Aldosterone plays an important role in the pathogenesis of cardiovascular and renal disease that is independent of angiotensin II. Mineralocorticoid receptors are expressed in nonepithelial tissues such as the heart and blood vessels. Although mineralocorticoid receptor antagonism reduces mortality in patients with congestive heart failure, the progestational and antiandrogenic side effects of the nonspecific mineralocorticoid receptor antagonist, spironolactone, have limited its usefulness in the treatment of cardiovascular diseases. This review examines the expanding role of aldosterone, including its broad spectrum of non-classical effects, and the recent clinical and experimental trials with the selective mineralocorticoid receptor antagonist, eplerenone. (Hypertens Res 2004; 27: 781–789)

Key Words: aldosterone, hypertension, mineralocorticoid receptor, eplerenone, angiotensin II

Introduction

Aldosterone plays an important role in the pathogenesis of cardiovascular disease that is independent of angiotensin II (ATII). For example, patients with primary aldosteronism, in which ATII levels are usually very low, have a higher incidence of left ventricular hypertrophy, and stroke than do patients with essential hypertension (1). A recent study performed in patients classified with New York Heart Association class III and IV cardiac failure showed a 30% reduction in morbidity and mortality with the addition of the mineralocorticoid receptor antagonist spironolactone to conventional therapy which included angiotensin converting enzyme (ACE) inhibitors, loop diuretics, and digoxin (2). This decrease occurred with an average dose of spironolactone that did not have significant hemodynamic effects. Experimental animal data support a role for aldosterone in mediating cardiovascular injury. In the stroke-prone spontaneously hypertensive rat (SHRSP), a genetic model of spontaneous hypertension, administration of spironolactone greatly attenuated cardiac hypertrophy (3). An important pathological effect of aldosterone in the heart has been reported in experimental models of mineralocorticoid hypertension (4). In these studies, prolonged exposure to aldosterone was associated with the development of myocardial hypertrophy and fibrosis.

In this review, I will consider the expanding role of aldosterone, including its broad spectrum of non-classical effects, and review the recent clinical and experimental trials with the selective mineralocorticoid receptor antagonist, eplerenone.

Classical Epithelial Effects of Aldosterone

The concentration of cortisol, the physiological glucocorticoid in humans, exceeds the circulating level of the mineralocorticoid aldosterone by 1,000-fold. The epithelial mineralocorticoid receptor (MR) is known to possess a similar in vitro affinity for cortisol and aldosterone. The mineralocorticoid target tissues metabolize glucocorticoids to less active compounds, by utilizing the enzyme 11β-hydroxysteroid dehydrogenase 2 (11β-HSD 2), thereby protecting the cytosolic MR (Fig. 1). The binding of aldosterone to the MR results in dissociation of the ligand-activated MR from a multipro-
tein complex containing molecular chaperones, translocation into the nucleus, and binding to hormone response elements in the regulatory region of target gene promoters.

In the distal nephrons of the kidney, induction of serum and glucocorticoid inducible kinase (sgk)-1, Kirsten RasA (Ki-RasA) and corticosteroid hormone-induced factor (CHIF) expression lead to the absorption of Na ions and water through the epithelial sodium channel (ENaC) and potassium excretion with subsequent volume expansion and hypertension (5). Final effectors of aldosterone action in epithelia have traditionally been considered to be the luminal ENaC and luminal K⁺ channel and the serosal Na⁺/K⁺-ATPase. Deletion of the α-ENaC gene in transgenic mice results in early neonatal death due to defective clearance of lung liquid, and partial restoration of the α-ENaC expression in these mice by transgenesis results in a phenotype very similar to that seen in pseudohypoaldosteronism (PHA) (6).

Na⁺/K⁺-ATPase activity is very sensitive to intracellular sodium concentrations; in isolated cortical tubules, the early Na⁺/K⁺-ATPase response to aldosterone is blocked by amiloride, suggesting that the increased activity is secondary to sodium influx at the apical membrane. In the late phase of the aldosterone response, levels of Na⁺/K⁺-ATPase mRNA, protein, and activity are increased. Other intrinsic membranes of epithelia in the gut and kidney are now recognized as final effectors. Aldosterone increases the activity of the luminal Na⁺/H⁺-exchanger (NHE3) in the proximal portion of the colon and the luminal thiazide Na⁺/Cl⁻ cotransporter (NCC) in the distal renal tubules. Increases in NHE3 and NCC translate into a sustained elevation of electroneutral Na⁺ reabsorption in the colon and kidney in response to volume contraction (7).

