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Smooth muscle cell mineralocorticoid receptors: role in vascular function and contribution to cardiovascular disease

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Abstract

The mineralocorticoid receptor (MR), a member of the steroid receptor family, regulates blood pressure by mediating the effects of the hormone aldosterone on renal sodium handling. In recent years, it has become clear that MR is expressed in vascular smooth muscle cells (SMC) and interest has grown in understanding the direct role of SMC MR in regulating vascular function. This interest stems from multiple clinical studies where MR inhibitor treatment reduced the incidence of cardiovascular events and mortality. This review summarizes the most recent advances in our understanding of SMC MR in regulating normal vascular function and in promoting vascular disease. Many new studies suggest a role for SMC MR-activation in stimulating vascular contraction, and contributing to vessel inflammation, fibrosis, and remodeling. These detrimental vascular effects of MR activation appear to be independent of changes in blood pressure and are synergistic with the presence of endothelial dysfunction or damage. Thus, in humans with underlying cardiovascular disease or cardiovascular risk factors, SMC MR activation may promote hypertension, atherosclerosis, and vascular aging. Further exploration of the molecular mechanisms for the effects of SMC MR activation has the potential to identify novel therapeutic targets to prevent or treat common cardiovascular disorders.

Keywords

aldosterone; mineralocorticoid receptor (MR); atherosclerosis; hypertension; inflammation; vascular remodeling; vascular aging

Introduction

The mineralocorticoid receptor (MR) is a member of steroid receptor family of hormone-activated transcription factors that includes the estrogen, progesterone, androgen and glucocorticoid receptors (GR). Like all steroid receptors, MR is an intracellular receptor that is activated by binding to its lipophilic ligand resulting in modulation of cellular function at

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least in part by modulating gene transcription. It has been known for more than half a century that MR is expressed in kidney epithelial cells and regulates blood pressure by binding to its ligand aldosterone (Aldo) and stimulating sodium retention [9]. In response to hypotension (or hyperkalemia), Angiotensin II (AngII) is generated and acts on type 1 angiotensin receptors (AT1R) on cells of the adrenal to promote Aldo synthesis and release. MR binds mineralocorticoids, like Aldo, and glucocorticoids, that circulate at much higher concentrations, with similar affinity but the enzyme 11β-hydroxysteroid dehydrogenase 2 (11βHSD2) locally inactivates corticosteroids in Aldo-responsive tissues such as the kidney. Ample clinical data supports a role for Aldo and MR in cardiovascular disease with elevated Aldo levels predicting increased risk of heart attack, stroke, and death [30, 52] and MR antagonist (MRA) drugs preventing mortality [65, 66, 96]. While much is known about MR function in the kidney, over the past two decades, it has become clear that MR also has extra-renal actions [32, 40, 47] that are less well understood. Clinical data supports that modulation of sodium handling alone does not fully explain the role of MR in development of hypertension, vascular dysfunction and cardiovascular disease [81, 82] and that the protective effects of MRA drugs are out of proportion to modest changes in blood pressure [65, 66, 96]. We and others have identified functional MR in human vascular smooth muscle cells (SMCs) [16, 31] supporting the potential for MR to modulate SMC function and contribute directly to vascular dysfunction, hypertension, and cardiovascular disease. This review will summarize recent advances in our understanding of the role of MR in vascular smooth muscle cell function with specific emphasis on how these findings might explain clinical observations and be used to inform future clinical trials and development of novel therapeutic strategies for cardiovascular disease.

SMC MR: an Aldo target that modulates SMC gene expression and function

Over 20 years ago, biochemical methods revealed the presence of mineralocorticoid receptors that bind to Aldo in the vasculature [16, 43] however, their function in this new target tissue was completely unknown. More recently, expression of MR and 11βHSD2 in human SMC has been confirmed along with the functional capacity of SMC MR to respond to Aldo at physiologically relevant concentrations to modulate SMC gene expression. Cytoplasmic MR can also activate rapid signaling pathways via so called “non-genomic” mechanisms that have recently been reviewed elsewhere and hence will not be a focus of this review [85, 92]. In cultured human coronary artery SMC we used a MR responsive element (MRE)-luciferase reporter adenovirus to demonstrate that MR-mediated gene transcription can be activated not only by Aldo, but also by AngII in an Aldo-independent manner [31]. Other steroid receptors can be activated in a ligand-independent manner via post-translational modification and the details by which AngII and other growth factors can directly activate SMC MR remain a topic of active investigation. The cross-talk between AngII and MR in the SMC is likely bidirectional with MR regulating AT1R expression and/or function and AngII modulating MR function (reviewed in [69]). This is likely also independent of local SMC Aldo production that was originally reported but has not subsequently been confirmed (reviewed in [47]).

