Brief Review

Role of Renin-Angiotensin-Aldosterone System Activation in Promoting Cardiovascular Fibrosis and Stiffness

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Abnormally increased arterial stiffness, cardiac stiffness/diastolic dysfunction, and associated functional changes are precursors and predictors for development of hypertension, coronary heart disease, heart failure, and renal disease.[1] Increased arterial stiffening, demonstrated clinically by an increase in pulse wave velocity (PWV), is associated with increased systolic and pulse pressures that lead to increased cardiac afterload, cardiac hypertrophy, and diastolic dysfunction.[1] In this regard, cardiac diastolic dysfunction is seen as an early cardiac structural and functional abnormality in diabetes mellitus, obesity, and aging and is termed clinically as heart failure with preserved ejection fraction (HFpEF), characterized by left ventricular (LV) hypertrophy, cardiac interstitial fibrosis, and concentric remodeling, and impaired diastolic relaxation.[1] Growing interest has been placed on relationships between activation of the renin-angiotensin-aldosterone (Aldo) system (RAAS), important in controlling sodium (Na^+)-potassium balance, fluid volume, and hemodynamic stability, and the pathogenesis of abnormal arterial and cardiac stiffness and associated cardiovascular disease (CVD).[1] Clinical trials have shown that RAAS blockade contributes to prevention of CVD and renal disease, including hypertension and heart failure.[2,3] However, the exact role and mechanisms by which an activated RAAS contributes to the development of cardiovascular fibrosis and stiffness are still not clear. Here, we focus on the role of the activated RAAS and provide a contemporary narrative of potential mechanisms by which activation of RAAS promotes increased arterial and cardiac fibrosis, stiffness, and CVD.

Clinical Importance of Increased Arterial and Cardiac Stiffness

Arterial Stiffness

In 2015, the American Heart Association Council for High Blood Pressure Research provided support for PWV as an appropriate method to measure arterial stiffness.[4] In 2007, based on published epidemiological studies without consideration of other factors influencing PWV, the European Society of Hypertension/European Society of Cardiology proposed that a threshold value (12 m/s) was indicative of abnormally increased arterial stiffness.[5] Also, providing support for the impact of an excessive increase in arterial stiffness, a meta-analysis of 17 longitudinal studies in 15 877 individuals showed that a 1 m/s increase in PWV increases the occurrence of CVD events by 14%, cardiovascular mortality by 15%, and all-cause mortality 15%.[6] Similarly, the Framingham Heart Study involving 2232 participants also found that increased arterial stiffness is an independent predictor of CVD-related morbidity and mortality in the general population, the elderly, and hypertensive patients.[7] Importantly, activation of RAAS contributes to excessive arterial stiffening, hypertension, and CVD while inhibition of Ang II (angiotensin II) and Aldo-activated receptors reduced PWV, excessive arterial stiffening, and CVD.[2,3] For example, in a meta-analysis of 3309 subjects, telmisartan and valsartan significantly reduced brachial-ankle PWV and valsartan significantly reduced carotid-femoral PWV.[2] Results from the SILVHIA study (Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol) showed that irbesartan improved arterial compliance without a change in inflammatory and endothelial markers.[8] A prospective study in 566 patients with uncontrolled hypertension found that spironolactone reduced arterial stiffness in parallel with treatment of hypertension.[9] Thus, an activated RAAS links excessive arterial stiffening and CVD. Of further importance, excessive arterial stiffening itself is the major cause of increased systolic blood pressure. Emphasizing this point, recent studies indicate that abnormal increases in arterial stiffness typically precede hypertension, and the arterial structural and functional abnormalities underlying increased arterial stiffness may exist before the development of hypertension.[10,11]

Cardiac Stiffness and Diastolic Dysfunction

Cardiac diastolic dysfunction is associated with coronary and cardiac tissue remodeling that leads to changes in mechanical structure and function.[1] Clinical findings include increased diastolic LV stiffness, prolonged isovolumic LV relaxation, and slowed LV filling.[1] Furthermore, increased cardiac mass and hypertrophy can engender a vicious cycle of greater LV filling pressures and impaired cardiac compliance, thus playing an important role in further promoting cardiac diastolic dysfunction and HFpEF.[1] The 2013 American College of Cardiology/American Heart Association heart failure management guidelines[12] and European Society of Cardiology recommendations[13] suggested that presence of LV diastolic dysfunction along with heart failure symptoms, with normal

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or near normal LV ejection fractions, consistent with the clinical picture of HFpEF. In the I-PRESERVE study (Irbesartan in Heart Failure With Preserved Ejection Fraction) involving 4128 patients, LV hypertrophy or concentric remodeling and diastolic dysfunction were found in the majority of patients with HFpEF.[14] Meanwhile, LV mass was independently associated with an increased risk of CVD morbidity and mortality.[14]

