of proinflammatory factors. Prothrombotic aldosterone actions include stimulation of plasminogen activator inhibitor-1, a finding that may contribute to extracellular collagen deposition because plasminogen activator inhibitor-1 inhibits the production of plasmin from plasminogen. The opportunity to advance the use of MR antagonists in prevention or management of coronary heart disease is a tantalizing proposal as mortality caused by myocardial infarction worsens in the presence of hyperaldosteronism.

Adrenal glands contain the genes and proteins for local tissue production of bioactive angiotensins. Cells in the zona glomerulosa contain the highest levels of renin activity and Ang II. The independence of this tissue system from that of the circulation or the kidney is based on the demonstration that renin activity is not suppressed in anephric rats, whereas increases in adrenal renin and aldosterone are independent of
plasma or kidney renin concentrations.65–67 The role of this adrenal RAAS remains to be elucidated. Nevertheless, the interdependent nature of the interplay between aldosterone and Ang II in the regulation of cardiovascular function entails both a stimulatory action of the peptide in the systemic release of aldosterone and the ability of aldosterone to increase the expression of angiotensin receptors in VSMCs.68 In VSMCs, intracellular mobilization of Ca2+ by Ang II is augmented in the presence of aldosterone via increased expression of extracellular signal–regulated protein kinase activation, radical oxygen species generation, and epidermal growth factor receptor transactivation.69,70 The aldosterone-induced activation of extracellular signal–regulated kinase, c-Jun N-terminal kinase, and nuclear factor κ-light-chain-enhancer of activated B cells has been reported to be dependent on the transactivation of AT1a, but not AT1b receptors.21,34 This interpretation agrees with 2 published studies showing that (1) eplerenone inhibits aldosterone vasoconstrictor actions and nongenomic effects on the sodium-proton exchanger activity or intracellular Ca2+ responses of rat mesenteric vessels71 and (2) aldosterone-mediated mesenteric artery vasoconstriction is blunted in AT1a knockout mouse.21,34,72 Mice in which a VSMC-specific deletion of MR (VSMC-MR) has been induced have lower blood pressure as they age.73 This occurs despite the absence of changes in sodium excretion, indicating that the kidney effects of aldosterone do not participate in these effects. There is also absence of vascular remodeling. Interestingly, the aged VSMC-MR mice exhibited a reduction in vascular myogenic tone and Ang II–dependent contraction, lar oxidative stress, contraction, and elevated blood pressure, and vascular MR-mediated vascular tone may participate in control of blood pressure as well as vascular aging.73 Salt excess seems to be important for aldosterone to exert its hypertensive action in rodents, especially in rats,74,75 whereas in mice even in the presence of salt, and despite powerful proinflammatory actions, aldosterone exerts little effect on blood pressure.59 The reason for this difference is unclear. However, the effect of salt on aldosterone effects may explain that in essential hypertensive patients with stage 1 hypertension, blockade of MR has a mild antihypertensive action, whereas in uncontrolled or in resistant hypertension, often associated with excess consumption of salt, the effects of MR blockade are much more powerful. Salt consumption by patients with hypertension and HF probably is a confounder of the efficacy of MR blockade in clinical trials.

### Translational Correlates
Dysregulation of MR participates in the pathogenesis of cardiovascular disease either via amplification of the proliferative,
proinflammatory, and prothrombotic effects of excess Ang II levels or excess secretion of aldosterone. Knowledge of the direct effects of hyperaldosteronism on cardiovascular function resulted from Conn’s report of the association of high blood pressure and hypokalemia in a woman with a diagnosis of primary aldosteronism. However, as early as 1937, Steigler and Reichstein had reported a syndrome of hypokalemia and hypertension associated with the administration of synthetic deoxycorticosterone. A syndrome resembling primary aldosteronism without elevated aldosterone levels is associated with inhibition of 11-β-hydroxysteroid dehydrogenase enzyme (11-β-OHSD). This apparent mineralocorticoid excess process results from increased binding of cortisol to MR because the 11-β-OHSD accounts for the conversion of cortisol to cortisone. Nevertheless, the contribution of aldosterone to cardiovascular pathology remained elusive until the publication of a sensitive radioimmunoassay and a more comprehensive understanding of its role in the control of electrolyte homeostasis. Availability of a measure of aldosterone activity provided a tool to seek the contribution of this hormone to the pathophysiology of essential hypertension, an effort that led to identification of subsets of hypertensive subjects where aldosterone was associated with sodium retention and hypertension. Williams and Hollenberg characterized a subset of essential hypertensive patients that they have named as nonmodulators. These individuals are now recognized to associate hypertension with salt sensitivity, insulin resistance, and RAAS gene polymorphisms. A state of hyperaldosteronism is also found in diseases associated with important alterations in body fluid volumes, particularly HF, cirrhosis accompanied by ascites, and the nephrotic syndrome.