Treatment of cultured human coronary artery SMC with Aldo also modulated expression of endogenous genes known to contribute to vascular inflammation and extracellular matrix modulation. Specifically, Aldo enhanced SMC expression of type 1 and 3 collagens, important mediators of vascular fibrosis, and of bone morphogenetic protein 2 (BMP2) and alkaline phosphate (ALP), mediators of vascular calcification [31]. Further exploration of the role of SMC MR using an in vitro vascular cell calcification model revealed that MR activation by Aldo or cortisol stimulates vascular ALP activity and SMC mineralization [33]. Recent work in a rat model of vascular calcification found that levels of both AngII and Aldo were significantly higher in calcified compared to control aorta providing *in vivo*

data supporting that vascular MR signaling is involved in vascular calcification [94]. Thus, ligand-activated SMC MR modulates expression of genes that promote vascular fibrosis and calcification. Vascular fibrosis is associated with vascular stiffness and hypertension and vascular calcification is a late stage finding in atherosclerosis, particularly in the elderly and in patients with renal failure, and is associated with the risk of MI and stroke (reviewed in [34]).

More extensive gene expression profiling of whole mouse aortas treated *ex vivo* with Aldo has established the early Aldo-regulated vascular transcriptome. Vascular Aldo-regulated genes are overrepresented in functional pathways important for vascular function including nitric oxide-mediated signaling, regulation of cell cycle, and extracellular matrix [61]. As the mouse aorta is largely composed of SMC, this expression profile represents predominantly expression changes in SMC mRNA. Newfell *et al* provided extensive further exploration of the mechanism of regulation by Aldo of seven genes including: connective tissue growth factor (CTGF), the metalothionine genes (MT1, MT2), Placental Growth Factor (PGF), and the FK506 binding protein 5 (FKBP5). In all cases regulation by Aldo in mouse vessels was completely prevented by MR and transcriptional inhibition, supporting a mechanism involving direct transcriptional regulation by vascular MR. Moreover, expression of the same genes in human aortic tissue from patients with atherosclerosis was decreased by 40–80% by the MRA spironolactone. In addition, most gene expression changes could be reproduced in cultured mouse aortic SMC and removal of the endothelium from whole vessels did not prevent regulation by Aldo, providing further support that these gene expression changes are mediated by SMC MR. Interestingly, for a subset of genes, denudation of the endothelium prior to Aldo treatment enhanced regulation by MR supporting the concept that the healthy endothelium attenuates or prevents some of the effects of SMC MR. Oxidative stress contributed to Aldo regulation of this subset of genes and while the mechanism is not clear, a contribution by vascular NADPH oxidases and protection by endothelial nitric oxide synthase is supported.

Thus, over the past decade it has become clear that vascular SMCs express MR that are capable of responding to Aldo, in some cases to cortisol, and to AngII to modulate transcription of genes that regulate SMC function. The healthy endothelium may modulate MR function in neighboring SMC, perhaps by modulating vascular oxidative stress. The role of these SMC MR-regulated genes and pathways in vascular pathology is reviewed below.

Mechanisms of SMC MR contribution to vascular remodeling

Vascular remodeling is the pathologic response of the vessel to damage and contributes to human ischemic vascular diseases. The vessel is composed of three layers, the inner intima composed of endothelial cells, the media composed of SMC, and the external adventitia containing fibroblasts and extracellular matrix. Remodeling occurs when the endothelial layer is damaged by insults from cardiac risk factors such as cigarette smoke, diabetes, and hypertension or by mechanical injury such as balloon angioplasty and stent implantation. This damage initiates a cascade of events that constitute the vascular injury response, resulting in the stimulation of vascular smooth muscle cells (VSMC) to migrate, proliferate, and produce extracellular matrix. Adverse vascular remodeling limits vascular lumen diameter and increases vascular stiffness thereby contributing to organ ischemia and to hypertension.