The important role of RAAS activation in the development of cardiac diastolic dysfunction and heart failure is supported in clinical trials using various RAAS inhibitors, including angiotensin-converting enzyme (ACE) inhibitors, Ang II receptor 1 (AT-1R) blockers, as well as mineralocorticoid receptor (MR) antagonists. Meta-analysis of RAAS blockade in 8152 patients with HFpEF showed that either ACE inhibition or AT-1R antagonism significantly lowered risks for heart failure hospitalization and cardiovascular mortality.[3] Furthermore, 6 months of aliskiren treatment decreased the stiffness of carotid arteries and improved LV end-systolic elasticity with maintenance of ventricular-arterial coupling without any effects on diastolic filling in elderly hypertensive patients with HFPEF.[15] A randomized double-blind trial where perindopril was administered to elderly people with chronic heart failure (PEP-CHF [Perindopril in Elderly People With Chronic Heart Failure]) showed that 1 year of treatment improved cardiac function, reduced the primary CVD outcome, and hospitalization for heart failure.[16] Furthermore, large randomized clinical trials, such as RALES (Randomized Aldactone Evaluation Study), EMPHASIS (Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure), and EPHESUS (Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study), have suggested that treatment with an MR antagonist decreases CVD morbidity and mortality in patients with heart failure.[17] Randomized controlled clinical trials in patients with cardiac diastolic dysfunction and HFpEF showed that MR antagonists reduced cardiac fibrosis and improved cardiac function. These results implicate that an activated RAAS signaling is an important contributor in the pathogenesis of arterial and cardiac stiffness and heart failure.[18]

Risk Factors in the Pathogenesis of Increased Arterial and Cardiac Stiffness

Genetic Determinants and Epigenetic Regulation

An association between genetic variation in components of the RAAS and the development of cardiovascular remodeling has been suggested by several studies.[19–21] A functional genetic polymorphism related to RAAS activation is indicated by significant association between genotypes at the angiotensinogen gene C-532T polymorphism and pulse pressure in 1425 individuals.[22] The AT-1R-153A/G polymorphism was significantly related to an abnormal increase in aortic stiffness after the age of 55 years, whereas the AT1 1166C allele related to excessive aortic stiffening at any age.[23] Furthermore, the D allele of the *ACE* gene has been associated with lower arterial stiffness,[24] and rs4343 and rs4291 polymorphisms of the *ACE* gene were associated with LV hypertrophy and HFpEF in a study of 176 hypertensive patients.[25] In the Framingham Heart Study, variation in Aldo biosynthesis could be attributed to *CYP11B2* gene polymorphisms.[26] *MR* gene polymorphisms are thought to regulate MR expression and activity. Indeed, the MRI180V (rs5522) variant affected blood pressure responses to enalapril treatment whereas MR-2GC polymorphism was involved in the spironolactone-induced increases in potassium levels in patients with heart failure.[27,28] Genetic variations in the AT-1R have also been suggested to impact the efficacy of antihypertensive agents in decreasing arterial stiffness as reflected by changes in PWV.[29] Thus, genetic variation in *RAAS* gene expression seems to impact arterial and cardiac stiffness and CVD, and this variance may be an important consideration in regard to genetic susceptibility and ultimately the development of precision medicine–based treatment approaches. It should, however, be acknowledged that other studies have questioned a direct relationship between polymorphisms and single-nucleotide polymorphisms in RAAS components and hypertension,[30] indicating the need for further research in this area.

Changes in DNA methylation patterns and histone structure seem to affect specific transcriptional mechanisms that can trigger cardiac pathologies.[31] For instance, histone acetylation plays a critical role in upregulation of insulin-like growth factor II receptor that is related to cardiac hypertrophy, fibrosis, and cardiomyocyte apoptosis.[32] Histone methyltransferase trithorax domain 1 mediates Ang II–induced endothelin-1 transcription and cardiac hypertrophy in mice.[33] Thus, these epigenetic modifications are involved on the regulation of cardiac structure and function. Titin is a sarcomeric protein that regulates cardiac stiffness through its isoforms, which include the larger and more compliant N2BA containing both N2A and N2B segments and the smaller N2B isoform containing only the N2B segment in heart.[34] Recent research indicates that RBM20 (RNA-binding protein 20), a titin splicing factor, is a major regulator of titin isoform transition and cardiac function.[35] In primary cultured neonatal rat cardiomyocytes expressing RBM20, triiodothyronine treatment increased the smaller N2B isoform expression which led to impaired cardiac relaxation.[36] In contrast, increased N2B expression did not occur in RBM20 knockout rats despite triiodothyronine stimulation.[37] However, in a mouse model of heart failure, deletion of the RNA recognition motif of the splicing factor RBM20 upregulated compliant titins and reduced diastolic fibrosis and stiffness,[38] suggesting that manipulation of the RBM20-based splicing system may have a beneficial role in improvement of cardiac diastolic function.

Insulin Resistance and Type 2 Diabetes Mellitus

Epidemiological studies have shown that insulin resistance is an independent risk factor for increased arterial and cardiac stiffness and HFpEF. In this regard, a cross-sectional study of 4701 men and women, aged 45 to 64 years, found that individuals with noninsulin-dependent diabetes mellitus, or borderline abnormal glucose intolerance, displayed abnormally increased arterial stiffness and that this abnormality occurred independent of arterial wall thickness.[39] Data from the Framingham Heart Study suggested that diabetic patients had a 2- to 8-fold increased of risk for developing heart failure and that 19% of diabetic individuals presented with heart failure symptoms.[40] A putative mechanism linking these observations is that insulin resistance and diabetes mellitus induce RAAS activation that promotes activation of the mammalian target of rapamycin/S6 kinase 1 signaling pathway, resulting in arterial and cardiac stiffness and HFpEF.[1]

