Effects of Aldosterone on the Vasculature

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Aldosterone is a steroid with mineralocorticoid activity produced mostly by the adrenal glomerulosa. Aldosterone may also be generated in the heart and blood vessels, although there is controversy on whether the amounts produced are physiologically relevant. The classical target of aldosterone is the distal convoluted tubule of the kidney, where it acts on cytosolic mineralocorticoid receptors (MRs) that translocate to the nucleus and via different mechanisms (serum and glucocorticoid-induced kinase-1 [SGK-1], neural precursor cell expressed developmentally downregulated 4 [nedd4], nedd4 isoform 2 [nedd4-2], K-Ras2A, and capsacin) modulating the epithelial sodium channel and renal outer medullary potassium channels to induce increased reabsorption of sodium and excretion of potassium, thus regulating sodium, potassium, and body fluid balance. Over the past few years it has become increasingly evident that aldosterone exerts powerful effects on blood vessels, independent of actions that can be attributed to blood pressure (BP) rise mediated via regulation of salt and water balance. Some deleterious consequences of aldosterone and, accordingly, some benefits derived from MR antagonism, may be BP dependent. However, the aldosterone effects on which this review will concentrate are the direct, BP-independent ones, although these may, indeed, contribute together with salt and water retention to BP elevation. The reader should be cautioned, however, that many of the vascular actions of aldosterone mentioned in this article were obtained with large unphysiological doses of aldosterone, which may raise questions regarding their physiological significance.

Aldosterone has been reported to be synthesized, MRs demonstrated, and the presence of the cortisol-inactivating enzyme 11β-hydroxysteroid-dehydrogenase-2 identified in blood vessels. However, the production of aldosterone by blood vessels and the heart remains controversial (see below). In addition to its classical genomic mechanisms, aldosterone exerts effects through rapid nongenomic pathways that may also be important in hypertension. Some studies suggest that aldosterone influences vascular contraction, as discussed below. Furthermore, aldosterone modulates membrane receptors and signaling molecules and influences the actions of a variety of agents to sensitize the vasculature to effects of various agents that induce vasoconstriction or result in direct effects on growth and remodeling. Vascular actions of aldosterone may be exerted on different layers of the blood vessel wall: on endothelium, smooth muscle cells of the media, or on the adventitial layer, as discussed below. The first 2 will be dealt with in succession, because there is little new knowledge on the actions of aldosterone on adventitia, which could, nonetheless, be important.

Endothelium

Aldosterone may affect endothelium-dependent dilatory or constrictor mechanisms, either directly or indirectly via angiotensin II (Ang II)–induced effects. Ang II induces endothelial dysfunction as a result of increased oxidative stress, which may scavenge NO resulting in decreased NO bioavailability. MR blockade with spironolactone improved the impaired acetylcholine-induced relaxation in Ang II–infused rats, suggesting that aldosterone induces actions attributed to direct effects of Ang II. Aldosterone infusion into rats impaired endothelium-dependent relaxation in association with increased oxidative stress in the vascular wall. This effect was reversed by the MR blocker spironolactone, as well as by endothelin receptor antagonism. Previous data demonstrated that mineralocorticoid infusion was associated with enhanced endothelin expression in the endothelium of large and small arteries. Spironolactone, which had beneficial effects when added to the therapy of heart failure patients in the Randomized ALdactone Evaluation (RALES) Study, improved endothelial function, suggesting a role for aldosterone as part of the activated renin–angiotensin–aldosterone system in the endothelial dysfunction of heart failure. These results may partially explain the beneficial effects of mineralocorticoid antagonist in chronic heart failure in the RALES study, as well as in the recent Eplerenone Post-acute myocardial infarction Heart failure Efficacy and SUrvival Study (EPHESUS) with the more selective MR blocker eplerenone in postmyocardial infarction subjects.

