Vascular Actions of Aldosterone

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Abstract

Aldosterone exerts direct effects on the vascular system by inducing oxidative stress, inflammation, hypertrophic remodeling, fibrosis, and endothelial dysfunction. Aldosterone exerts its effects through genomic and nongenomic pathways in a mineralocorticoid receptor (MR)-dependent or independent manner. Other aldosterone receptors such as GPR30 have been identified. A tight relation exists between the aldosterone and angiotensin II pathways, as well as with the endothelin-1 system. There is a correlation between plasma levels of aldosterone and cardiovascular risk. Recently, an increasing body of evidence has underlined the importance of aldosterone in cardiovascular complications associated with the metabolic syndrome, such as arterial remodeling and endothelial dysfunction. Blockade of MR is an increasingly used evidence-based therapy for many forms of cardiovascular disease, including hypertension, heart failure, chronic kidney disease, and diabetes mellitus.

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Introduction

Aldosterone is a steroid hormone synthesized by the adrenal glomerulosa in response to various stimuli including angiotensin II, high potassium levels, and adrenocorticotrophic hormone. Aldosterone classically acts on sodium reabsorption at the level of the distal nephron through its interaction with the mineralocorticoid receptor (MR) and activation of the apical epithelial sodium channel [1]. The link between aldosterone and hypertension was clearly established many years ago by Conn [2] in secondary forms of hypertension but is also present in essential hypertension, since mild elevations of aldosterone levels have been shown in patients with essential hypertension [3].

Beside its effect on sodium reabsorption and hypertension, aldosterone is involved in various biological processes at the level of the vasculature, where it induces inflammation, oxidative stress, endothelial dysfunction, and vascular remodeling. In this review we will detail the molecular mechanisms involved in the vascular effects of aldosterone, the cross talk with angiotensin II and endothelin-1 (ET-1) pathways, and the genomic versus nongenomic effects of aldosterone (fig. 1).

Fig. 1. Effects of aldosterone on the vascular system. Aldosterone is mainly synthesized by the adrenal gland in response to angiotensin II, potassium (K⁺), and adrenocorticotrophic hormone (ACTH). At the level of the vessel, aldosterone induces inflammation and oxidative stress, hypertrophic remodeling, and endothelial dysfunction. Aldosterone acts through its effect on MR and another type of receptor, the G protein-coupled receptor 30 (GPR30), via genomic and non genomic pathways. Aldosterone induces endothelial dysfunction through a mechanism involving

reduction of nitrite oxide bioavailability. At the level of endothelial cells, aldosterone exerts a direct effect on glucose 6 dehydrogenase (G6PD), and epidermal growth factor (EGFR), increasing their volume and stiffness. Finally, aldosterone acts on endothelial progenitor cells (EPCs) reducing vascular homing, migration, differentiation, and proliferation. T regulatory cells reduce the vascular effects of aldosterone. Perivascular fat synthesizes aldosterone and influences the vascular wall in an MR-dependent manner.

Effects of Aldosterone on Vascular Remodeling

Role of Aldosterone in Cardiovascular Remodeling Induced by Angiotensin II

Aldosterone induces vascular fibrosis and hypertrophic remodeling particularly in the presence of salt [4, 5]. In animal models of hypertension, such as spontaneously hypertensive rats, vascular fibrosis has been prevented by administration of spironolactone, an MR antagonist, independently of blood pressure reduction [6]. Cardiovascular remodeling effects usually attributed to angiotensin II are significantly reduced by the use of MR blockers. Indeed, in Sprague-Dawley rats infused with angiotensin II, spironolactone reduced systolic blood pressure and blunted the hypertrophic remodeling characterized by an increase in the wall-to-lumen ratio in small resistance arteries [7].

