

*Critical Review***Pathophysiological Roles of Aldosterone and Mineralocorticoid Receptor in the Kidney**Kazi Rafiq¹, Hirofumi Hitomi¹, Daisuke Nakano¹, and Akira Nishiyama^{1,*}¹Department of Pharmacology, Kagawa University Medical School,
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Abstract. Aldosterone, a steroid hormone, has traditionally been viewed as a key regulator of fluid and electrolyte homeostasis, as well as blood pressure, through the activation of mineralocorticoid receptor (MR). However, a number of studies performed in the last decade have revealed an important role of aldosterone/MR in the pathogenesis of renal injury. Aldosterone/MR-induced renal tissue injury is associated with increased renal inflammation and oxidative stress, fibrosis, mesangial cell proliferation, and podocyte injury, probably through genomic and non-genomic pathways. However, our preliminary data have indicated that acute administration of aldosterone or a selective MR antagonist, eplerenone, does not change blood pressure, heart rate, or renal blood flow. These data suggest that aldosterone/MR induces renal injury through mechanisms that are independent of acute changes in systemic and renal hemodynamics. In this review, we will briefly summarize the roles of aldosterone/MR in the pathogenesis of renal injury, focusing on the underlying mechanisms that are independent of systemic and renal hemodynamic changes.

Keywords: aldosterone, mineralocorticoid receptor (MR), renal injury, eplerenone, renal blood flow

1. Introduction

Aldosterone, a circulating mineralocorticoid hormone, plays a central role in controlling epithelial sodium channel activity in the distal nephron in the kidney, thereby regulating sodium balance, body fluid, and blood pressure (BP) (1, 2). Aldosterone is predominantly produced in the adrenal cortex, which is stimulated by corticotrophic releasing hormone, adrenocorticotrophic hormone, potassium, and angiotensin II. However, several studies have also revealed extra-adrenal production of aldosterone. The enzyme aldosterone synthase, CYP11B2, has been identified in the heart, blood vessels, and brain (3–5). Recently, Nishikawa et al. (6) reported that human glomerular mesangial cells produced aldosterone via steroidogenic enzymes such as p450_{scc}, 3 β -hydroxysteroid dehydrogenase, 21-hydroxylase, and CYP11B2, suggesting

the local production of aldosterone in the kidney. Aldosterone is classically known to bind to the cytosolic mineralocorticoid receptor (MR) in epithelial cells and aldosterone-bound MR translocates to the nucleus to promote protein synthesis. It has also been suggested that aldosterone, via rapid non-genomic pathways, regulates vasoconstriction (7) and c-Src activation in vascular smooth muscle cells (8). Although intrinsic glucocorticoids (cortisol in human and corticosterone in rodents) also show high affinity to the MR, aldosterone specially induces physiological responses through the MR by 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2)-dependent regulation. 11 β -HSD2 transforms glucocorticoids into inactive metabolites (cortisone in human and 11-dehydrocorticosterone in rodents) and thereby inhibits glucocorticoids from binding to the MR (9, 10).

Beyond its physiological role in renal sodium reabsorption, aldosterone elicits renal tissue injury, independently of BP changes. In rats, chronic aldosterone/salt treatment induces proteinuria, glomerular mesangial injury, and tubulointerstitial fibrosis (9, 11). These aldosterone-induced renal injuries were prevented by MR antagonists, suggesting the involvement of locally expressed

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MR. The potential contribution of aldosterone/MR to the progression of renal injury is also supported by the substantial expression of the MR in renal mesangial cells (12), renal fibroblasts (13), and podocytes (14, 15). Clinical studies have shown that the incidence of proteinuria or albuminuria is higher among patients with primary aldosteronism than among patients with essential hypertension (16–18). Among patients with primary aldosteronism (19), chronic kidney disease (CKD) (20), or diabetic nephropathy (21), plasma aldosterone levels are positively correlated with urinary protein excretion levels and negatively correlated with glomerular filtration rate. Furthermore, the addition of MR antagonists to either angiotensin-converting enzyme inhibitors or angiotensin II-receptor blockers reduces albuminuria in patients with type 2 diabetic nephropathy (22) or CKD (20). Aldosterone has been reported to be involved in renal injuries, including renal inflammation, oxidative stress, fibrosis, mesangial cell proliferation, and podocyte injury in various animal models (15, 23–26). In this review, we will briefly summarize the role of aldosterone/MR in the pathogenesis of renal injury, focusing on the underlying mechanisms that are independent of systemic and renal hemodynamic changes.