Chronic treatment with aldosterone resulted in impaired acetylcholine-induced relaxations of aorta in both Wistar-Kyoto and spontaneously hypertensive rats (SHRs) and increased aortic cyclooxygenase (COX)–2 protein expression associated with enhanced acetylcholine-induced aortic production of 13,14-dihydro-15-keto prostaglandin (PG)F2α, PGE2, and 6-keto-PGF1α, whereas in SHRs, acetylcholine only stimulated generation of 6-keto-PGF1α. Aldosterone may, thus, produce endothelial dysfunction through COX-2 activation in normotensive and hypertensive rats. PGI2 may be involved in...
endothelial dysfunction in SHR, whereas other COX-derived agents may play a role in endothelial dysfunction in normotensive rats.

Aldosterone causes nongenomic vasoconstriction by activating phospholipase C in pregglomerular afferent arterioles. The endothelium may modulate this vasoconstrictor effect by NO release. Aldosterone induced a dose-dependent vasoconstriction in microperfused rabbit afferent arterioles, which was enhanced by disrupting the endothelium. Inhibition of NO synthase induced a similar effect, which was inhibited by chelerythrine, a protein kinase (PKC) inhibitor. Thapsigargin, or dantrolene, which blocks inositol 1,4,5-triphosphate (inositol triphosphate)–induced intracellular calcium release, attenuated the effect of aldosterone. Thus, these data show that endothelium-derived NO modulates the vasoconstrictor actions of aldosterone in pregglomerular afferent arterioles that are mediated by the activation of inositol triphosphate and PKC pathways.

Using atomic force microscopy, Oberleithner et al demonstrated that aldosterone induced increases of the cell nucleus of endothelial cells that could reach 15% to 28% of total cell volume in <10 minutes, effects which disappeared within 30 minutes. They postulated that aldosterone-induced nuclear swelling was a rapid genomic effect, because receptors translocated from the cytoplasm into the nucleus, and gene transcription followed, with a return of nuclear volume to normal when mRNA was exported into the cytoplasm. These authors concluded that endothelial responses to aldosterone could not be divided into acute nongenomic (<10 minutes) and sustained genomic (>10 minutes) effects, because rapid genomic effects also occurred. They also demonstrated that cultured human umbilical vein endothelial cells responded to aldosterone with sodium and water entry and swelling that was blocked by spironolactone. Swollen aldosterone-treated cultured human umbilical vein endothelial cells or dantrolene, which blocks inositol 1,4,5-triphosphate (inositol triphosphate)–induced intracellular calcium release, attenuated the effect of aldosterone. Therefore, these data show that endothelium-derived NO modulates the vasoconstrictor actions of aldosterone in pregglomerular afferent arterioles that are mediated by the activation of inositol triphosphate and PKC pathways.

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Another recent interesting vascular effect demonstrated on rings of rat aorta was a paradoxically favorable effect of aldosterone that attenuated α-adrenergic vasoconstriction through effects on the endothelium mediated by increased endothelial NO synthase activation stimulated via phosphati-
clearly established.11,35–37 This may occur more frequently in the presence of high salt, which sensitizes the cardiovascular system to the nefarious cardiovascular inflammatory effects of aldosterone. Inflammatory responses have increasingly been associated with mechanisms involved in the pathophysiology of cardiovascular disease.38 The mineralocorticoid-induced vascular and cardiac inflammatory response includes the upregulation of mediators such as nuclear factor κB and activated protein 1, adhesion molecules such as vascular cell adhesion molecule 1 and intercellular adhesion molecule 1, and endothelin 1.39

Several years ago we demonstrated that mineralocorticoids, such as deoxycorticosterone in vivo,40 and later aldosterone in vivo and in vitro41 upregulated angiotensin receptors in VSMCs (Figure 1). Other investigators extended these findings, demonstrating that signaling of Ang II was amplified by exposure of VSMCs to aldosterone.42–44 More recently, it has been demonstrated that aldosterone upregulates components of the renin–angiotensin system, specifically angiotensin-converting enzyme (ACE), resulting in increased local generation of Ang II (Figure 1).45 Furthermore, aldosterone exerts direct effects on signaling in VSMCs, upregulating mitogen-activated protein (MAP) kinases,46–48 local generation of Ang II (Figure 1).45 Furthermore, aldosterone exerts direct effects on signaling in VSMCs, upregulating mitogen-activated protein (MAP) kinases,46–48 and vascular remodeling, and their improvement under eplerenone, particularly under a high-salt diet, underlines the severity of hypertension, endothelial dysfunction, and cardiac and vascular remodeling, and their improvement under eplerenone, particularly under a high-salt diet, underlines the pathophysiological involvement of aldosterone in salt-sensitive hypertension.