Aldosterone Signaling Pathways in Vascular Smooth Muscle Cells

In vitro, aldosterone (10⁻⁷ M) exerts a direct effect on signaling in vascular smooth muscle cells (VSMCs) involving extracellular signal-regulated kinase (ERK) [8],

mitogen-activated protein kinase (MAPK) [8], the tyrosine kinase c-Src [9], and c-jun N terminal kinase (JNK) and participates in epidermal growth factor receptor (EGFR) transactivation [8]. Aldosterone also significantly increased phosphorylation of myosin phosphate target subunit-1 (MYPY1), a marker of Rho-kinase activity and the amount of GTP-Rho. As a consequence, aldosterone induced VSMC stress fiber formation and migration. All of these effects were blocked by eplerenone, a selective MR blocker, and Y27632, a specific Rho-kinase inhibitor [10].

Effect of Aldosterone on Arterial Mechanical Properties

Aldosterone infusion (1 µg/h) into uninephrectomized Sprague-Dawley rats receiving a high-salt diet increased large artery stiffness with an increase in pulse pressure, and the carotid elastic modulus. These functional changes were associated with increased aortic fibronectin expression with no change in elastin or collagen density. This phenotype was completely blunted by eplerenone, an MR antagonist [11]. In hypertensive patients, an inverse correlation was found between the plas-

ma aldosterone concentration (PAC) (mean $\pm$ SD: 13.6 $\pm$ 4.5 ng/100 ml in normotensive patients and 11.5 $\pm$ 6.6 in hypertensive patients) and large vessel compliance, independently of age and blood pressure [12]. In addition, a polymorphism of CYP11B2 was associated with higher aortic stiffness in hypertensive patients [13].

Role of Salt in Aldosterone-Induced Vascular Disease

The effects of aldosterone on the cardiovascular system are dependent on salt co-administration or pre-existing vascular injury. Indeed, in uninephrectomized rats fed a low-salt diet, aldosterone infusion (0.75 μ g/h) did not induce cardiac fibrosis [14]. Vascular function and remodeling in patients with Gitelman or Bartter syndrome characterized by a renal loss of sodium were similar to those measured in normotensive controls despite a high level of aldosterone associated with these diseases (PAC, mean $\pm$ SD: 0.18 $\pm$ 0.02 nM in controls and 0.77 $\pm$ 0.09 nM in patients with Gitelman syndrome) [15]. These studies underlined the importance of salt in the sensitization of vascular tissues to aldosterone effects.

Effects of Aldosterone on Vascular Inflammation and Oxidative Stress

It is well demonstrated now that aldosterone induces oxidative stress and inflammation in the vascular wall, in endothelial cells, and in VSMCs [16]. Part of the effects of angiotensin II on the vasculature is due to aldosterone since selective aldosterone blockade by an MR antagonist blunted perivascular inflammation characterized by macrophage infiltration, and reduced the expression of inflammatory markers, cyclooxygenase-2 and osteopontin, in coronary arteries of rats infused with angiotensin II [17].

In rats, aldosterone and salt infusion induced an increase in NADPH oxidase subunit expression in an MR-dependent manner. Aldosterone infusion in Nox2-deficient mice was not associated with an increase in NADPH oxidase activity as was observed in wild-type controls, which underlines the role of Nox2 in aldosterone-induced oxidative stress in the vascular system [18].

Signaling Pathways Involved in Aldosterone-Induced Oxidative Stress

In vitro, in VSMCs, aldosterone (10^{-7} M) induced a time-dependent increase in NADPH-oxidase activity through c-Src activation since this effect was significantly reduced in VSMCs from c-Src $\pm$ mice [19]. Recently, Callera et al. [20] demonstrated that aldosterone-induced

c-Src activation involved lipid rafts/caveolae via the platelet-derived growth factor receptor. In endothelial cells, aldosterone (10^{-8} – 10^{-6} M) induced superoxide generation through Rac1 activation of NADPH oxidase in an MR-dependent manner [21].