2. Aldosterone/MR and inflammation in the kidney

Accumulating evidence has suggested that renal inflammation plays a pivotal role in the progression of aldosterone/MR-induced glomerulosclerosis and tubulointerstitial fibrosis (27–29). Aldosterone/MR induces phosphorylation of serum and glucocorticoid regulated kinase 1 (SGK1), which in turn phosphorylates and inactivates the nuclear factor of κ light polypeptide B, resulting in the activation of nuclear factor κ B (NF κ B) (27, 30). The activated NF κ B then enhances the transcription of inflammatory cytokine genes such as intercellular adhesion molecule 1 and connective tissue growth factor (CTGF) (30). In addition, increased expression of the proinflammatory cytokines osteopontin, monocyte chemoattractant protein-1, interleukin-1b, and interleukin-6 was observed in the kidneys of aldosterone-infused rats, and this was markedly attenuated by eplerenone, a selective MR blocker (27).

3. Aldosterone/MR and reactive oxygen species (ROS) in the kidney

Several studies have shown that ROS are important mediators of aldosterone/MR-induced renal injury (15, 25). Chronic infusion of aldosterone increased renal ROS formation via NADPH oxidase activation (25, 31, 32). Nishiyama et al. (25) showed that aldosterone/salt-in-

duced renal injury was associated with increased thiobarbituric acid-reactive substance (TBARS) content, a marker of ROS production, and mRNA levels of NADPH oxidase components, p22phox, Nox-4, and gp91phox, in renal cortical tissues. Treatment with eplerenone blocked the aldosterone-induced increases in TBARS levels and NADPH oxidase subunit expression in the kidney. Interestingly, tempol, a superoxide dismutase mimetic, normalized renal ROS expression and prevented the progression of proteinuria and renal tissue injury in these animals (11, 25). These data suggest that ROS are essential factors in mediating aldosterone/MR-induced renal injury.

In rat mesangial cells (RMCs), aldosterone directly stimulates superoxide anion generation, which is accompanied by an increase in NADPH oxidase activity and translocation of p47phox and p67phox, cytosol components of NADPH oxidase, to the cell membrane (33). These *in vitro* findings are consistent with the hypothesis derived from animal studies (11, 34, 35) that aldosterone stimulates ROS generation through NADPH oxidase-dependent mechanisms. Recent studies have shown that aldosterone induces mesangial cell apoptosis and that the administration of an antioxidant or a MR antagonist attenuates the proapoptotic effects of aldosterone (36). In addition, Huang et al. (32) showed that aldosterone dose-dependently increased ROS formation in mesangial cells, which was blocked by an MR antagonist, an inhibitor of complex I of the mitochondrial respiratory chain, or an inhibitor of NADPH oxidase. These findings suggest that mitochondria and NADPH oxidase are the major sources of aldosterone-dependent renal ROS production.

4. Aldosterone/MR and renal fibrosis

In vitro studies have shown that aldosterone stimulates collagen synthesis via MR-mediated extracellular signal-regulated kinases (ERK)1/2 activation in renal fibroblasts (13). Chronic treatment with aldosterone/salt resulted in severe tubulointerstitial fibrosis with increases in renal collagen content and ERK1/2 activity in rats. Furthermore, these effects of aldosterone were prevented by concurrent treatment with eplerenone (25). These data are consistent with those obtained *in vitro* (13) that aldosterone/MR contributes to the pathogenesis of tubulointerstitial fibrosis through ERK1/2-dependent collagen accumulation.