Cardiovascular Effects of Aldosterone

Vascular Effects of Aldosterone

The renin-angiotensin-aldosterone system plays an important role in the control of blood pressure and the water electrolyte balance. It has been shown that all components of the renin-angiotensin system are expressed in both the vascular wall and heart and function on an autocrine-paracrine level (8). The physiological functions of such locally acting tissue renin-angiotensin have been postulated, but are still subject to further investigation.

The mineralocorticoid aldosterone is classically involved in the regulation of sodium and water homeostasis and thus participates in the regulation of blood pressure. Aldosterone is involved in vascular smooth muscle hypertrophy and can cause vascular matrix impairment and endothelial dysfunction. Tobian and Redleaf (9) proposed that aldosterone influences salt and water balance in vascular smooth muscle cells (VSMC) and thereby affects vessel lumen size. Kornel et al. (10) presented evidence that glucocorticoids and mineralocorticoids control the contractility of VSMC. This control is affected via glucocorticoid and mineralocorticoid receptors on the VSMC. Farquharson and Struthers (11) reported that spironolactone increases endothelial nitric oxide (NO) bioactivity by the large margin of 94%, which is much greater than any other therapy—e.g., ACE inhibitors and statins usually improve endothelial dysfunction by only 25%–35%. Improving endothelial dysfunction is important, because such improvement is likely to be associated with a reduced future incidence of cardiovascular events.

Aldosterone can be synthesized locally in tissues such as those of blood vessels, heart and brain (12–14). Synthesis at
extra-adrenal sites appears to be regulated by the renin-angiotensin system (15). MR has been identified not only in the epithelial cells of the kidney but also in blood vessels, the heart, and the brain. We reported that aldosterone increased 3H-leucine incorporation in these VSMC; this incorporation is inhibited by a specific aldosterone antagonist (16). These findings have led investigators to propose an autocrine or paracrine role for aldosterone. However, Rocha et al. (17) reported that the coronary vascular inflammatory response induced by aldosterone infusion was absent in adrenalectomized rats. These findings, incidentally, provide compelling evidence against the idea that aldosterone synthesized in the myocardium plays pathophysiological roles, as suggested by Funder (18).

Rapid Aldosterone Action

In contrast to the classical effects of aldosterone on its nuclear receptor, a nongenomic effect of aldosterone, that is, activation of the sodium/hydrogen antiporter occurs within seconds to minutes in VSMC. Inhibitors of transcription or translation do not affect this rapid aldosterone action, demonstrating that the nongenomic effects are mediated by additional signal transduction pathways. Other short-term aldosterone effects include intracellular Ca^{2+} increase, inositol triphosphate turnover, protein kinase C (PKC), and Na^{+} · K^{+} -adenosine triphosphatase activation (19). Table 1 gives an overview. Direct evidence for nongenomic aldosterone action without involvement of the MR is drawn from a study done with cultured skin cells from MR-knockout mice (20). A rapid increase in intracellular Ca^{2+} induced by 10 nmol/l aldosterone was still present in MR−/− mice. Some of the effects are mediated, in part, through the activation of the enzyme 11β-HSD (21), and some effects are mediated by the interaction of aldosterone and other growth factors, such as ATII (22). Further studies will be needed to unravel the mechanisms of these nongenomic effects.

### Aldosterone and Cardiovascular Disease

Mineralocorticoid receptors are present in cardiac myocytes as well as in blood vessels. Peripheral infusion of aldosterone in rats with a high sodium intake causes cardiac hypertrophy and fibrosis independent of effects on blood pressure (23). MR antagonism with spironolactone or eplerenone prevents aortic and myocardial fibrosis in rat models even in the absence of blood pressure effects (24, 25). The mechanism(s) through which aldosterone causes cardiac hypertrophy and fibrosis are the subject of ongoing investigation. ATII has been shown to elicit a hypertrophic response in neonatal rat cardiac myocytes and a mitogenic response in neonatal rat cardiac fibroblasts. The growth-related cellular actions of ATII are mediated by the type 1 ATII receptor (AT1R) subtype and upregulation of AT1R occurs in response to hypertrophic changes (26). Aldosterone increases AT1R density and messenger RNA (mRNA) accumulation in.
necrotic changes. Vascular cytokine activation, inflammatory damage and induced by aldosterone is a secondary event in response to this model. These findings suggest that the cardiac fibrosis blood pressure and attenuates the inflammatory changes in cellular adhesion molecule-1. Eplerenone partially decreases teopontin, macrophage chemoattractant protein-1, and intra-