Impact of Aldosterone-Induced Oxidative Stress on Vascular Remodeling

Increased oxidative stress secondary to aldosterone infusion has consequences on vascular remodeling. Indeed, in Wistar rats receiving aldosterone infusion (0.75 μ g/h) and 1% NaCl in the drinking water, administration of apocynin, an NADPH oxidase inhibitor, prevented hypertrophic remodeling and vascular fibrosis and suppressed the expression of transforming growth factor- β_1 , type I and III collagen, and monocyte chemoattractant protein-1 [16]. In addition, cardiac fibrosis induced by aldosterone in wild-type mice was blunted in Nox2-deficient mice [18]. Oxidative stress induced by aldosterone also upregulated the expression of oxidized low-density lipoprotein receptor-1 (Olr-1). Olr-1 is a member of the C-type lectin family known to act as a cell-surface receptor for oxidized lipoprotein. This observation provides a link between aldosterone and the development of atherosclerosis [16].

Aldosterone Effect of Vascular Inflammation

Aldosterone infusion (600 μ g/kg/day) into experimental animals resulted in vascular infiltration of monocytes, macrophages, and lymphocytes [22]. In human coronary endothelial cells, aldosterone (1–100 nM) promoted ICAM-1 transcription and leukocyte adhesion in an MR-dependent manner [23]. In response to aldosterone (0.75 μ g/h), vascular inflammation is partly dependent on oxidative stress [24]. Interestingly, increased oxidative stress and vascular inflammation are the first effects of aldosterone in vessels. Indeed, in uninephrectomized rats receiving aldosterone and salt 1%, a vascular increase in oxidative stress and inflammation, characterized by expression of intracellular adhesion molecule-1, monocyte chemoattractant protein-1, and tumor necrosis factor- α , preceded coronary artery fibrosis [24], which could be prevented by treatment with pyrrolidine dithiocarbamate or N-acetylcysteine [24].

Recently, we showed that adaptive immunity plays a pivotal role in aldosterone-induced vascular injury. In mice, adoptive transfer of CD4 $^{+}$ CD25 $^{+}$ (T regulatory) lymphocytes prevented the vascular effects of aldosterone infusion characterized by endothelial dysfunction, hypertrophic remodeling, aortic superoxide production, and inflammation [22].

Effects of Aldosterone on the Endothelium

Aldosterone affects endothelial function in humans with primary hyperaldosteronism and in various animal models of hypertension through mechanisms involving inflammation and oxidative stress and through direct effects on the electrolyte composition of endothelial cells and on endothelial progenitor cell (EPC) function.

Effect of Aldosterone on Endothelial Function

In a clinical study, endothelial function, evaluated by measurement of flow-mediated dilation, was significantly impaired in 36 patients with primary hyperaldosteronism (PAC, mean $\pm$ SD: 20.4 $\pm$ 12.8 ng/dl) compared to 44 patients with essential hypertension (9.5 $\pm$ 6.6 ng/dl). A 3-month treatment with spironolactone was associated with a significant improvement in endothelial function [25].

Aldosterone infusion in various models of mice [22] and rats [26] induced a significant decrease in endothelium-dependent relaxation in large [26] and small arteries [22]. In addition, part of the effects of angiotensin II on endothelial function can be attributed to aldosterone. Indeed, in Sprague-Dawley rats infused with angiotensin II, treatment with the MR antagonist spironolactone partially reversed endothelial dysfunction and abolished angiotensin II-increased oxidative stress [7].

Mechanisms Involved in Aldosterone-Induced Endothelial Dysfunction

Aldosterone induces an increase in oxidative stress in VSMCs, which may play a role in endothelial dysfunction through a reduction in nitric oxide (NO) bioavailability. Generation of oxygen species and the activity of NADPH oxidase were increased in mice [22] and rats [16] infused with aldosterone in an MR-dependent manner. In patients with chronic heart failure, treatment with spironolactone improved endothelial function measured at the level of the forearm by plethysmography, in association with increased NO bioavailability [27]. Interestingly, in a model of mice with cardiomyocyte-specific overexpression of aldosterone synthase (1.7-fold increase in PAC in the heart, no change in plasma), despite the absence of structural or functional alterations at the level of the heart, impairment in coronary endothelial-dependent relaxation was observed [28]. The alteration of endothelial function was mostly due to a decrease in NO availability [28]. In this model, coronary endothelial dysfunction was associated with an increase in cardiac reactive oxygen species production and Nox expression and activ-