Rho-kinase is an important molecule that mediates various cellular functions such as contraction, adhesion, proliferation, motility or migration, cellular morphology, growth control, and cytokinesis (37, 38). Several studies have demonstrated the potential involvement of Rho-kinase in the pathogenesis of renal injury (39, 40). In RMCs, aldosterone induces myofibroblastic transdiffer-

entiation and collagen gene expression via Rho-kinase dependent pathways (41). In uninephrectomized rats treated with aldosterone/salt, severe tubulointerstitial fibrosis, and inflammation are associated with increases in renal expression of transforming growth factor- β (TGF- β) and CTGF, as well as increased activities of smad2/3 and Rho-kinase (42). Furthermore, the administration of a Rho-kinase inhibitor, fasudil, attenuated aldosterone-induced tubulointerstitial fibrosis, inflammation, the expression of TGF- β and CTGF, and the activity of smad2/3 without affecting BP. These data support a role of the Rho-kinase-dependent signaling pathway in aldosterone-induced kidney injury.

5. Aldosterone/MR and glomerular mesangial injury

Severe glomerular proliferation was found to be associated with the activation of renal tissue ERK1/2 in rats chronically treated with aldosterone and salt (25). Furthermore, aldosterone-induced mesangial injury and ERK1/2 activation were prevented by eplerenone, suggesting that the glomerular mesangium is a potential target for injuries induced by aldosterone via activation of locally expressed MR. Indeed, MR is abundant in the cytoplasm of cultured RMCs (12). In RMCs, application of aldosterone caused cell proliferation and deformability, which were prevented by pretreatment with eplerenone or an ERK inhibitor (12). These results indicate that aldosterone directly induces RMC proliferation and deformability through MR- and ERK1/2-dependent pathways. In mesangial cells, aldosterone increased ROS production, thus activating the epithelial growth factor receptor (EGFR), which activates the Ras/MAPK and PI3K/Akt pathways and cell proliferation (32). Aldosterone induced EGFR phosphorylation in a dose-dependent manner, and these effects of aldosterone were blocked by eplerenone (32).

During the development of CKD, the epithelial-mesenchymal transition (EMT) plays an essential role in extracellular matrix expansion and myofibroblastic changes in the glomerular mesangium (43). We recently showed that aldosterone induces hypertrophy and increases α -smooth muscle actin expression in RMCs (41), both of which are involved in the progression of EMT (44). These data are consistent with the hypothesis that aldosterone induces mesangial cell EMT, leading to glomerular matrix expansion and sclerosis.

6. Podocyte injury and aldosterone

Glomerular podocytes (glomerular visceral epithelial cells) have interdigitating foot processes that are connected to each other by slit diaphragms composed of

nephrin, podocin, and other molecules (45). Podocytes thus serve as the final filtration barrier to prevent leakage of plasma proteins (45 – 47). Several studies have shown that podocytes are a target of aldosterone and the MR (11, 15, 48). Shibata et al. (15) showed that chronic infusion of aldosterone induced hypertension with massive proteinuria and glomerular podocyte injury in uninephrectomized rats. They also showed that podocyte injury was associated with reduced glomerular expression of nephrin and podocin in uninephrectomized aldosterone-infused rats (49). Treatment with tempol or eplerenone significantly reduced intrarenal ROS and attenuated podocyte injury and proteinuria in aldosterone-infused rats (15). Moreover, treatment with tempol or eplerenone markedly attenuated podocyte injury and proteinuria in other rodent models of hypertensive glomerulosclerosis (14, 48, 50). We also showed that eplerenone attenuated podocyte injury and proteinuria in type 2 diabetic rats (51). Preliminary experiments have shown that aldosterone significantly protracts wound healing in cultured mouse podocytes (52), although the precise molecular mechanisms are not yet clear.