Many studies in cultured VSMC have demonstrated a direct mitogenic and pro-fibrogenic effect of Aldo that is mediated by SMC MR and is synergistic with other SMC mitogens including AngII, platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) [20, 31, 53–55, 95]. The mechanism involves rapid non-genomic signaling via MAPK and

the c-Src/RhoA pathways as well as genomic effects that promote expression of genes involved in vascular cell proliferation, migration, and matrix modulation (reviewed in [47]). In addition, multiple animal models support that Aldo exacerbates vascular remodeling after injury and that these effects are reversed by MR antagonist [47, 58, 67, 78, 86, 88]. As one clinically relevant example, the MRA eplerenone attenuated constrictive remodeling and vascular collagen accumulation in pig coronary arteries after angioplasty and stenting [84, 91]. New evidence suggests that vascular remodeling may involve a balance between GR and MR signaling. Using a mouse model of femoral artery wire angioplasty, Inqbal *et al* demonstrate that GR and MR have opposing effects on neointimal proliferation following vascular injury with GR activation or MR inhibition reducing vascular remodeling and GR inhibition promoting it [29]. These effects did not depend on the presence of 11βHSD2 and were therefore likely receptor-rather than ligand-mediated. However, despite ample evidence for a role for MR in adverse vascular remodeling *in vivo*, the detailed molecular mechanism and the direct role of SMC MR are only beginning to be elucidated.

Recently, using a mouse model of localized wire-induced carotid endothelial injury, our lab demonstrated that Aldo infusion, at a low dose that does not raise blood pressure, enhances vascular SMC proliferation and fibrosis specifically at the site of injury but not in the contralateral uninjured vessel. We concluded that Aldo acts independent of blood pressure and synergistically with endothelial injury to promote vascular remodeling [32]. From our investigation of vascular MR-regulated genes, we focused on the vascular endothelial growth factor family member, placental growth factor (PGF), as a potential mediator of Aldo-induced vascular remodeling. PGF is a vascular MR-regulated gene and PGF expression and release from mouse vessels is further enhanced by Aldo when the endothelium is denuded, a condition that also permits Aldo-induced expression of the specific PGF receptor VEGFR1 [32]. Using mice genetically deficient in PGF, we showed that PGF is necessary for Aldo-induced carotid SMC proliferation and fibrosis following wire injury. Moreover, in human aortic tissue from patients with atherosclerosis (but not in healthy human vessels), Aldo regulates PGF and VEGFR1 expression and MR antagonism reduces PGF expression and release by 80 percent [32]. Thus the PGF/VEGFR1 pathway is a renal-independent mechanism for the vascular protective effects of MR antagonists in animal models and potentially also in humans [32]. While we believe that PGF is regulated by MR in vascular SMC, proof of a direct role for SMC MR in vascular remodeling awaits studies in SMC-specific MR knockout mice.

Evidence for a direct effect of Aldo on vascular structure in humans is supported by studies demonstrating that patients with primary aldosteronism have significantly increased vascular thickening compared to other patients with similar degrees of hypertension without aldosterone excess [8, 27, 72]. Excess Aldo is also responsible8, for arterial stiffness and carotid artery fibrosis in these patients [93]. A recent randomized trial in patients with chronic kidney disease demonstrated that MR antagonist treatment reduced the progression of carotid vessel remodeling [87]. The vascular beneficial effects of MRA have been attributed to decreased blood pressure but the benefits seen in such clinical studies are far greater than expected from very modest blood pressure reductions with these drugs. Thus, MR activation contributes to vascular remodeling by acting synergistically with endothelial damage, AngII, and growth factor signaling to promote VSMC proliferation, migration, and extracellular matrix deposition. Blockade of MR may exert beneficial effects by preventing SMC MR-regulation of genes that promote vascular proliferation and fibrosis including PGF, VEGFR1 and others that remain to be fully explored. As the details of the molecular mechanisms by which MR promotes vascular remodeling are clarified, these new pathways will provide novel therapeutic targets to prevent adverse vascular remodeling in humans.