Obesity

Obesity and associated activation of the RAAS promotes cardiovascular remodeling and stiffness and predicts the risk of subsequent morbidity and mortality.[41] Both Ang II and Aldo are synthesized by adipose tissue, especially visceral adipose tissue.[42] A recent population study found that skinfold thickness was a predictor of abnormally increased arterial stiffness in 100 patients diagnosed as having essential hypertension.[43] The data from the Framingham Offspring and Third Generation cohorts in a total of 3001 individuals indicated that periaortic adipose tissue volume was associated with increased arterial stiffness even after adjusting for sex, age, and other CVD risk factors.[44] A further Framingham Heart Study report indicated that body mass index was a strong independent risk factor in the development of cardiac hypertrophy, diastolic dysfunction, and heart failure.[45] Furthermore, each increment of 1 in body mass index increased the occurrence of heart failure 5% for men and 7% for women after adjustment for other risk factors, including dyslipidemia, diabetes mellitus, and hypertension,[46] suggesting that obesity may contribute to cardiovascular remodeling and stiffness through increased activation of adipose tissue and systemic RAAS.

Hypertension

Hypertension is associated with impaired vascular function that is characterized by component cell dysfunction, increased vascular contraction, fibrosis, and arterial remodeling.[47] Established essential hypertension is associated with increased arterial stiffness and peripheral resistance. For example, 1 study found that there was an increase in forearm vascular resistance in young normotensive men with first-degree relatives experiencing hypertension.[48] At the molecular and cellular levels, changes in cytoskeletal organization, cell-to-cell connections, cell calcification, inflammation, matrix protein composition, collagens, and fibronectin are involved in the hypertension-induced arterial fibrosis, remodeling, and stiffness.[49] The pathophysiological changes include narrowing of arteries and reduced vasodilator capacity.[48]

High blood pressure is also associated with cardiac structural and functional changes including LV hypertrophy. Typically, hypertension increases hemodynamic overloading and wall stress that lead to the parallel addition of sarcomeres, widening of the cardiac myocytes, and consequent of concentric hypertrophy.[1] One study showed that young to middle-aged patients genetically predisposed for essential hypertension display an increased augmentation index and LV mass index,[50] suggesting that a hypertensive phenotype is an important contributor to the development of cardiac stiffness and diastolic dysfunction.

Aging

Framingham study data indicated that advancing age was significantly associated with higher carotid-femoral PWV and mean arterial pressure in healthy populations.[51] Recent data also indicate that aging is also an important factor in the prevalence of HFpEF.[52] Previous clinical research showed that the early cardiac diastolic filling rate gradually slows after age 20 years and is reduced to 50% by 80 years of age.[52] Furthermore, the cardiomyopathy of aging is characterized by myocardial fibrosis, hypertrophy, and a predisposition toward cardiomyocyte apoptosis and autophagy.[53] The specific abnormalities that predispose to in cardiovascular stiffness and associated dysfunction include ventricular artery stiffening, cardiac calcium dysregulation, and decreased β-adrenergic reserve, all important contributors to the development of HFpEF in aging.[54] That these age-related abnormalities are associated with RAAS activation is supported by a recent study showing that compared with younger mice those aged 24 months, expressions of Ang II and the AT-1R were increased whereas that of the Mas receptor and AT-2R were decreased.[55] Meanwhile, progression of vascular and cardiac fibrosis and stiffness as well as a decline in exercise capacity with aging were prevented in the vascular smooth muscle cell (VSMC) gene MR deletion mouse model.[56] These studies underscore the role of an activated RAAS in promoting age-related increases in vascular and cardiac stiffness.

Sexual Dimorphism in RAAS Activation and Associated Vascular and Cardiac Dysfunction

Recent studies highlight a sex difference in RAAS activation. For instance, increased AT-2R expression contributes to the cardiovascular protection in premenopausal women.[57] Furthermore, reduced AT-2R expression may contribute to the CVD risk after the onset of menopause in women.[57] In a cross-sectional community study of 2042 subjects, men had a higher systolic blood pressure until middle age compared with women, but this was not the case in postmenopausal women.[58] Typically, the female heart is characterized by development of concentric hypertrophy that is linked to an increase in wall thickness, cardiac stiffness, and initial preservation of ejection fraction. In contrast, the male heart, as well as the hearts of female mice with metabolic disease, develop a more eccentric hypertrophy that is associated with progressive LV dysfunction and heart failure.[59] This helps explain the fact that while CVD risk is decreased in premenopausal women, this protection is lost in states of obesity, diabetes mellitus, and postmenopausal status.

Mechanisms by Which RAAS Activation Induces Arterial and Cardiac Stiffness

Stiffness of cardiovascular tissues is a complex property determined by cellular components, extracellular matrix (ECM), and matrix-cell interactions.[60] This involves both active and passive tissue/cellular properties[60] and is also impacted by factors, such as activation of the sympathetic nervous system (SNS), input from immune cells, and levels of oxidative stress. Such determinants of arterial and cardiac stiffness are briefly described below in the context of RAAS involvement. For more general information on determinants of vascular stiffness, the reader is referred to several recent reviews.[61–63]

Local RAAS and Cross-Talk Between Aldo and Ang II Signaling in Cardiovascular Tissue