Although most studies in hypertensive subjects have not demonstrated elevations of plasma aldosterone except in few patients, quartiles of plasma levels of aldosterone in the Framingham study were directly associated with blood pressure levels. Moreover, treatment with MR blockers lowers blood pressure in patients with hypertension, and particularly in resistant hypertension. The MR blocker spironolactone prevented vascular fibrosis independently of blood pressure reduction in spontaneously hypertensive rats. The more selective but less potent MR blocker eplerenone also reduces small artery fibrosis and stiffness in stage 1 essential hypertension, possibly contributing to the antihypertensive actions of MR antagonism as well as improvement of tissue perfusion. Spironolactone has also been shown to block some of the effects of Ang II, indicating that aldosterone mediates some actions often attributed to direct effects of the peptide. The effects with MR blockers are mimicked by aldosterone synthase inhibitors, indicating that spironolactone and eplerenone effects are probably mediated by blockade of aldosterone actions.

The important role of aldosterone in the regulation of sodium and potassium balance led to the exploration of the potential clinical benefits that could be derived from blocking the MR. The synthesis of the first MR antagonist (spironolactone) by the chemists at Searle laboratories lagged for years behind the identification of aldosterone. As commented by Delyani, the drug was initially labeled as a potassium-sparing diuretic given that at that time the known aldosterone actions were limited to epithelial ion transport. Spironolactone (7-acetate of the γ-lactone of 17-hydroxy-7-mercapto-3-oxo-17-α-pregn-4-ene-21-carboxylic acid) acts as a competitive antagonist of the MR (but also other steroid
receptors such as the androgen receptor, leading to both its adverse side effects and also some additional therapeutic indications as a consequence of its antiandrogenic effects). The drug is rapidly cleared from the circulation (T1/2 10 minutes) to 2 principal metabolites (canrenoate and canrenone).99,100,102 The most compelling results for the participation of aldosterone in the pathogenesis of cardiovascular disease derived from the use of MR antagonists in HF. The rationale for the exploration of blocking MRs in HF was based on the observation that ACE inhibitors had only a transient Ang II suppression action and that the same could be demonstrated for plasma aldosterone.102 It was therefore reasoned that addition of spironolactone on top of background ACE therapy could have a greater impact on the progression of HF. The Randomized ALdactone Evaluation Study (RALES) trial established the benefits of aldosterone antagonists in the evolution of severe HF (New York Heart Association class III/IV).103 In this trial, 1663 subjects with a diagnosis of severe HF (ejection fraction <35%) were randomized to conventional therapy (ACE inhibitor, loop diuretic and digoxin in most subjects) plus placebo or the combined use of conventional therapy plus spironolactone (25 mg/d). The trial was interrupted after a mean follow-up period of 24 months because an interim analysis showed a significant 35% reduction in all causes of death in the spironolactone-treated group compared with the placebo-mediated subjects.103 The beneficial effects of spironolactone were associated in this trial with significant reductions in the frequency of hospitalization for worsening HF and HF symptomatology. Because the greatest benefits were observed in subjects in whom spironolactone was administered on top of background ACE inhibitor therapy, it is clear that combined suppression of Ang II formation and aldosterone production was enhanced through blockade of the MR. The pleiotropic actions of aldosterone on the cardiovascular system, particularly the aldosterone-mediated collagen synthesis that contributes to adverse left ventricular remodeling, prompted an examination of the action of MR antagonists in subjects’ postmyocardial infarction. The trial was in part based on finding that adrenalectomy or eplerenone attenuated the adverse cardiac remodeling in an Ang II model of experimental hypertension.75 The Eplerenone Post-myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) determined the effect of the MR antagonist eplerenone on morbidity and mortality among patients with acute myocardial infarction.104 Eplerenone (pregn-4-ene-7,21-dicarboxylic acid, 9,11-epoxy-17-hydroxy-3-oxo, γ-lactone, methyl ester) is a selective MR antagonist devoid of the progestational and antiandrogenic side effects of spironolactone and shows minimal activity on P450 enzymes. During a mean 16-month follow-up, patients with acute myocardial infarction complicated by left ventricular dysfunction and HF assigned to eplerenone and associated optimal medical therapy demonstrated significantly reduced morbidity and mortality.104 The beneficial effect of aldosterone blockade in the evolution of HF can be shown even in those subjects with milder forms of the syndrome. This was found in another trial that recruited New York Heart Association class II systolic HF subjects. Patients enrolled in the Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF) study showed a reduced rate of hospitalization and death when treated with eplerenone.105,106