Increasing evidence indicates that aldosterone elicits vascular effects through nongenomic signaling pathways.50–52 Phosphorylation of c-Src and p38MAP kinase phosphorylation, NADPH oxidase activation, and protein synthesis were dose-dependently stimulated by aldosterone in VSMCs.53 These responses were abrogated by eplerenone and almost abolished by PD2, a selective c-Src inhibitor. Aldosterone-induced collagen synthesis was significantly reduced by a p38MAP kinase inhibitor. Aldosterone increased phosphorylation of c-Src, p38MAP kinase, and cortactin, a Src-specific substrate, in wild-type VSMCs, but not in c-Src-deficient VSMCs. These processes may play an important role in profibrotic actions of aldosterone and are depicted in Figure 2. Big MAP kinase 1 (BMK1) is a newly identified MAP kinase that has been shown to be involved in cell proliferation, differentiation, and survival. Ishizawa et al54 demonstrated that aldosterone stimulated proliferation and dose-dependently activated BMK1 in rat aortic SMCs, effects inhibited by eplerenone. These effects were not inhibited by cycloheximide, suggesting that they were nongenomic. Dominant-negative MAP kinase kinase (MEK)5, which regulates BMK1, and the MEK inhibitor PD98059 inhibited, in part, aldosterone-stimulated cell proliferation. This showed that aldosterone-induced rapid nongenomic effects may participate in mechanisms of cell proliferation in cardiovascular disease.

Min et al55 examined the cross-talk of growth-promoting signaling between aldosterone and Ang II in VSMCs (Figure 2). Treatment with low doses of aldosterone (10−12 mol/L) and Ang II (10−10 mol/L) significantly enhanced DNA synthesis, whereas aldosterone or Ang II alone at these doses did not affect VSMC proliferation. This effect of combined aldosterone and Ang II was inhibited by olmesartan, an Ang type 1 (AT1) receptor blocker, by spironolactone, an MEK inhibitor, PD98059, or an epidermal growth factor receptor tyrosine kinase inhibitor, AG1478. Aldosterone with Ang II at concentrations that were individually inefficacious increased extracellular signal-regulated kinase activation and Ki-ras2A expression, and reduced mitogen-activated protein kinase phosphatase-1 (MKP-1) expression. The decrease in MKP-1 and increase in Ki-ras2A expression were restored by PD98059 or AG1478 (summarized in Figure 2). These results suggest that aldosterone exerts a mitogenic effect synergistic with Ang II and that blockade of both MR and Ang II may provide enhanced protection from vascular remodeling, as already reported by Iglarz et al.56

Ang II and aldosterone stimulate MAP kinase and ROS signaling.57,58 Jaffe and Mendelsohn57 showed that Ang II directly activates MRs in human coronary and aortic VSMCs (Figure 2). The presence of 11-β-hydroxysteroid-dehydrogenase-2, necessary for mineralocorticoid action, was also demonstrated. Ang II activation of MRs was a direct effect, independent of aldosterone generation, which represents an example of receptor transactivation by the AT1 receptor. Genes regulated by the MR stimulated by Ang II may contribute to vascular changes associated with aging, potentially different from the ones stimulated directly by the AT1 receptor as pointed out in the Editorial accompanying the article.59 Thus, these studies extend previous in vivo evidence of Ang II and aldosterone synergistic effects in the vascula-
Integrative Molecular Physiology of Vascular Effects of Aldosterone: Role in Atherosclerosis