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Aldosterone and Renal Dysfunction

Clinical studies have demonstrated a relationship between augmented levels of aldosterone and renal deterioration (30). Aldosterone/salt-induced hypertension has been used as a model of renal disease (31). The renal damage that develops in this model is characterized histopathologically by severe glomerular injury with vascular fibrinoid necrosis and thrombotic microangiopathy, leading to renal fibrosis. Several lines of experimental evidence confirm that blockade of aldosterone, independent of renin-angiotensin blockade, reduces proteinuria and nephrosclerosis in the SHRSP (32). Recently, Blasi et al. (33) reported that aldosterone/salt-induced renal injury and fibrosis have inflammatory components involving macrophage infiltration and cytokine up-regulation, which were prevented by the aldosterone blockade.

Clinical studies using aldosterone blockers to attenuate chronic renal injury have also been reported. Chrysostomou and Becker (34) administered spironolactone to 8 proteinuric patients who had been receiving enalapril for at least 1 year. At the end of a 4-week period, an additional 54% reduction in the 24-h protein excretion rate, without any change in creatinine clearance or plasma potassium levels, was observed. More recently, Sato et al. (35) identified 13 patients with type II diabetes mellitus who were treated with an ACE inhibitor but whose aldosterone levels increased after initial suppression (i.e., aldosterone escape). When spironolactone was added to the ACE inhibitor treatment for 24 weeks in these patients, albuminuria and left-ventricular mass both decreased. However, there have been reports that spironolactone induced severe and life-threatening hyperkalemia when used in combination with ACE inhibitors (36). Therefore, patients at risk of hyperkalemia will need to be followed closely when prescribed these medications.

Pharmacology of Eplerenone

Eplerenone exerts the selective aldosterone blockade with specificity for the MR. Its chemical structure differs from that of the non-selective aldosterone antagonist spironolactone in that the 17thioacyl group is replaced with a carboxymethoxy group. For example, in rats the IC50 of spiron-
onone for the aldosterone receptor was 360 nmol/l, whereas the IC50 values for the androgen, progesterone, and estrogen receptors were >10,000 nmol/l (37). Eplerenone is cleared primarily via metabolism by CYP4503A4 to inactive metabolites, with an elimination half-life of 4 to 6 h. Eplerenone is 50% to 75% as potent as spironolactone in human studies (37).

Clinical Efficacy

Hypertension

Clinical studies support the concept that eplerenone is effec-
tive for the treatment of hypertension without exhibiting anti-androgenic adverse effects. In patients with mild-to-moderate hypertension, eplerenone produced dose-dependent reduction in systolic and diastolic blood pressure (SBP/DBP) compared with placebo (38). The efficacy of eplerenone 50–200 mg/day was similar to that of enalapril 10–40 mg/day (39) or as amlopidine 2.5–10 mg/day (40). Several studies have also demonstrated that eplerenone provides end-organ protection. In patients with hypertension and left-ventricular hypertrophy, eplerenone 100 mg/day produced reductions in left-ventricular mass similar to that produced by enalapril 40 mg/day. The concomitant use of both agents produced an additional reduction in left-ventricular mass (41). In patients with hypertension, eplerenone 50–200 mg reduces proteinuria as measured by the urinary albumin/creatinine ratio (UACR). In one study, eplerenone decreased UACR by 21.6% in patients with essential hypertension compared with a decrease of 18.2% for those receiving losartan 50–100 mg/day or an increase of 5.2% for those receiving placebo (42). In all studies, the incidence of adverse effects with eplerenone was similar to that of placebo, with no reports of gynecomastia. This drug is also effective for low renin hypertension and possesses similar efficacy in white, hispanic and black people (43). Levy et al. (44) recently reported that 80% of hypertensive patients achieved the target blood pressure levels (responders) by eplerenone, and there was no difference in plasma potassium levels between responders and nonresponders who showed no reduction of blood pressure by treatment with eplerenone. The response in terms of blood pressure does not correlate with changes in serum potassium, which may mean that eplerenone lowers blood pressure by acting largely at sites other than epithelial sites of electrolyte transport.