In addition to the classical systemic effects of RAAS, the role of local regulation of RAAS signaling within cardiovascular tissues is increasingly being recognized. Tissue RAAS has been demonstrated in the heart, vasculature, and perivascular

adipose tissue.[62] Furthermore, cell-specific RAAS signaling is evident in VSMCs and endothelial cells (ECs), cardiomyocytes, macrophages, and adipocytes.[64] As both ECs and VSMCs express the enzyme, 11-β hydroxysteroid dehydrogenase, MR signaling is specific for Aldo and not mediated via glucocorticoids.[1] Importantly, and in relation to the current discussion, regulation of RAAS in the vasculature is impacted by diet, hyperinsulinemia, and hyperglycemia all of which likely contribute to the high cardiovascular risk of obesity and insulin resistance.[1,65] Beyond tissue- and cell-specific considerations, further complexity arises from interactions that occur between elements of the RAAS.[66,67] Improvement of Ang II–induced vascular and diastolic dysfunctions by MR antagonism and suppression of Aldo-induced dysfunctional vascular responses by AT-1R blockers in vitro and in vivo are consistent with cross-talk between Aldo and Ang II signaling.[62,68] Although such interactions have been largely related to VSMC function, recent studies demonstrate additive roles for AT-1R and MR activation in vascular remodeling and endothelium dysfunction.[69,70] Importantly, study of the local effects of RAAS signaling in cardiovascular tissues, including both cardiovascular cells and tissue stiffness, has been facilitated recently by the availability of cell-specific knockout animal models.[56,71,72]

Sodium Homeostasis and the EC Sodium Channel

Increased renal Na^+ reabsorption and impaired pressure natriuresis are major contributors to the development of high blood pressure and are associated with increased Ang II and Aldo levels. Both Ang II and Aldo can promote Na^+ and fluid retention through activation of renal AT-1R and MR receptors, respectively. RAAS activation promotes epithelial Na^+ channel (ENaC) activity with ENaC, thereby promoting reabsorption of Na^+ in the collecting ducts of the kidney.[73] Importantly, ENaC is also expressed in nonepithelial cells, including ECs and VSMCs. Recently, considerable interest has focused on the role for endothelial ENaC (EnNaC) activation in the development of increased arterial stiffness.[72] Similar to ENaC, MRs are expressed in ECs, VSMCs, cardiomyocytes, fibroblasts, and immune cells and have been suggested to contribute to increases in both vascular and cardiac fibrosis/stiffness. MR activation in ECs also plays an important role on initiating cardiac fibrosis and diastolic dysfunction by activating EnNaC which leads to development of a stiff endothelium and impaired eNOS (endothelial nitric oxide [NO] synthase) activation.[72,74] As in the kidney, RAAS activation induces serum and glucocorticoid-regulated kinase 1 activation which impairs ENaC ubiquitination/degradation, leading to its accumulation in the plasma membrane, thus contributing to net increases in Na^+ channel activity.[72] In this regard, ECs are normally protected by a well-developed glycocalyx, which acts, in part, to limit the access of Na^+ into ECs and to promote vasodilation.[75] Ang II and Aldo increase EnNaC activity by several mechanisms. For example, both Ang II and Aldo enhance serum and glucocorticoid-regulated kinase 1 and inhibit EnNaC ubiquinization/degradation by upregulation of E3 ubiquitin ligase (Nedd4-2), resulting in increased EnNaC expression and plasma membrane abundance in ECs that leads to enhanced Na^+ entry, polymerization of G-actin to F-actin, reduction of endothelium eNOS activity, NO production, and the development of vascular stiffness (Figure 1).[75,76] Recent data from our laboratory suggest that Ang II increases EnNaC activity, in part, through increased oxidative stress. This is evidence by our preliminary data showing decreased EnNaC activity by antioxidant tempol in control mice but not in EnNaC knockout mice. Furthermore, our recent investigation has demonstrated both Ang II and Aldo induce an increase in inward Na^+ current and endothelium stiffness that were blunted in endothelial-specific EnNaC α subunit knockout mice (Figure 2). Consistent with this scenario, our recent studies in obese mice fed a Western diet (high in fat and fructose) have shown that inhibition of ENaC with a low dose of amiloride, an EnNaC inhibitor, decreased oxidative stress, arterial fibrosis, aortic PWV, and aortic stiffness without affecting blood pressure.[77] Interestingly, in this model of