Aldosterone, as already mentioned, increases tissue ACE activity and upregulates angiotensin receptors. This suggests the potential for a vicious cycle in which Ang II, through the AT1 receptor, stimulates the production of aldosterone, which, in turn, leads to an increase in tissue ACE activity, an additional increase in Ang II, and, therefore, an additional elevation in aldosterone levels. Because Ang II and aldosterone may enhance LOX-1 receptor expression, MR blockade added to an ACE inhibitor and/or an angiotensin receptor blocker could decrease oxidized low-density lipoprotein LDL cholesterol and ROS, with a consequent increase in NO bioavailability and beneficial effects on atherosclerosis. Eplerenone reduced oxidative stress and atherosclerosis progression in apolipoprotein E−/− mice. Aldosterone given to apolipoprotein E−/− mice increased macrophage oxidative stress and atherosclerosis, whereas MR blockade or an AT1 receptor blocker reduced the atherogenic effects of aldosterone. Keidar et al showed that aldosterone increased macrophage-oxidized LDL cholesterol concentration and atherosclerotic plaques in the apolipoprotein E−/− mouse. The combination of an MR blocker and an ACE inhibitor or angiotensin receptor blocker was the most effective antitherapeutic therapy. In a study of monkeys fed a high-cholesterol diet, MCP-1 and malondialdehyde-modified LDL were suppressed in the eplerenone-treated animals. Intravascular ultrasound demonstrated that the aortic intima/media ratio was dose-dependently reduced in monkeys treated with eplerenone. Impaired endothelium-dependent relaxation was improved by eplerenone. ACE activity, which was increased in controls, was reduced by eplerenone. Aldosterone decreased the expression of AT1 receptors in a model of hind-limb ischemia, which provides additional evidence of a relationship to NO bioavailability and atherogenesis. Aldosterone also increased and MR blockade reduced vascular inflammation and metalloproteinase-2 and -9 activation, suggesting that aldosterone may play a role in plaque rupture. These effects are
not limited to the vasculature, because many of the signaling events, as well as the proinflammatory and atherogenic actions of aldosterone, are also exerted on other organs, such as the kidney, where MR blockade may be protective. Renoprotective effects of eplerenone may, in part, be associated with inhibition of LOX-1-mediated adhesion molecules and the PKC–MAP kinase–p90RSK pathway, as well as improvement of endothelial function.

A question that has been addressed only in part so far is whether the increased potassium excretion and shift into the cell that aldosterone elicits with associated hypokalemia may be partially responsible for some of the effects of aldosterone on the cardiovascular system. It is well known that thiazide diuretic–induced hypokalemia is associated with increased cardiovascular risk in hypertensive patients. However, aldosterone-mediated changes in potassium homeostasis do not appear to contribute to cardiac necrosis in experimental models. Although some recent studies have suggested that increased endothelium-derived plasminogen activator inhibitor (PAI) 1, for example, could result from changes in potassium balance rather than direct effects of the renin–angiotensin–aldosterone system, MR blockade was shown to prevent effects of activation of the renin–angiotensin–aldosterone system on PAI 1 antigen in normotensive subjects and to improve fibrinolytic balance in hypertensive subjects independent of potassium changes. Whether other effects of aldosterone can be attributed to potassium shifts remains to be established, but there is evidence that potassium administration does lower BP and protects from vascular injury. Thus, changes in potassium balance may mediate some vascular effects of aldosterone, but this remains to be unambiguously demonstrated.

Conclusions
There is increasing literature suggesting that aldosterone exerts important physiologically and/or pathophysiologically relevant effects on the cardiovascular system and on different organs including the brain, in contrast to the classical notion that mineralocorticoids were only involved in body electrolyte and water homeostasis mediated by the kidney. Increased mechanistic knowledge of this critical mediator and its many targets will contribute to our ability to act therapeutically to benefit patients with cardiovascular disease, including hypertension, ischemic heart disease, stroke, heart failure, and renal disease. The current understanding of the increasingly appreciated involvement of aldosterone in hypertension acting through BP elevation, vascular damage, and cardiac fibrosis and the realization that aldosterone has complex interactions with Ang II have provided justification for the use of combined angiotensin and mineralocorticoid blockade in the treatment of hypertension and cardiac failure.

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