7. Effects of aldosterone/MR on renal hemodynamic parameters in vivo

As described above, in addition to the MR-mediated genomic effects of aldosterone, non-genomic effects of aldosterone have also been described (7, 8, 53, 54). Although it has been suggested that the non-genomic effects of aldosterone are mediated via MR (12, 55), MR-independent mechanisms have also been reported to mediate the non-genomic effects of aldosterone (53, 56). It has also been reported that aldosterone induces rapid effects within minutes at its target organs, including the kidney, heart, and vasculature (57). Arima et al. (56) performed studies in isolated afferent and efferent arterioles and showed that aldosterone caused vasoconstriction of afferent and efferent arterioles through MR-independent mechanisms. They also showed that endothelium-derived nitric oxide (NO) modulates the vasoconstrictor response to aldosterone in afferent arterioles (58). In contrast, Uhrenholt et al. (55) performed similar experiments and reported that aldosterone rapidly caused afferent arteriolar vasodilation. Furthermore, aldosterone-induced vasodilation of afferent arterioles was blocked by an MR antagonist, suggesting the involvement of MR in the rapid aldosterone-dependent vasodilation of glomerular afferent arterioles. Clinical studies have shown that intravenous injection of aldosterone increases systemic vascular resistance within 5 min in healthy volunteers without changes in systolic BP (SBP) or heart rate (HR) (59, 60). In addition, non-genomic cardiovascular effects of aldos-

terone on the adrenergic system have also been reported (61). In contrast, Gunaruwan et al. (62) reported that acute aldosterone infusion caused no detectable changes in forearm vascular tone. Thus, the findings from studies of the rapid and non-genomic effects of aldosterone on systemic and renal hemodynamics are still contradictory.

Therefore, we examined the acute effects of aldosterone/MR on systemic and renal hemodynamic parameters in rats. We intravenously infused aldosterone at doses of 0.1, 1, 10, and 100 $\mu\text{g}/\text{kg}$ for 1 min through the femoral vein in anesthetized Dahl salt-sensitive rats, Dahl salt-resistant rats, and Sprague-Dawley (SD) rats treated with nitro-L-arginine methyl ester (L-NAME). Rats were anesthetized with Inactin (100 mg/kg, i.p.) and body fluid volume was maintained by intravenous infusion of isotonic saline at a dose rate of 2 mL/h. SBP and HR were monitored through a femoral artery. A Doppler flow probe (HDP 10.20R; Crystal Biotech, Northborough, MA, USA) was placed around the renal artery and renal blood flow (RBF) was continuously monitored, as previously described (63). After completing the surgical procedures, the rats were left alone for 60 min to stabilize SBP, HR, and RBF. After intravenous infusion of aldosterone, SBP, HR, and RBF were monitored for 10 min at each dose ($n = 6$ for each). As shown in Fig. 1, intravenous infusion of aldosterone did not affect SBP, HR, or RBF, even at high doses in Dahl salt-sensitive rats fed a high-salt or normal-salt diet. Similar results were obtained in Dahl salt-resistant rats fed a high-salt diet. An earlier clinical study showed that aldosterone did not cause rapid renal vasoconstriction in humans, but during

infusion of an NO synthase inhibitor, N^G -monomethyl-L-arginine, aldosterone did act as a potent renal vasoconstrictor (64). Therefore, studies were also performed in L-NAME (50 mg/L in drinking water for 1 week)-pretreated SD rats. However, we found no significant changes in SBP, HR, or RBF in response to intravenous aldosterone infusion (Fig. 1).

Next, we examined the rapid effects of MR blockade in hypertensive rats. Experiments were performed in anesthetized spontaneously hypertensive rats (SHR) and in Dahl salt-sensitive rats fed a high-salt diet ($n = 6$ rats per group). All surgical and experimental procedures were similar to those described above. SBP, HR, and RBF were measured 3 min before and after the intravenous infusion of vehicle (Na_2Ca_3 and DMSO mixture) and eplerenone. In these experiments, intravenous infusion of eplerenone did not elicit any rapid changes in SBP, HR, or RBF, even at high doses (Fig. 2). These data suggest that the rapid actions of aldosterone/MR do not play a role in the regulation of systemic and renal hemodynamic parameters in rats.