The landmark studies using MR antagonists in HF have in part overshadowed the potential use of these agents in a broader patient population, such as individuals with cardiac arrhythmias, myocardial ischemia secondary to cardiac fibrosis, and diastolic dysfunction.107 Spironolactone can prevent cardiac collagen deposition in 2 experimental rat models of hypertension independent of the drug effect on blood pressure and cardiac hypertrophy.107,108 Translational outcomes of these experimental studies are contained in a study where spironolactone improved echocardiographic measurements of myocardial relaxation in patients with exertional dyspnea and abnormal left ventricular filling patterns.109 These important findings need further confirmation as diastolic dysfunction associated with HF and preserved ejection fraction has no known effective treatment.110,111 The Trial of Aldosterone Antagonist Therapy in Adults with Preserved Ejection Fraction Congestive Heart (TOPCAT) has addressed the potential benefit of spironolactone on 3445 patients with symptomatic HF and HF and preserved ejection fraction.112,113 However, results have been disappointing because only hospitalization for HF was reduced by MR antagonism, whereas other end points such as the primary composite outcome of death from cardiovascular causes, aborted cardiac arrest, or hospitalization for the management of HF did not differ statistically with placebo.114

Other areas where additional research is needed relate to the observation that spironolactone suppresses QT-dispersion115 and sudden death in subjects that were enrolled in the RALES and EPHESUS trials. It is not known whether these antiarrhythmic actions of aldosterone antagonism are related to conservation of total body potassium and magnesium or reversal of cardiac fibrosis. An attractive alternative hypothesis for the antiarrhythmic effects of MR antagonists has been provided by studies using mice with conditional cardiac-specific overexpression of the human MR.116 In this experimental model, prolongation of ventricular repolarization and presence of severe ventricular arrhythmias because of overexpression of the human MR were prevented by spironolactone administration.116 In addition, aldosterone arrhythmogenic actions may relate to myocyte gap junction remodeling through its dual effects on the expression of connexin 43,55,117 This is clearly an area of potential fruitful research.

**Summary**

Aldosterone’s main physiological function maintains sodium homeostasis through its direct action on controlling sodium excretion at the level of the distal tubules via activation of the apical epithelial sodium channel and the basolateral Na+/K+ ATPase pump.118 An excess production/release of aldosterone in relation to the salt status induces a genomic and nongenomic effect that in experimental animals leads to vascular remodeling, endothelial dysfunction, and cardiorenal adverse remodeling including the structural and functional consequences of increased collagen deposition. In humans, the nongenomic actions of aldosterone through MR are somewhat more varied as vasoconstriction, vasodilatation, or no effects have been observed.119 Amphibaric actions of aldosterone in humans may be related to doses used, the baseline plasma aldosterone levels,120
as well as salt status. The more chronic effects of aldosterone play a critical role in contributing to target organ damage associated with hypertension, HF, myocardial infarction, and chronic renal failure.\textsuperscript{18} MR antagonists are powerful treatment agents having a marked beneficial effect in HF progression and resistant hypertension, and in experimental animals, in ath
erosclerosis. The pleiotropic actions of this mineralocorticoid hormone in human primary essential hypertension and the cardiometabolic syndrome await further investigation as emerging evidence demonstrates that the use of MR antagonists may be associated with potentially impressive outcomes.\textsuperscript{35,38}

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Disclosures
None.

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