ity. Treatment with the antioxidant vitamins E and C and by the Nox inhibitor apocynin (also considered a superoxide scavenger) prevented endothelial dysfunction in this model [29]. Another mechanism of aldosterone-induced endothelial dysfunction implicates glucose-6-phosphate dehydrogenase (G6PD) activity, a critical regulator of the intracellular redox state. Indeed, it has been shown that aldosterone (10^{-9} – 10^{-7} M) decreases endothelial G6PD expression and activity in vitro. In vivo, aldosterone infusion also decreased vascular expression of G6PD in an MR-dependent manner. Vascular gene transfer of G6PD restored endothelial dysfunction induced by aldosterone [30]. Altogether, these studies underline the deleterious effect of aldosterone on endothelial function through a mechanism involving oxidative stress generation.

Recently, Griol-Charhbil et al. [31] underlined the role of EGFR in aldosterone-mediated endothelial dysfunction. In the *waved 2* mouse, a model characterized by a mutation in the EGFR gene altering the receptor kinase domain and reducing the functional activity of EGFR, functional consequences of aldosterone infusion (60 μ g/kg/day) on endothelial function were attenuated [31].

The participation of prostacyclin in aldosterone-induced endothelial dysfunction at the level of the aorta was shown in normotensive (Wistar Kyoto) and hypertensive (spontaneously hypertensive) rats. More precisely, aldosterone induced endothelial dysfunction through cyclooxygenase-2 activation in normotensive and hypertensive conditions. Prostacyclin was the main factor implicated in endothelial dysfunction in hypertensive rats [26].

Effect of Aldosterone on Mechanical Properties of Endothelial Cells

Using atomic force microscopy, Oberleithner's group have demonstrated that aldosterone exerts a direct effect on endothelial cells, increasing the volume through an MR-dependent mechanism that implicated the amiloride-sensitive sodium-proton exchanger [32, 33], resulting in increased stiffness of endothelial cells [34], MR-dependent cell growth [35], and reduced NO release by endothelial cells [36]. Altogether, these results show that aldosterone exerts a direct effect on the electrolyte composition of endothelial cells that influences their mechanical and functional properties.

Effect of Aldosterone on EPCs

Recently, Thum et al. [37] showed that treatment of EPCs with aldosterone induced nuclear translocation of

MR and impaired EPC functions such as differentiation, migration, and proliferation. In mice, aldosterone infusion impaired EPC homing to vascular structures and vascularization capacity in an MR-dependent manner [37]. Interestingly, they also showed that in patients with primary hyperaldosteronism, EPCs had a reduced migratory potential. Treatment with spironolactone for 4–6 weeks significantly improved EPC migratory function [37]. In another clinical study comparing 113 patients with primary hyperaldosteronism (PAC, mean $\pm$ SD: 47.4 ± 36.8 ng/dl) to 55 patients with essential hypertension (PAC, mean $\pm$ SD: $24.2.4 \pm 16.4$ ng/dl), a reduction of circulating EPCs and endothelial colony-forming units was seen in patients with primary hyperaldosteronism. The preoperative number of EPCs predicted the curability of hypertension after adrenalectomy [38].

Cross Talk between Aldosterone and Angiotensin II

An increasing body of evidence suggests that cross talk between aldosterone and angiotensin II pathways takes place in VSMCs. Aldosterone and angiotensin II exert a synergistic effect on proliferation, senescence, and migration.

Effect of Angiotensin II on Aldosterone Secretion

Aldosterone increases the expression of angiotensin receptors in vivo [39, 40] and in vitro [40]. On the contrary, angiotensin II stimulates aldosterone synthesis by the adrenal gland. There is also controversial data suggesting the existence of local synthesis of aldosterone in the vasculature after stimulation by angiotensin II. Hatakeyama et al. [41] showed that VSMCs and endothelial cells express CYP11B2, the key enzyme involved in aldosterone synthesis, as well as MR. In vitro, angiotensin II administration to VSMCs had a proliferative effect which was blunted by ZK 91587, an MR antagonist, suggesting local synthesis of aldosterone by VSMCs [41]. Direct evidence of aldosterone synthesis in rat mesenteric arteries has been shown using a reverse-phase high-performance liquid chromatographic system [42]. More recent studies have failed to show aldosterone synthase mRNA and aldosterone synthesis in human endothelial cells in response to angiotensin II, ACTH, and potassium, and in the presence of deoxycorticosterone [43]. In adrenalectomized rats, Fiebeler et al. [44] showed a strong reduction in cardiac aldosterone suggesting that more than 99% of cardiac aldosterone was not produced locally. Thus, local synthesis of aldosterone in the vascular sys-