8. Conclusions

Here, we have discussed the role of aldosterone/MR as a mediator of renal injury. In some pathophysiological conditions, aldosterone/MR exerts direct deleterious effects on the kidney. As shown in Fig. 3, aldosterone/MR directly contributes to the progression of tubulointerstitial fibroblasts, glomerular mesangial cells, and podocyte injury. However, our preliminary data indicate that the rapid actions of aldosterone/MR may not be involved in

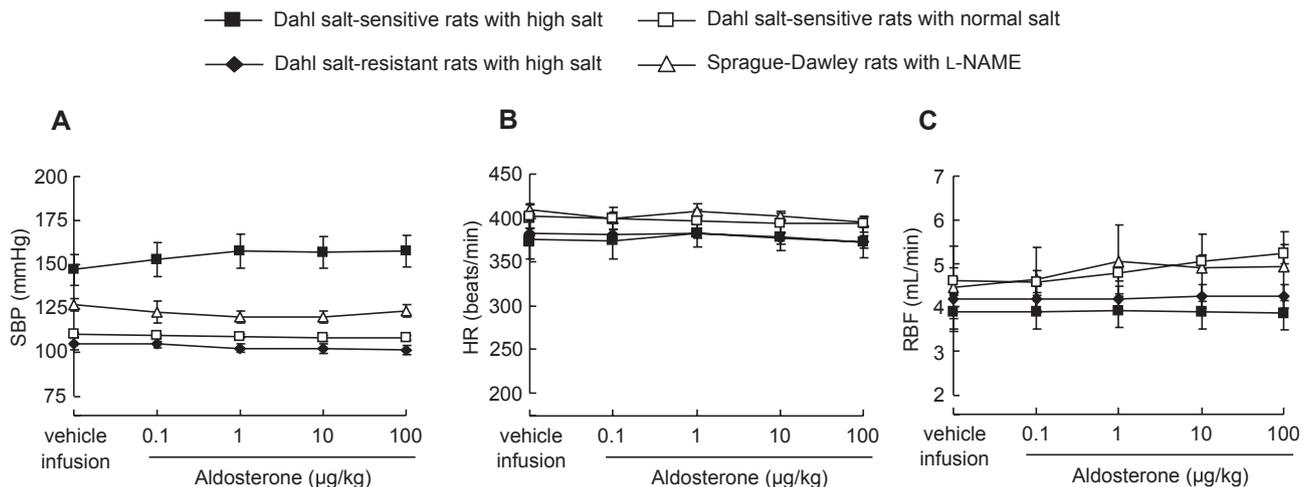


Fig. 1. Effects of intravenous infusion of aldosterone on systolic blood pressure (SBP) (A), heart rate (HR) (B), and renal blood flow (RBF) (C) in anesthetized rats. Intravenous infusion of aldosterone does not change SBP, HR, or RBF in Dahl salt-sensitive rats fed a high-salt or normal-salt diet, Dahl salt-resistant rats fed a high-salt diet, or nitro-L-arginine methyl ester (L-NAME)-treated Sprague-Dawley rats. All data are expressed as means \pm S.E.M. ($n = 6$ per group).