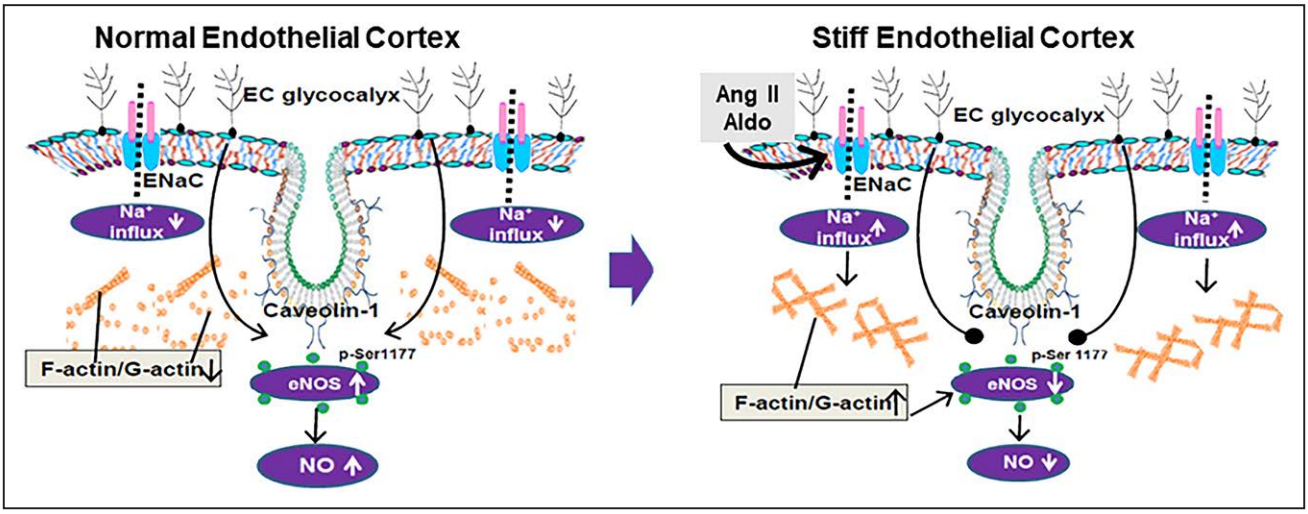

Figure 1. Interactions between endothelial cell (EC) glycocalyx, endothelial Na^+ channel (EnNaC), and eNOS (endothelial nitric oxide synthase) contribute to increases in endothelial stiffness. ECs are protected by a well-developed glycocalyx that restricts the access of Na^+ into the EC and maintains a relative state of vasodilation. Both Ang II (angiotensin II) and aldosterone (Aldo) increase EnNaC membrane abundance in ECs which promotes Na^+ entry into the endothelium and triggers the polymerization of G-actin to F-actin, resulting in a reduction of eNOS activity and nitric oxide (NO) production and a subsequent increase in vascular stiffness.

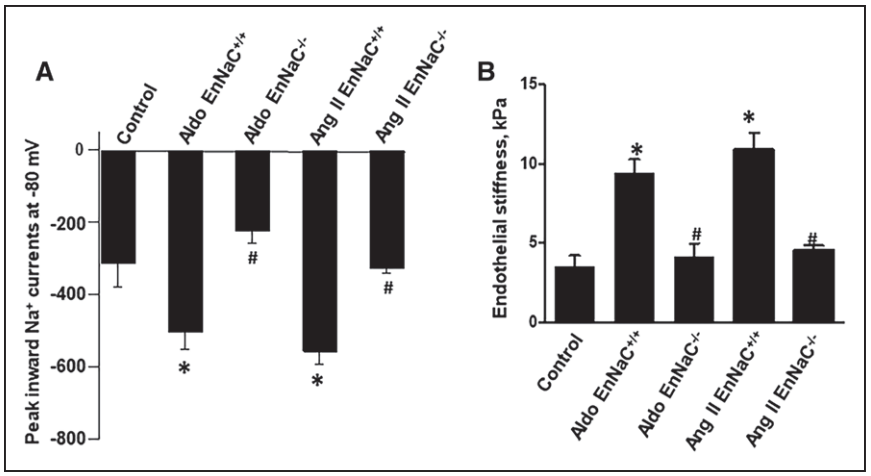

Figure 2. Aldosterone (Aldo), Ang II (angiotensin II), and epithelial Na^+ channel (ENaC) potentiate endothelial cell (EC) Na^+ currents and promote increased endothelium stiffness. Endothelial Na^+ channel (EnNaC; α-subunit) knockout ($EnNaC^{-/-}$) and wild-type ($EnNaC^{+/+}$) mice were treated with Aldo (250 μg/kg per day)/Ang II (500 ng/kg per minute), or vehicle, via osmotic minipumps for 3 wk. Lung ECs were isolated, subjected to short-term culture, and Na^+ currents were recorded using whole-cell patch clamp. Ex vivo aortic endothelium stiffness was determined by atomic force microscopy. **A**, $EnNaC^{-/-}$ inhibited Aldo and Ang II infusion-induced increases in inward Na^+ current, n=7 to 14 cells. **B**, $EnNaC^{-/-}$ inhibited Aldo and Ang II infusion-induced increases in aortic endothelium stiffness, n=3 to 4. *P<0.05 compared with control $EnNaC^{+/+}$ group. #P<0.05 compared with Aldo/Ang II+ $EnNaC^{+/+}$ group.

diet-induced obesity, inhibition of EnNaC with amiloride also reduced coronary endothelium permeability, decreased cardiac macrophage infiltration, and M1 inflammatory polarization, along with attenuated inflammation and oxidative stress.[74] Collectively, these data suggest that ECMR (endothelial cell mineralocorticoid receptor)-mediated EnNaC activation is an important contributor in the development of arterial and cardiac stiffness (Figures 1 and 2).

VSMCs and ECM

VSMCs, as the major cellular component of the arterial wall, are increasingly being recognized as dynamically contributing to overall vessel stiffness through their level of active tone, interactions with ECM proteins, and possibly through their own intrinsic stiffness properties.[78] In addition, VSMCs contribute to mechanical properties of the vessel through structural remodeling as a result of changes in cellular phenotype and production of ECM proteins.[60] Recent studies have shown that VSMCs acutely alter their adhesive interactions with the ECM during active contraction and relaxation. Furthermore, modulation of the elasticity of the cortical cytoskeleton occurs in parallel with the changes in adhesion.[79,80] Thus, in response to acute Ang II application, VSMCs increase their number of integrin-mediated adhesions to the ECM protein fibronectin and increase cellular stiffness.[79,80] MR blockade prevents vascular remodeling, increased VSMC number, and excessive arterial stiffening in diabetic mice.[81] Interestingly, aging is associated with stiffening of VSMCs, as reflected by an increase in elastic modulus, and increased expression of cellular actin and β 1 integrin.[78,82] The age-related effects on VSMC adhesion and stiffness are potentiated by the coexistence of hypertension.[83]