SMC MR in vascular inflammation and atherosclerosis

Atherosclerosis is a chronic inflammatory condition of the vasculature characterized by lipid-rich plaques within the arterial wall (reviewed in [21]). Plaque formation begins with endothelial damage caused by traditional cardiovascular risk factors including hypertension, diabetes, and smoking. In response to these stimuli circulating leukocytes become activated, migrate, adhere to, and enter the vessel wall, and release inflammatory chemokines and cytokines that recruit additional leukocytes and promote fibrosis, lipid accumulation, and SMC proliferation. This results in a plaque with a thrombogenic core of lipids and inflammatory cells covered by a fibrous cap of SMC and matrix. Vascular inflammation further enhances protease activity thereby promoting matrix degradation and plaque rupture. Plaque rupture and associated thrombosis is the cause of most MIs and strokes. Ample clinical data demonstrate that circulating Aldo levels are independent predictors of atherosclerosis complications [23, 71]. Patients with primary hyperaldosteronism have a 4- and 6-fold increased risk of stroke and MI respectively when compared to patients with the same degree of hypertension without Aldo excess [30]. In patients with atherosclerosis, higher serum Aldo levels—even within the normal range—predict a substantial increase in subsequent MI or death [52]. Conversely, drugs that prevent Aldo production (angiotensin converting enzyme inhibitors or angiotensin receptor blockers) or that inhibit Aldo action (MRA) reduce the risk of cardiovascular events and death out of proportion to small reductions in blood pressure (reviewed in [47]). Thus, clinical data support blood pressure-independent effects of Aldo to promote atherosclerosis and plaque rupture in humans (reviewed in [23]).

Animal models of atherosclerosis confirm that Aldo enhances, and MR antagonists prevent, plaque progression *in vivo* [35, 36, 68], but once again, the molecular mechanisms and a direct role for SMC MR have not been elucidated. Aldo has been shown to enhance vascular and macrophage oxidative stress in the apolipoprotein E knockout mouse (ApoE-KO), a well validated model of lipid-induced atherosclerosis that faithfully mimics the human pathology. We have recently confirmed that Aldo infusion at a dose that does not produce sustained blood pressure elevation, promotes atherosclerotic plaque formation, again implicating non-renal MR activation. Aldo specifically enhances early plaque formation in the aortic arch and great vessels, regions of turbulent rather than laminar blood flow [49]. These regions of turbulent blood flow are associated with plaque formation in humans and with expression of pro-atherogenic genes in human vascular cells [13]. These same flow-regulated pro-atherogenic genes (CTGF, MT1, PGF) are also vascular MR-regulated genes with enhanced regulation in the aortic arch relative to the descending aorta [61]. These findings support these genes as potential mediators of Aldo-induced atherosclerosis and suggest a new role for Aldo and SMCMR in determining plaque distribution in animals and in humans.

MR activation in ApoE KO mice, achieved by either Aldo infusion [49] or by 11βHSD2 deletion causing persistent MR activation by cortisol [14], also promotes an unstable plaque phenotype with increased plaque lipid and inflammatory cell content. In humans, such inflamed, lipid laden plaques with thin fibrous caps are associated with plaque rupture and cardiovascular ischemia. Aldo infusion in the ApoE KO mouse specifically promotes vascular monocyte and T cell infiltration prior to accelerating plaque burden only in regions destined to develop Aldo-enhanced atherosclerosis [49]. This suggests the possibility that by inducing vascular inflammation, MR activation might promote unstable atherosclerosis.

In advanced atherosclerosis, SMCs are thought to stabilize plaques by synthesizing collagen and other matrix proteins, preventing plaque rupture [19]. In the setting of mechanical vascular injury, SMC MR-activation increases extracellular matrix production and vascular