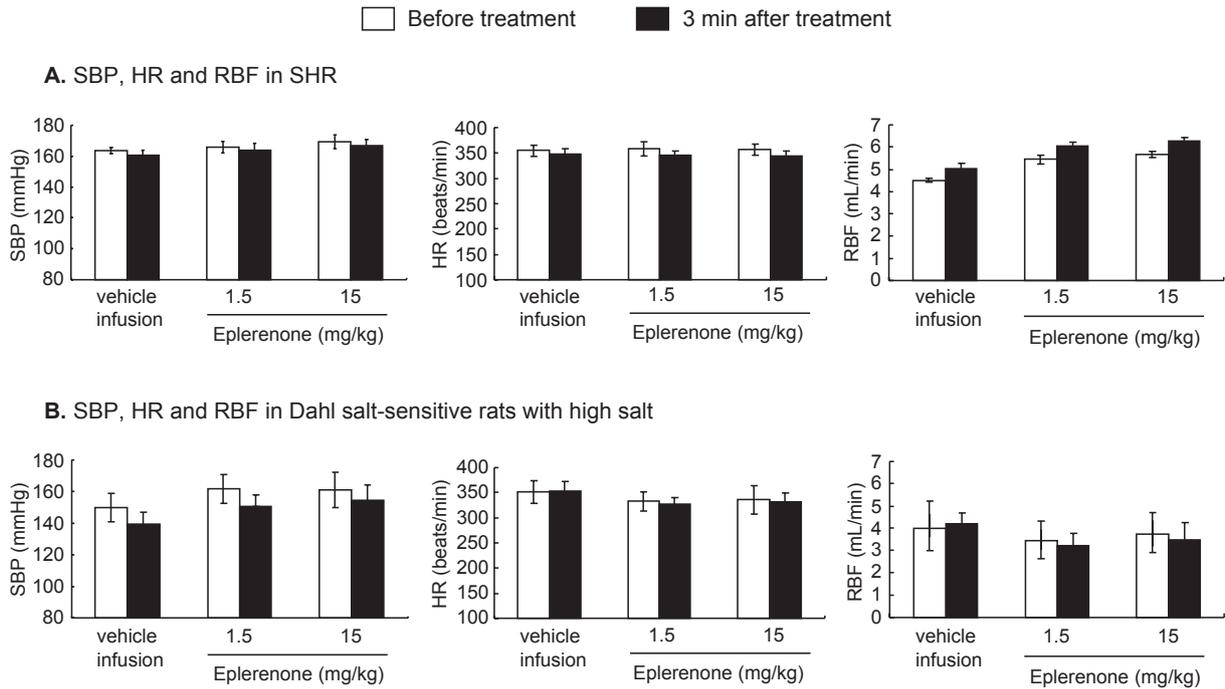


Fig. 2. Effects of intravenous infusion of eplerenone on SBP, HR, and RBF in anesthetized spontaneous hypertensive rats (SHR) (A) and Dahl salt-sensitive hypertensive rats fed a high-salt diet (B). Intravenous infusion of eplerenone does not change SBP, HR, or RBF. All data are expressed as means \pm S.E.M. (n = 6 per group).

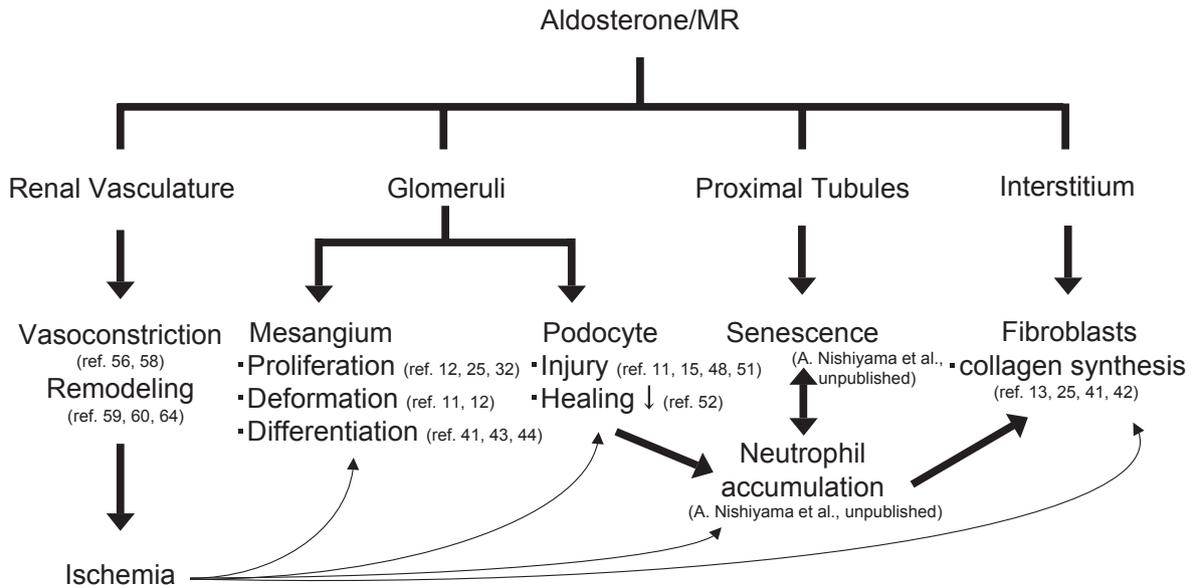


Fig. 3. Possible mechanisms involved in renal injury induced by aldosterone/MR.

the regulation of systemic or renal hemodynamic parameters. Clearly, further studies are needed to determine the precise mechanisms underlying aldosterone/MR-mediated renal injury.

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