Integrin-ECM adhesive interactions also play a role in Ang II/Aldo-mediated proliferation of VSMCs.[84] In particular, there is a requirement for α5β1 and α1β1 integrins in Ang II activation of extracellular signal-regulated kinase signaling and cellular proliferation.[84] Ang II further causes VSMCs to synthesize ECM proteins (including collagen and fibronectin) and activation of the ECM-modifying matrix metalloproteinases.[61] However, SMCMR (smooth muscle cell mineralocorticoid receptor) knockout prevents Aldo and salt-induced expression of α5-subunit integrin and arterial stiffening in conduit arteries.[85] These mechanisms likely contribute to the more chronic modulation of vascular wall plasticity and potentially contribute to changes in the mechanical properties of the vessel. Collectively, these studies also underscore the importance of interactions between cellular components of the vessel wall and the ECM. Importantly, ECM-VSMC signaling occurs bidirectionally; that is, exhibiting both inside-out and outside-in characteristics. Furthermore, chronic modification of the vessel wall, as reflected by stiffening, has the potential to modify cellular signaling through receptors, such as the AT-1R, as it has been shown to be mechanically activated[86–88] and that such activation impacts VSMC actin polymerization.[87]

Sympathetic Nervous System

Interactions between an activated RAAS and SNS have been well studied in the setting of CVD. Recent studies have provided evidence that activation of RAAS induces cardiovascular dysfunction through enhanced SNS activity. Although Ang II does not cross the blood-brain barrier, Ang II is sensed by the area postrema and subfornical organ that exist outside the blood-brain barrier and provide information to the hypothalamus and brain stem under normal physiological circumstances.[89] However, increased Ang II levels have been reported to increase permeability of the blood-brain barrier and activate the SNS, which then results in increased renin secretion and Na^+ retention.[89] Aldo also plays a key role in central SNS activation, and this is closely related to the salt-induced hypertension. Aldo infusion enhances muscle SNS activity and impairs baroreflex responses,[90] whereas inhibition with the MR antagonist spironolactone prevents chlorthalidone-induced activation of the SNS activation in patients with hypertension.[91]

Oxidative Stress

Considerable evidence supports the notion that activation of the RAAS induces oxidative stress, which subsequently contributes to cardiovascular inflammation, fibrosis, and dysfunction. One of the most important enzymes involved in generating reactive oxygen speciesis nicotinamide adenine dinucleotide phosphate oxidase. Nicotinamide adenine dinucleotide phosphate oxidase can be activated by both Ang II and Aldo through several mechanisms. Aldo increases expression of p47phox through both AT-1R–dependent and MR-dependent mechanisms and expression of nicotinamide adenine dinucleotide phosphate oxidase 2 isoform and the p22phox subunit through an MR-dependent mechanism in rat

vascular tissues.[92] The signaling pathways of cyclooxygenase 2, the mitochondrial monoamine oxidase, and p66Shc signaling are involved in Ang II–induced activation of nicotinamide adenine dinucleotide phosphate oxidase induction and oxidative stress in cardiovascular tissue.[93] Recent data suggest that increased reactive oxygen species inactivates NO limiting NO bioavailability, thereby impairing endothelium-dependent relaxation.[94] Our recent studies have shown ECMR activation induces oxidative stress, vascular fibrosis, stiffness, and impairment of flow-mediated mesenteric artery relaxation in conjunction with reduced eNOS activation and bioavailable NO.[72] Oxidative stress–induced decreases in bioavailable NO leads to coronary functional changes that promote cardiac stiffness and diastolic dysfunction.[74] To this point, the cytoskeletal protein titin regulates the molecular spring in the sarcomere, and reduced bioavailable NO increases the ratio of stiff titin isoform N2B/N2BA (compliant) expression and contributes to cardiomyocyte stiffness and diastolic dysfunction.[1] Reduction in bioavailable NO also increases intracellular calcium and calcium sensitization in cardiomyocytes by inhibition of cyclic guanosine monophosphate/protein kinase G signaling.[1] Indeed, transgenic renin overexpression leads to increased titin-based stiffness and resulted diastolic dysfunction in hypertensive mRen2 rats.[95]

Inflammation

Vascular and cardiac inflammations are important contributors to the development of arterial and cardiac stiffness and associated dysfunction. Increased EC stiffness in coronary artery branches results in release of adhesion molecules and chemokines, which promote monocyte adherence to, infiltration, and MΦ polarization in cardiac tissue. Ang II and Aldo increase the expression of proinflammatory cytokines, including intercellular adhesion molecule-1, vascular cell adhesion molecule 1, tumor necrosis factor-α, interleukin 6, as well as interleukin 8.[74] A generally accepted concept is that activated nuclear factor κ B (NF-κB) is involved in mediating arterial and cardiac stiffness. Typically, while p65 and p50 subunits of NF-κB are maintained in the cytoplasm, IκB α-subunit and phosphorylation of NF-κB lead to translocation of the heterodimer to the nucleus and expression of proinflammatory cytokines.[1] Inhibition of RAAS has been shown to suppress NF-κB activation and prevent diabetes mellitus–induced cardiac diastolic dysfunction.[96] The TLR4 (toll-like receptor 4) is a major component of pathogen-associated molecular recognition receptors and promotes cardiovascular inflammatory responses. Ang II infusion activates TLR4 signaling and induces NF-κB activation and several proinflammatory genes, leading to endothelial dysfunction and vascular remodeling.[97] Ang II also activates MCP-1 (monocyte chemoattractant protein-1) and dysfunction via TLR4 signaling.[98] *TLR4* gene deletion has been shown to prevent isoproterenol-induced cardiac proinflammatory responses and fibrosis.[99] T lymphocytes and T cell–derived cytokines play an important role in the development of hypertension and CVD.[100,101] RAG-1$^{-/-}$ mice, lacking T- and B-lymphocytes, have been found to exhibit blunted responses to Ang II and associated arterial stiffness and hypertension.[100,101] In contrast, deficiency of T-regulatory cells exaggerates Ang II–induced tissue oxidative stress, inflammation, endothelial injury, excessive arterial stiffening, and hypertension.