**Congestive Heart Failure**

The Eplerenone Neurohormonal Efficacy and Survival Trial (EPHESUS) was designed to evaluate the effect of the addition of eplerenone (25–50 mg/day) to standard therapy with ACE inhibitors, AT1R antagonists, β-blockers, digoxin, and diuretics on the primary end points of all-cause mortality and the time to first occurrence of either cardiovascular mortality or morbidity leading to hospitalization in 6,632 patients with LV dysfunction (ejection fraction <40%) after a recent (3 to 14 days) myocardial infarction (45). The recently published results indicate that addition of eplerenone significantly reduced the relative risks for death (a reduction of 15%), cardiovascular death (17%), and hospitalization for cardiovascular events (13%) compared with placebo-treated patients. The decreased risk of death was due in large part to a 21% decreased risk of sudden death from cardiovascular causes. The incidences of gynecomastia and impotence in men and breast pain in women were identical between the eplerenone group and placebo group.

**Diabetic Nephropathy with Hypertension**

Recent clinical studies have indicated that aldosterone blockade may confer an antiproteinuric effect in diabetic patients. Epstein et al. (46) studied the effects of eplerenone-induced selective aldosterone blockade on protein excretion in patients with type 2 diabetes mellitus with hypertension. By week 8, eplerenone significantly reduced proteinuria, and by week 24 the incidence of proteinuria was reduced by to 62% in the eplerenone monotherapy group, compared with 45% in the enalapril group and 74% in the combination therapy group. Reductions in SBP and DBP were similar among the groups. This suggests that the antiproteinuric effect of eplerenone was independent of blood-pressure lowering.

**Effects of Eplerenone on the Circulating Renin-Angiotensin-Aldosterone System**

Dose-related increases in active plasma renin and aldosterone were seen 12 to 24 h after eplerenone administration. The changes in PRA and aldosterone during the 400 mg/day dosing were equivalent to those observed by treatment with spironolactone 50 mg/day; the aldosterone response to 200 mg/day eplerenone was significantly greater (38). Addition of 50 to 100 mg daily eplerenone to treatment with an ACE inhibitor or AT1R antagonist increased active plasma renin by 92.5% and 95.9%, respectively, and serum aldosterone by 70.3% and 60.4%, respectively (47). In an experimental rabbit model of hypercholesterolemia, eplerenone induced approximately 66% decrease in the amount of mRNA of mineralocorticoid receptor compared with the controls (48).

**Experimental Studies**

**Anti-Inflammatory Effect**

Aldosterone produces a vascular and perivascular inflammatory response in the context of a high salt intake, followed by perivascular and interstitial fibrosis. It is now clear that this inflammatory response is a direct humoral effect, rather than secondary to hemodynamic changes. Rocha et al. (17) demonstrated that eplerenone could prevent ATII/salt-induced vascular inflammation in the rat heart. The protective effect of eplerenone was associated with a down-regulation of the inflammatory mediators cyclooxygenase 2 and osteopontin. Young and Funder (49) reported that eplerenone could reverse established cardiac inflammation and fibrosis induced by mineralocorticoid excess and high salt. In the kidney of the aldosterone/salt-induced hypertensive rats, eplerenone also improved inflammatory components involving macrophage infiltration and cytokine up-regulation (53).
Table 2. Pleiotropic Effects of Eplerenone

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| Diabetic nephropathy with hyperten-
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| fects                           |
| 2. Experimental studies           |
| Anti-inflammatory effect          |
| Improvement of vascular endothelial dysfunction |
| Protection of the kidney         |
| Others: improvement of aortic stiff-
| ness; attenuation of constrictive remodeling after coronary artery angioplasty; decreased ischemic and hemorrhagic changes in the brain |

### Improvement of Vascular Endothelial Dysfunction

Nitric oxide, a known vasodilatory and platelet anti-aggregatory agent, is considered cardioprotective under various circumstances. Aldosterone inhibits nitric oxide formation and inducible nitric oxide synthase mRNA in cultured VSMCs stimulated by the cytokine interleukin-1β (50). Eplerenone improved endothelial dysfunction in early atherosclerosis induced by a high cholesterol diet in New Zealand rabbits (48). This result was associated with a reduction in the production of oxygen radicals and a reduced activity of NADH and NADPH oxidase in aortic segments of the eplerenone-treated group.

### Protection of the Kidney

Rocha et al. (31) have reported that administration of eplerenone to saline-drinking SHRSP markedly attenuated progressive arteriopathy and associated proteinuria compared with vehicle-treated animals without appreciably influencing blood pressure. They also reported that treatment with eplerenone reduced proteinuria and attenuated elevation of the expression of the cytokines osteopontin, monocyte chemoattractant protein, interleukin-1β and interleukin 6 in the kidney of aldosterone/salt-treated rats (17). Thus the attenuation of aldosterone-mediated vascular inflammation likely represents one of the mechanisms by which eplerenone confers renal protection.