fibrosis. Prior to lesion formation, however, vascular SMCs have been shown to upregulate expression of adhesion molecules and cytokines, particularly in the setting of pro-atherogenic hemodynamic flow [22,64]. Thus, SMCs could contribute to leukocyte recruitment during early atherogenesis, while adopting more protective mechanisms as the disease progresses. In support of this hypothesis, we recently demonstrated that conditioned media from human coronary artery SMCs promote monocyte chemotaxis (even without Aldo), suggesting a novel role for SMC in recruiting leukocytes to specific locations in the vasculature [49]. Moreover, when SMC were exposed to Aldo, there is increased production of soluble factors that promote monocyte chemotaxis *in vitro*. This effect is blocked when SMCs are co-treated with the MR antagonist spironolactone, directly implicating human SMC MR in vascular leukocyte recruitment. When monocytic VEGFR1 receptors were blocked with antibodies, SMC MR-induced leukocyte chemotaxis was prevented. Moreover, in vivo, genetic loss of PGF imparted resistance to early Aldo-dependent plaque inflammation in ApoE KO mice [49]. Indeed, atherosclerotic human aorta specimens explanted from patients undergoing coronary artery bypass surgery reveal upregulation of the PlGF/VEGFR1 axis upon treatment with Aldo [32]. In agreement with these findings, both MR antagonism [70] and inhibition of PlGF/VEGFR1 signaling [74] specifically inhibit the early phases of atherosclerosis in animal models. These data suggest that SMC MR-enhanced vascular inflammation may be an initiating step in atherogenesis and that MR antagonists may be a useful therapy for MI and stroke prevention. Future studies with mice specifically deficient in SMC MR will be needed to formally test this possibility *in vivo*.

SMC-mediated neovascularization also contributes to plaque destabilization [25]. Plaque microvessels are poorly formed and are prone to leakage and injury, thereby serving as potential initiation sites for rupture and thrombosis. Since VEGFR1 activation induces macrophage polarization towards the angiogenic M2 phenotype [73] it is possible that SMC MR-mediated PGF secretion contributes to plaque neovascularization that ultimately destabilizes plaques. While a switch to a proliferative phenotype from the normal, contractile state has long been considered a hallmark of SMC function during atherogenesis (reviewed in [17], few studies characterizing the role of MR in SMC phenotype switching in atherosclerosis have been conducted. Similarly, the source of SMC within atherosclerotic plaques remains controversial [63].

Does SMC MR contribute directly to blood pressure regulation?

A primary role for the kidney in blood pressure regulation that has been uncontested for decades has been recently challenged by the idea that primary abnormalities of vascular function could also directly contribute to blood pressure regulation [50]. Mice deficient in the MR in all tissues die in the neonatal period from salt wasting, which is consistent with the known role of the MR in regulating vascular volume [6, 7]. However, mice with renal tubule–specific MR deficiency survive unless challenged with low-salt conditions [75, 76], supporting the concept that loss of extra-renal MR contributes to the hypotension and mortality associated with complete MR deficiency. Whether direct effects of SMC MR activation on vascular reactivity can translate into alterations in systemic blood pressure has only recently been explored. Vascular contraction is initiated by a rise in VSMC intracellular calcium, which occurs as a result of either extracellular calcium entry into the cell via calcium channels following membrane depolarization or mobilization of intracellular calcium stores following activation of membrane receptors by contractile agonists (reviewed in [79]). When intracellular calcium rises, myosin light chain kinase (MLCK) is activated and phosphorylates myosin light chains (MLCs), which causes SMC contraction. Conversely, vascular relaxation is mediated by dephosphorylation of MLCs, a process that is regulated by NO activation of guanylyl cyclase (GC) and by additional calcium signaling pathways (reviewed in [26]). Previous work on the role of SMC MR has

demonstrated that MR activation increases vascular reactive oxygen species (ROS) and decreases bioavailable NO and thus would be expected to promote VSMC contraction by decreasing GC activity. In addition, MR activation in cultured VSMC has recently been shown to reulst in posttranslational modification of soluble GC making it unresponsive to NO and to directly promote calcium mobilization, two mechanisms that would be expected to directly promote SMC contraction [39, 46, 51]. Numerous studies in whole blood vessels also show that SMC MR activation leads to vasoconstriction (reviewed in [47]) and whether this regulation of vascular reactivity can translate into alterations in systemic BP is just beginning to be investigated.

Many studies in rat models of hypertension demonstrate benefits of chronic MR antagonism in lowering blood pressure and improving hypertension outcomes [2, 4, 38, 57, 78, 97]. A newer study found that treatment with spironolactone lowers portal hypertension by reducing fibrosis and inhibiting intrahepatic vasoconstriction via down-regulation of ROCK-2 activity and activation of the NO/PKG pathway [44]. Another recent study evaluated the effects of the novel non-steroidal mineralocorticoid receptor (MR) antagonist SM-368229 and found that it strongly attenuated the progression of hypertension and exerted cardiorenal protection in Aldo/salt-treated hypertensive rats [59]. Similarly, SM-368229 treatment was able to reduce blood pressure in spontaneously hypertensive rats [59]. However, none of these studies directly implicate SMC MR. To address this, we recently developed a mouse model deficient in SMC MR to help understand the direct contribution of vascular MR to blood pressure regulation [48].