Cell-specific MR signaling is implicated in MΦ-mediated inflammatory responses, cardiovascular fibrosis, and diastolic dysfunction. For instance, macrophage-specific MR knockout mice administered deoxycorticosterone/salt exhibit normal inflammatory cell recruitment into the heart but are protected from both the full inflammatory and tissue fibrosis responses after cardiac MΦ recruitment that occurs in wild-type littermates.[102] One study showed a role for ECMR signaling in the initiation and early progression of deoxycorticosterone/salt-induced cardiac tissue fibrosis, inflammation, and remodeling through the regulation of chemoattractant signals and ECM turnover.[103] Our recent investigation has shown that consumption of a diet high in saturated fat and refined carbohydrates induces an increase in CD11b, a total macrophage marker in cardiac tissue, and cell-specific ECMR knockout prevents this abnormality.[104]

Vascular and Cardiac Fibrosis

Ang II and Aldo, acting through the AT-1R and MR, respectively, induce fibrosis, in part, by increasing growth factor β 1 (transforming growth factor-β1)/Smad signaling.[1] To this point, transforming growth factor-β1 is expressed in ECs, VSMCs, macrophages, as well as myofibroblasts. Enhanced transforming growth factor-β1 expression and activation are well described to promote synthesis and accumulation of ECM protein.[1] ECM proteins include collagen and elastin that are regulated, in part, by matrix metalloproteinases. Activated matrix metalloproteinases degrade elastin, collagen, and other ECM proteins, leading to cardiovascular remodeling and fibrosis.[1,105] Recently, increased activity of tissue TG2 (transglutaminase 2), an ECM scaffold protein and cross-linking enzyme, was associated with increased cross-linking of collagen, thereby increasing ECM stiffness and cardiovascular dysfunction.[106] TG2 also activates transforming growth factor-β1/Smad signaling to further promote collagen synthesis and fibrosis.[106] This suggests that TG2 activation induced abnormally increased arterial stiffness by promoting VSMC proliferation and matrix remodeling.[106] Consistent with this, inhibition of TG2 decreases cardiac hypertrophy, fibrosis, and diastolic dysfunction in a pressure overload mouse model.[107] Interactions occur between bioavailable NO and TG2, whereby impaired bioavailability of NO is accompanied by impaired S-nitrosylation of TG2 subsequently increasing cellular TG2 secretion/activation which, in turn, leads to increased cross-linking of collagen and elastin.[108] In addition to the actions of TG2, the cross-linking enzyme lysyl oxidase promotes connective tissue cross-linking and thus matrix remodeling and arterial and cardiac stiffness.[109] Overexpression of lysyl oxidase has been shown to accelerate cardiac remodeling and potentiate Ang II–induced cardiac hypertrophy, fibrosis, and impaired relaxation.[109] In contrast, inhibition of lysyl oxidase prevents Ang II–induced arterial remodeling, stiffness, and hypertension.[110] Interestingly, TRAF3 (tumor necrosis factor receptor-associated factor 3) interacting protein 2, a redox-sensitive cytoplasmic adaptor molecule, mediates Aldo-induced cardiac hypertrophy and fibrosis by enhancing expression of NF-κB,

matrix metalloproteinase 2, lysyl oxidase, and inflammatory cytokine.[111] These profibrotic molecules are all important contributors to activated RAAS-induced cardiovascular fibrosis, remodeling, and stiffness.

Dysregulation of Autophagy

Autophagy is essential to maintain cellular homoeostasis and has a protective role in cardiovascular tissue.[112] Under pathophysiological conditions, upregulation of autophagy is an adaptive response.[112] Thus, autophagy is a well-conserved physiological process whereby long-lived proteins and damaged cytoplasmic organelles are degraded with the resultant byproducts used in energy supply and nutrient replenishment.[1] In this regard, activation of RAAS and subsequent hypertension-mediated LV hypertrophy often occur by increases in protein synthesis that form new sarcomeres, thereby promoting cardiac remodeling.[113] Furthermore, isoprenaline, a β-adrenergic agonist, has been shown to reduce the autophagic vacuoles of cardiomyocytes.[114] Meanwhile, suppression of autophagy was noted in pressure-overloaded mouse hearts.[115] Reduction of autophagy might result in the accumulation of harmful proteins, damaged mitochondria, and cardiac dysfunction. Thus, transient suppression of autophagic activity might serve as an early mechanism contributing to the adaptive increase in myocardial pressure overload. With respect to the RAAS and autophagy, reductions in autophagy-related gene 7 in VSMCs have been found to exacerbate Ang II–induced aortic remodeling.[116] Consistent with this concept, activation of autophagy attenuates Ang II–induced cardiac fibrosis.[117] Furthermore, interaction of sirtuin 3 and forkhead boxO transcription factors 1 signaling pathways enhances autophagy and prevents Ang II–induced myocardial hypertrophy and cardiac remolding.[118] Meanwhile, chronic Aldo stimulation increased the ratio of LC3-II/LC3-I expression, which is an indicator of autophagosome formation. These abnormalities were associated with an increase in with cardiac wall thickness, collagen deposition, interstitial fibrosis, and impairment of diastolic dysfunction,[119] suggesting that dysregulation of autophagy contributes to cardiovascular fibrosis and stiffness.