### Other Beneficial Effects on the Vasculature

Increased stiffness of large arteries is the major factor of increasing SBP and pulse pressure in subjects with hypertension. It also has been shown to be a significant and independent marker of cardiovascular risk. Lacolley et al. (51) have demonstrated that eplerenone blunted the increase of arterial stiffness associated with fibronectin accumulation by aldosterone infusion in rats. Ward et al. (52) reported that eplerenone attenuated constrictive remodeling after coronary artery angioplasty by mechanisms involving reduction in collagen accumulation, which thus appears to be an important contributor to constrictive remodeling of angioplastied coronary arteries. Rocha et al. (32) examined the role of aldosterone in the development of cerebral lesions of SHRSP. Treatment with eplerenone markedly reduced severe cerebral vascular and parenchymal lesions characteristic of ischemic and hemorrhagic stroke in SHRSP independent of blood-pressure lowering. Table 2 summarizes the pleiotropic effects of eplerenone.

### Side Effects

#### Hyperkalemia

Treatment with eplerenone causes a dose-dependent increase in the mean change in serum potassium levels from baseline from 0.08 mmol/l at the dose 100 mg/day to 0.36 mmol/l at the 400 mg/day dose (53). The median increase in serum potassium observed in the RALES trial, in which spironolactone was given to patients receiving ACE inhibitors as well as loop diuretics, was 0.3 mmol/l (2). The frequency and severity of hyperkalemia during eplerenone administration are expected to be increased in patients with renal insufficiency, diabetes, and microalbuminuria. Rates of hyperkalemia, defined as a serum potassium level ≥ 5.5 mmol/l, as a function of calculated creatinine clearance have been analyzed and were 2.6%, 5.6%, and 10.4% in patients with baseline creatinine clearances > 100 ml/min, 70 to 100 ml/min, and < 70 ml/min, respectively (37). In a study of patients with type 2 diabetes and microalbuminuria, the frequency of hyperkalemia was 33% in patients receiving eplerenone 200 mg/day and 38% in patients receiving eplerenone and the ACE inhibitor enalapril (37). However, hypokalemia contributes to the pathogenesis of cardiovascular disease, and many cardiovascular disorders and drugs aggravate hypokalemia. It is important to note that those patients on a potassium-sparing diuretic, such as spironolactone or eplerenone, had a reduction in mortality both from progressive heart failure and sudden cardiac death in comparison to those on a regular diuretic (54).

#### Sex-Hormone-Related Side Effects

Compared to spironolactone, eplerenone has little affinity for other steroid hormone receptors. The rates of gynecomastia, mastodynia, or either in men were 0.7%, 1.3%, and 1.6%. By comparison, the rate of gynecomastia in the RALES trial, in which the mean time of follow-up was 24 months, was 10%, and the rate of gynecomastia in men with essential hypertension treated with spironolactone has been reported as 6.9%. In trials of eplerenone, the rate of abnormal vaginal bleeding in females was 0.8% and similar to that seen in subjects given active treatment other than spironolactone.
Laboratory Adverse Events

Mild dose-dependent increases in cholesterol and mild elevated serum transaminases (0.66% of patients) were reported. Ketoconazole induced a 5-fold increase in eplerenone area-under-the concentration (AUC), while less potent inhibitors of CYP3A4 (such as verapamil, erythromycin, fluconazole, and saquinavir) increased the eplerenone AUC approximately 2-fold (37).

Conclusion

Aldosterone is now well recognized to induce a variety of actions that lead to progressive target organ damage in the heart, vasculature, and kidneys. Eplerenone is a new selective mineralocorticoid receptor antagonist with decreased progential and antiandrogenic side effects compared with spironolactone. Eplerenone effectively decreases mortality and morbidity in patient with left-ventricular dysfunction and heart failure after an acute myocardial infarction. Eplerenone also effectively reduces blood pressure compared with agents such as enalapril, losartan, and amlodipine. Although eplerenone improves microalbuminuria in patients with diabetic nephropathy, the possibility of hyperkalemia as a side effect must be considered, particularly in patients with renal insufficiency. The potential importance of pleiotropic effects of this agent should be clarified in clinical studies.

References

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