tem has not been demonstrated in a rigorous way, or the amount produced is very low and its physiological local effect remains to be demonstrated.

Aldosterone and Angiotensin II Cross Talk on VSMC Function

Aldosterone exerts a synergistic effect with angiotensin II on VSMC proliferation [45, 46]. Low doses of aldosterone (10^{-12} M) and angiotensin II (10^{-10} M) alone had no effect on VSMC proliferation, whereas the combination significantly increased DNA synthesis. This synergistic effect on proliferation implicates the angiotensin II type 1 receptor, MR, MAPK/ERK1/2, and EGF receptor tyrosine kinase, since proliferation was blunted by specific inhibitors of each of these components. Interestingly, treatment of VSMCs with aldosterone and angiotensin II at a low dose induced two peaks of ERK phosphorylation: the first one within 5 min, compatible with a non-genomic action, and the second one between 2 and 4 h later, compatible with a genomic pathway. The first peak of ERK phosphorylation was angiotensin II type 1 receptor and EGF receptor dependent, whereas the second one also implicated MR. The late peak of ERK phosphorylation was dependent on Ki-ras2A activity.

Min et al. [47] also showed that angiotensin II and aldosterone exert a synergistic effect on senescence through a mechanism involving oxidative stress and Ki-ras2A. Low doses of angiotensin II (100 pM) and aldosterone (1 pM) separately had no effect on VSMC senescence, whereas in combination an increase in SA- β -gal-positive cells and expressions of p21, p53, p16, and p27, which are markers of senescence, were observed. This effect was attenuated by knocking down the expression of Ki-ras2A and by the inhibition of oxidative stress by an antioxidant, N-acetyl-L-cysteine (NAC), or superoxide dismutase.

Synergistic effects of aldosterone and angiotensin II were also involved in migration [48]. Montezano et al. [48] showed that VSMC migration was stimulated by co-administration of a low dose of aldosterone (0.1 nM) and angiotensin II (0.1 nM), whereas angiotensin II or aldosterone alone at this low dose had no effect. This process implicated c-Src pathways that regulated RhoA/Rhokinase, and was dependent on transactivation of EGFR and PDGFR.

Recently, Batenbourg et al. [49] demonstrated that aldosterone potentiated the constrictor effect of angiotensin II on human coronary microarteries at nanomolar levels depending on G protein-coupled receptor 30 (GPR30) and EGFR transactivation. Interestingly, at micromolar levels, aldosterone abolished the angiotensin II-

Fig. 2. Genomic and nongenomic action of aldosterone. Aldosterone diffuses across the cytoplasmic membrane of vascular smooth cells and binds to the MR, inducing dimerization and translocation to the nucleus, where the complex binds to the glucocorticoid response element, and induces the expression of Sgk-1. Sgk-1 increases Ki-Ras2A, which in turn activates ERK1/2 (extracellular stimulus regulating kinase). Nongenomic pathways are MR dependent or independent. Aldosterone, in an MR-dependent pathway, induces rapid activation of JNK, p38 MAPK, and ERK1/2 through the transactivation of EGFR and c-Src (a nonreceptor tyrosine kinase). c-Src induces ROS (reactive oxygen species) production, which activates ROCK and MAPK. Aldosterone, through its interaction with GPR30, activates ERK1/2 nongenomically without intervention of MR.

induced contraction through a mechanism involving endothelial NOS phosphorylation [49].

Mazak et al. [8] showed that aldosterone (10^{-7} M) and angiotensin II (10^{-7} M) had synergistic effects on ERK1/2 and JNK phosphorylation, which was dependent on ROS generation.

