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 capsaicin) 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 antagonism 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)F$_{2\alpha}$, PGE$_2$, and 6-keto-PGF$_{1\alpha}$, whereas in SHRs, acetylcholine only stimulated generation of 6-keto-PGF$_{1\alpha}$. Aldosterone may, thus, produce endothelial dysfunction through COX-2 activation in normotensive and hypertensive rats. PGI$_2$ 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 preglomerular afferent arterioles.18 The endothelium may modulate this vasoconstrictor effect by NO release.19 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 preglomerular afferent arterioles that are mediated by the activation of inositol triphosphate and PKC pathways.

Using atomic force microscopy, Oberleithner et al20 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.21 Swollen aldosterone-treated endothelial cells shrunk when amiloride was applied at concentrations that do not inhibit the sodium-proton exchanger (NHE) or with cariporide, a selective inhibitor of NHE, indicating that a sodium channel similar to the epithelial sodium channel of the distal nephron was involved in this aldosterone effect. Amiloride hyperpolarizes the cell as a result of the blockade of sodium channels, inducing chloride and water efflux, and cell shrinking. Oberleithner et al20 suggested that because the surface of the endothelium is huge, aldosterone may reduce serum concentrations of potassium, which was associated with endothelium-dependent aortic NAD(P)H oxidase activity and reactive oxygen species (ROS) generation, and MR blockade improved endothelial function in Ang II–infused rats10 and in a high-lipid diet rabbit model.25

Recent data suggest that aldosterone may elicit both vasoconstrictor and vasodilatory effects in humans. Schmidt et al26 examined 48 healthy male volunteers in a randomized, placebo-controlled, double-blind crossover trial to investigate the rapid nongenomic, vascular effects of aldosterone in humans. Aldosterone increased forearm blood flow. Superimposed on aldosterone, Nω-monomethyl-ω-arginine induced a greater vasoconstriction, sodium nitroprusside a reduced vasoconstriction, and phenylephrine an enhanced vasoconstriction within minutes compared with placebo. Aldosterone through rapid nongenomic effects on the endothelium may, thus, increase NO release and relax blood vessels, whereas it may act on vascular smooth muscle cells (VSMCs) to induce contraction, which agrees with recent in vitro data already mentioned.23

Smooth Muscle Cells

Aldosterone may be produced in cardiomyocytes in the heart4,28 and VSMCs, although it is not established whether concentrations achieved are high enough to exert local effects.30 Indeed, in the rat heart, for example, there is little evidence that aldosterone synthase (CYP11B2) and 11β-hydroxylase (CYP11B1) genes are expressed, because there is either extremely low expression of mRNA or protein for steroidogenic enzymes or they are not found at all.31 Cardiac and vascular MRs have also been demonstrated.5 Aldosterone induces fibrosis in heart, blood vessels, and kidney, particularly in the presence of high salt.12,32–34 In addition, actions that are usually attributed to direct effects of Ang II, such as vascular remodeling, endothelial dysfunction via increased oxidative stress, and inflammation of the vascular wall and the heart, may, in fact, be mediated at least in part by aldosterone.10 Systolic BP increased by Ang II was reduced by spironolactone. In mesenteric small arteries studied on a pressurized myograph, the media/lumen ratio was increased and acetylcholine-mediated relaxation impaired by Ang II, and both were partially improved by spironolactone. Aortic NAD(P)H oxidase activity increased by Ang II was reduced by spironolactone. Plasma thiobarbituric acid–reactive substances (a marker of oxidative stress), higher in Ang II–infused rats, were normalized by spironolactone. These results suggested that aldosterone mediates some actions of Ang II usually attributed to direct actions of the peptide.

A proinflammatory cardiovascular and renal response to mineralocorticoids and particularly to aldosterone has been
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

In salt-loaded stroke-prone spontaneously hypertensive rats (SHRSPs) fed high- (4.2%), normal- (0.28%), or low-salt (0.03%) diets with or without eplerenone, BP rise was prevented by eplerenone in the salt-loaded rats, with little effect on control and low-salt SHRSPs.49 The remodeling of resistance arteries and endothelial dysfunction induced by salt were prevented by eplerenone. Effects of aldosterone in SHRSPs depend on 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 PP2, 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.

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.58 Thus, these studies extend previous in vivo evidence of Ang II and aldosterone synergistic effects in the vascular-
ture. Xiao et al suggested that steroid production by VSMCs plays a critical role in response to Ang II and that aldosterone is required for the full proliferative response to Ang II, which could occur via modulation of expression and function of AT1 receptors.

Fujita et al infused aldosterone into the left anterior descending coronary artery of dogs, resulting in a dose-dependent reduction of coronary blood flow in both nonischemic and ischemic hearts. Fractional shortening and lactate extraction decreased. Decreases in coronary blood flow were reduced by coadministration of GF109203X, an inhibitor of PKC, but not by spironolactone. Thus, aldosterone non-genomically induces vasoconstriction via PKC-dependent pathways. Similar results have also been obtained recently in human coronary arteries obtained from heart valve donors. In aldosterone-potentiated coronary artery Ang II responses in proportion to extracellular signal–regulated kinase 1/2 phosphorylation and PKC activation. These effects were not blocked by spironolactone or eplerenone, suggesting that they are nongenomic.

Nongenomic effects of aldosterone on VSMC NHE have also been proposed. Both short- and long-term VSMC exposure to aldosterone increased NHE activity. Gene transcription (actinomycin D) and protein synthesis inhibitors (cycloheximide) had no effect on short-term actions but blocked long-term effects. Spironolactone and a glucocorticoid receptor antagonist (RU38486) did not influence short-term but inhibited long-term aldosterone actions. PKC inhibitors and downregulation of PKC by phorbol ester inhibited the short- and long-term effects of aldosterone. Neither mineralocorticoid nor glucocorticoid receptors are involved in NHE effects. This action was inhibited by colchicine, which indicates involvement of the cytoskeleton. Long-term aldosterone effects on VSMC NHE are mediated by mineralocorticoid and glucocorticoid receptors and involve gene transcription and protein synthesis. Both short- and long-term actions require PKC activation.

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 antiatherogenic 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 PKCζ–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.

Acknowledgments
The work cited was supported by grants 13570 and 37917 and a Group Grant to the Multidisciplinary Research Group on Hypertension, all from the Canadian Institutes of Health Research.

References


Effects of Aldosterone on the Vasculature
Ernesto L. Schiffrin

Hypertension. 2006;47:312-318; originally published online January 23, 2006;
doi: 10.1161/01.HYP.0000201443.63240.a7
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/47/3/312

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located,
click Request Permissions in the middle column of the Web page under Services. Further information about
this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/