MicroRNAs (miRNA)

miRNAs are short (≈22 nucleotides in length) single stranded noncoding RNAs that act as negative regulators of gene expression through mRNA degradation or inhibition of translation.[120,121] Over- and underproduction of various miRNAs has been implicated in several disease states. Specifically, recently considerable interest has been focused on possible roles for miRNAs in abnormally increased arterial stiffness and remodeling.[120,121] Relevant to this, miRNAs have been shown to impact a variety of signaling pathways in both ECs and VSMCs, as well as in immune cells such as macrophage.[120,121] Moreover, recent data indicate that miRNAs regulate RAAS and RAAS-associated physiological and pathophysiological processes. In kidneys of human subjects with hypertension, miR-663 controlled renin expression and overexpression of renin mRNA induced the different expression of miR-21, -126, -181a, -196a, -451, -638, and -663.[122] In a prospective, single-center, randomized, double-blind clinical trial, aliskiren, a direct renin antagonist, decreased expression of miR-106b-5p, -27a-3p, and -18b-5p that are related to atherosclerotic plaque progression.[123] Meanwhile, miR-124 and -135a showed a potential negative role in MR expression.[124]

Evidence also exists for components of the RAAS system regulating miRNA transcription. For example, AMP-activated protein kinase suppressed endothelial ACE expression by upregulation of miR-43/145.[125] Chronic Ang II infusion increased miR-132 and -212 in the heart, aorta, and kidney of rats with cardiac hypertrophy and hypertension.[126] It has been shown that activation of the VSMC MR suppresses miR-155 leading to upregulation of signaling via L-type Ca^{2+} channels and the AT-1R.[127] This MR-mediated effect was exacerbated in aging and attenuated by cell-specific MR knockout.[127] Similarly, overexpression of miR-155 has been shown to effectively inhibit Ang II–induced myocardial hypertrophy by targeting the AT-1R.[128] MiR-34b/c inhibited Aldo-induced VSMC calcification and stiffness.[129] Collectively, these studies provide an insight into possible mechanisms of interaction of RAAS and miRNA in arterial and cardiac stiffness and CVD.

Therapy in Arterial and Cardiac Stiffness

Carotid-femoral PWV is measured by obtaining a recording of the arterial pulse wave at the common carotid and the femoral by a noninvasive method, and this is recommended by the American Heart Association Council for High Blood Pressure Research,[4] as well as in the European expert consensus document.[130] Echocardiography, computed tomography, and cinematic magnetic resonance imaging have also been applied to detect changes of cardiac structure and function.[131] Studies showing reversal of excessive carotid arterial stiffening after renal transplantation in patients with chronic kidney disease[132] and adrenalectomy in subjects with Aldo producing adenomas[133] are consistent with the likely efficacy of treatment interventions that reduce Aldo signaling. Lifestyle modifications including those involving aerobic exercise, sodium restriction, and healthy diet have been reported to be effective intervention methods in prevention and treating excessive arterial stiffening, hypertension, and heart failure.[134,135] Pharmacological interventions, diuretics, ACE inhibitors, AT-1R, and MR antagonists have shown some effect in decreasing excessive arterial and cardiac stiffening.[9,15,136–139] The Joint National Committee-8 and the European Society of Hypertension recommend that MR antagonists may be considered as the fourth-line drug in the treatment of the resistant hypertension.[140] Clinical trials also suggest that MR antagonists present an effective approach in patients with heart failure.[17]

Conclusions and Future Perspectives

Activated RAAS is an important contributor to changes in arterial and cardiac stiffness, characterized by elevated PWV, increased myocardial stiffness, abnormalities of cardiac diastolic relaxation, and later by hypertension and clinical heart failure. Risk factors, such as genetic determinants, insulin resistance, diabetes mellitus, obesity, aging, and sexual dimorphism, are associated with the development and progression of cardiovascular fibrosis, stiffness, and

CVD. From a mechanistic point of view, sodium homeostasis, EC/VSMC dysfunction, and EnNaC activation of AT-1R and MR receptors lead to fibrosis, and it is likely that dysregulation of autophagy and miRNA are involved in these pathophysiological changes. New drugs targeting underlying inflammation, oxidative stress, and breakers of collagen cross-links, but they are still in early stages of clinical therapy. To date, there is not been large clinical trials that provide the required evidence for the treatment/reversal of excessive arterial stiffening, cardiac diastolic dysfunction, and HFpEF. Using miRNAs as biomarkers to detect arterial and cardiac stiffness may provide a potential new strategy for the early diagnosis and prevention of arterial and cardiac stiffness, hypertension, and heart failure. Further studies will be important to better understand the precise mechanisms by which RAAS promotes abnormally increased arterial and cardiac stiffness.

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Disclosures

None.

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