In VSMCs, we have provided direct evidence of the involvement of angiotensin II type 1 receptor in aldosterone signaling pathways. Aldosterone-induced activation of ERK1/2, JNK, and NF- κ B was reduced in VSMCs from AT1a-deficient mice or in VSMCs which had undergone knock-down for AT1a receptor [50].

These experimental data emphasize the cross talk between aldosterone and angiotensin II pathways, and provide physiological arguments for the use of MR antagonists in addition to angiotensin converting enzyme inhibitors or angiotensin II receptor blockers in hypertensive patients.

Cross Talk between Aldosterone and ET-1

An increase in ET-1 expression has been shown in the deoxycorticosterone (DOCA)-salt hypertension rat model [51, 52]. It is well established that ET-1 is implicated in the vascular remodeling process. Endothelium-restricted

overexpression of human prepro-ET-1 mouse exhibited inward hypertrophic remodeling of resistance arteries, and vascular inflammation and endothelial dysfunction, in the absence of blood pressure rise [53]. Accordingly, ET_A receptor antagonists prevented blood pressure rise and vascular remodeling in aldosterone-infused rats [4, 5] and in DOCA-salt-treated spontaneously hypertensive rats [54, 55]. An ET_A blocker also blunted aldosterone-induced vascular inflammation [56]. These studies, however, do not establish if there is a direct cross talk between ET-1 and aldosterone or a physiological antagonism induced by the ET_A blocker. The report from Stow et al. [57] that aldosterone modulates steroid receptor binding to the ET-1 gene (*edn1*) are in agreement with a direct link. These experiments underline the importance of the endothelin system in aldosterone-induced vascular injury.

Nongenomic Effects of Aldosterone

Aldosterone exerts some effects on the vascular system with a time course which is not compatible with transcriptional mechanisms and may thus involve nongenomic mechanisms. These short-term actions involve variations in intracellular electrolyte concentration, the activity of the sodium-proton exchanger, inositol triphos-

phate (IP₃) production, and vascular reactivity. Genomic effects are all mediated via the activation of the MR whereas nongenomic effects may be MR dependent or MR independent involving other receptors such as GPR30 (fig. 2).

Nongenomic Effects of Aldosterone on the Vascular Tone

In clinical studies, aldosterone injection in healthy subjects was associated with an increase in vascular resistance within the first 10 min [58, 59], with a rapid decrease in forearm blood flow between 4 and 8 min [60]. A rapid decrease in forearm blood flow was also observed in treated chronic heart failure after aldosterone infusion [61]. In healthy subjects, aldosterone infusion (0.5 mg) did not significantly affect renal plasma flow, whereas co-infusion with N(G)monomethyl-L-arginine (L-NMMA) that inhibits NO synthesis unmasked the effect of aldosterone on the renal circulation and induced a decrease in renal plasma flow and an increase in renal vascular resistance which was significantly higher than with L-NMMA alone ($1,588 \pm 237$ vs. 614 ± 240 dyn·s·cm⁻⁵; $p = 0.014$, respectively) within 30 min after injection. This is compatible with a nongenomic effect [62]. In accordance with the observations made in humans, Liu et al. [63] described a rapid and opposite effect of aldosterone on the vascular reactivity of aortic rings isolated from Wistar rats, which could be dependent or not on the presence of an intact endothelium. On aortic rings with intact endothelium, aldosterone (0.01 nM) induced a 25% reduction of phenylephrine-mediated constriction compared to controls in 10 min. This effect was MR dependent since it was attenuated by spironolactone. On the contrary, in denuded aortic rings, aldosterone induced a monophasic dose-dependent enhancement of vasoconstrictor responses. In conclusion, aldosterone induces a constrictor effect, which is attenuated by intact endothelial function. This protective effect of endothelium is partly due to an increase in NO synthase activity which is phosphatidylinositol 3-kinase (PI3K) dependent [63].

Receptors Involved in Nongenomic Effects of Aldosterone

In intact mesenteric vessels isolated from male Sprague-Dawley rats, aldosterone (10 nM, 10 min) potentiated the constrictor response to norepinephrine and elicited a direct constrictor effect within the first 10 min [64]. A direct constrictor action of aldosterone (10^{-13} – 10^{-16} M, <10 min) was also observed in mouse mesenteric vessels [65]. Interestingly, contradictory effects of MR

antagonists spironolactone and eplerenone on the direct constrictor action of aldosterone have been described, from complete inhibition [64] to the absence of blockade [65], suggesting that different receptors might be involved.

Yamada et al. [65] showed that the rapid constrictor effect of aldosterone on mesenteric vessels was unaffected by MR antagonists but was blunted by the AT₁ blockers candesartan or valsartan and by the transglutaminase inhibitors cystamine and monodansyl cadaverine. These results emphasize the potential role of intracellular transglutaminase activity and AT₁ dimer formation on the direct and rapid vascular action of aldosterone [65].

Recently, Gros et al. [66] identified a new receptor involved in nongenomic effects of aldosterone, i.e. the 7-transmembrane-spanning, GPR30. They demonstrated that aldosterone induced a rapid (15 min) and concentration-dependent increase in phospho-ERK in endothelium-denuded aortic rings, which was partly attenuated by the MR antagonist eplerenone and by the GPR30-selective antagonist G15 [66].

Mechanisms Involved in Aldosterone-Induced Rapid Vascular Constriction

Aldosterone-induced rapid vascular constriction involves various mechanisms. In intact mesenteric vessels, aldosterone induced a rise in intracellular pH, and the direct constrictor effect was blunted in the presence of amiloride, an Na⁺-H⁺ exchanger inhibitor [64]. In vitro on VSMCs, aldosterone (1 nM) stimulated the activity of the sodium-proton exchanger since ²²Na influx increased after 4 min, an effect blocked by amiloride, a specific inhibitor of the Na⁺-H⁺ exchanger [67]. The activation of the Na⁺-H⁺ exchanger by aldosterone in VSMCs was associated with an increase in IP₃ levels and was inhibited by the specific inhibitors of phospholipase C neomycin and U-73122 [67]. Interestingly, in this study, MR antagonists did not block this effect of aldosterone [67]. These experiments suggest that in VSMCs, aldosterone induces an increase in the activity of the Na⁺-H⁺ exchanger through a mechanism involving phospholipase C and IP₃ generation through MR-independent pathways [67, 68]. In addition to increased IP₃ levels and Na⁺-H⁺ exchanger activation, aldosterone induced an immediate rise in intracellular calcium, which was also MR independent since it was not blunted by spironolactone [68]. Finally, aldosterone induced myosin light chain phosphorylation. Myosin light chain phosphorylation started at 10 min with 1 nM of aldosterone and was MR [69] and GPR dependent [66].

Aldosterone exerts also a direct and rapid effect on p38 MAPK and NADPH oxidase activity through a c-Src-dependent pathway in VSMCs. This effect was abrogated by the MR blocker eplerenone, by PP2, a c-Src inhibitor, and in c-Src-deficient mice [19]. Interestingly, in VSMCs from hypertensive rats, upregulation of the c-Src signaling pathway was observed in response to exogenous aldosterone [9]. These processes may play a role in the fibrotic response to aldosterone.

Nongenomic pathways have also been involved in aldosterone-induced VSMC proliferation. Indeed, Ishizawa et al. [70] demonstrated that aldosterone induced a dose-dependent activation of big MAP kinase 1, an MAP kinase implicated in cell proliferation, differentiation, and survival, in rat aortic VSMCs. This effect was blunted by eplerenone but not by cycloheximide, suggesting a nongenomic process [70].

Tight Relation between Aldosterone, Vascular Dysfunction, and Fat

Aldosterone is increased in metabolic syndrome and obese patients and is also a predictor of incident metabolic syndrome as shown in the longitudinal follow-up of 2,292 participants of the Framingham Offspring Study [71]. An independent association between body weight and PAC has been shown in overweight patients with [72] or without hypertension [73]. Interestingly, plasma aldosterone decreases after weight loss [74]. Increased aldosterone levels in obese patients could be attributed to circulating factors which stimulate aldosterone synthesis by the adrenal gland such as insulin [75], C1q TNF-related protein [76], exogenous fatty acid oxidation products [77], and adipokines [78]. Local synthesis of aldosterone by adipocytes could also participate in the hyperaldosteronism of the metabolic syndrome and/or in obese patients. Indeed, aldosterone can now be considered a novel adipocyte-derived factor since direct evidence of aldosterone synthesis by adipocytes has been shown. Adipocytes possess aldosterone synthase and produce aldosterone in an AT₁ receptor-dependent manner [79].

Aldosterone synthesis by adipocytes influences vascular function. Briones et al. [79] showed that mesenteric arteries from db/db mice had an impairment of acetylcholine-induced relaxation when perivascular fat was preserved, and it was blunted by the MR antagonist eplerenone.

Adipocyte-conditioned medium obtained from differentiated 3T3-L1 adipocytes activated MAPK signaling

in VSMCs leading to inflammatory and profibrotic responses. MR antagonism blunted adipocyte-conditioned medium stimulation of p38 MAPK and ERK1/2 phosphorylation [80]. Aldosterone induces insulin resistance in rat VSMCs through a mechanism involving insulin growth factor-1 receptor (IGF1R) [81]. Aldosterone increased insulin-induced ERK1/2 phosphorylation and α -smooth muscle actin expression, which was significantly attenuated by eplerenone or picropodophyllin, an IGF1R inhibitor [81]. In addition, aldosterone decreased insulin receptor substrate-1 expression via c-Src and reactive oxygen species stimulation by proteasome-dependent degradation in VSMCs [82].

Pharmacological Effects of Blockade of Aldosterone Action in Patients

The advantage of aldosterone blockade in patients has been shown with the two drugs that block MR, i.e. spironolactone and eplerenone, in congestive heart failure. Low doses of these drugs (25–50 mg/day) in addition to renin-angiotensin system blockers (RAS) and β -blockers improved the cardiovascular morbidity and mortality of patients with class II–IV chronic heart failure (RALES trial in chronic heart failure and EPHESUS in heart failure after myocardial infarction) [83, 84]. In hypertensive patients, eplerenone has been shown to significantly improve the stiffness of small resistance arteries [85]. Small clinical studies have shown a beneficial effect of the use of eplerenone or spironolactone on proteinuria and chronic kidney disease progression [1]. Though useful in resistant hypertension, in the presence of renal failure there is the problem of handling hyperkalemia, which becomes a limitation for the use of these agents. The use of spironolactone is limited by its lack of specificity for MR. Spironolactone also binds to the progesterone and androgen receptors and long-term use is associated with gynecomastia and menstrual irregularities. In addition, the use of MR blockers leads to an increase in aldosterone concentration, which may exert deleterious effects on the cardiovascular system through non-MR-dependent pathways. New drugs targeting aldosterone synthase have been developed more recently, such as LCI699. The main limitation of this agent is that a partial inhibition of the glucocorticoid axis was also observed in 20% of treated patients with primary hyperaldosteronism [86] or in essential hypertension [87]. Second generation aldosterone synthase inhibitors with higher specificity for the enzyme should limit this adverse effect.

Conclusion

Aldosterone exerts direct effects on the vascular system by inducing oxidative stress, inflammation, hypertrophic remodeling, and fibrosis. Aldosterone exerts its effects through genomic and nongenomic pathways in an MR-dependent or independent manner. Other aldosterone receptors such as GPR30 have been identified. A tight relation exists between aldosterone and angiotensin II pathways underlying the pharmacological interest of blockade of both pathways. Recently, an increasing body of evidence has underlined the importance of aldosterone in cardiovascular complications associated with the metabolic syndrome, such as arterial remodeling and endothelial dysfunction. Blockade of MR is an increasingly

used evidence-based therapy for many forms of cardiovascular disease. However, the absence of specificity of some of the agents and the potential for development of hyperkalemia when renal function is impaired results in side effects that somewhat limit their use.

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