Like humans, blood pressure rises with age in control, SMC-MR intact mice but this age-associated increase in blood pressure is lost in SMC-MR deficient mice. The vasoreactivity and tone of resistance vessels (rather than large conduit vessels like the aorta) is thought to be critical in the modulation of total vascular resistance that contributes to systemic blood pressure. Resistance vessels from aged control mice developed augmented agonist-induced contraction with aging but this enhanced contraction with aging was absent in vessels from SMC MR deficient mice. These resistance arteries from aged SMC MR deficient mice had similar structure and stiffness as those from control mice, but they developed significantly less spontaneous myogenic tone, supporting the notion that SMC MR contributes to vascular tone and blood pressure regulation in aged mice independently of structural changes in the vasculature. These blood pressure phenotype in the SMC-MR deficient mice is independent of sodium intake and renal MR function is intact supporting a renal-independent role for SMC MR in blood pressure regulation. Voltage-gated calcium channels have a well-characterized role in the development of myogenic tone. The expression and activity of the Cav1.2 subunit of the L-type calcium channel was reduced in vessels from SMC MR deficient mice suggesting that SMC-MR regulation of vascular calcium channels may participate in age-associated alterations in myogenic tone, agonist-induced contraction, and blood pressure.

We have also previously demonstrated that MR in human SMC can be directly activated by AngII [31]. New evidence for crosstalk between MR activation and AngII signaling comes from studies in Aldo synthase deficient mice. In these studies Aldo deficiency (or treatment with an MR antagonist) prevented angiotensin II-induced cardiac, renal, and vascular injury [45]. This work does not specifically implicate SMC MR signaling, however we recently discovered that many of the detrimental effects of AngII on the vasculature are mediated by SMC MR. AngII infusion causes significant hypertension, vascular contraction, and vascular oxidative stress, all of which are attenuated in young mice lacking SMC MR and prevented in aged SMC-MR deficient mice [48]. These data support the concept that SMC MR mediates AngII-induced vascular damage and inhibition of MR could prevent the adverse sequelae and may even be more beneficial than other renin-angiotensin-system antagonists.

SMC MR as a global regulator of vascular aging

Cardiovascular disease incidence rises substantially with aging and remains the leading cause of morbidity and mortality in Western countries despite a growing number of effective treatments and preventative therapies. Hypertension is an extremely prevalent aging-associated cardiovascular risk factor affecting two thirds of people over 60 years old and almost 80 percent of those over 80 [18, 28]. This rise in BP with age contributes substantially to the incidence of heart attack, stroke, atrial fibrillation and kidney and heart failure in the aging population worldwide [18, 42]. MRA therapy has been effective in the treatment of congestive heart failure and hypertension in several clinical trials [3, 12, 15] and has clearly been shown to decrease cardiovascular risk and significantly impact morbidity and mortality. Recent advances in our understanding of the specific role for SMC MR signaling in aging may help elucidate the beneficial effects of MRA therapy.

We recently discovered that many aspects of cardiovascular aging are prevented in our model of mice lacking MR in the SMC. As described above, these mice do not experience the rise in BP or enhanced vascular contraction associated with age. The attenuated AngII signaling in SMC MR deficient mice is also noteworthy because AngII-signaling is enhanced in the aging vasculature [11, 83, 89, 90]. In rodents AT1R antagonism prevents many aspects of vascular aging and chronic AT1R blockade or $AT1_AR$ deletion prolongs lifespan [5, 10, 41] supporting a critical role for AngII-signaling in vascular aging. Thus emerging data suggests that SMC MR mediates AngII-induced vascular damage and a better understanding of the crosstalk between the SMC MR and AT1R pathways could further elucidate mechanisms of vascular aging.

With aging, the structure of the arterial wall also changes with increased fibrosis, enhanced inflammation, and calcification of the vessel wall. The intima-medial thickness triples between the ages 20 and 90 [56, 62]. These age-related changes result in increased arterial stiffness leading to increases in systolic blood pressure and poor organ perfusion [80]. One prior study in cultured SMC showed that MR expression and signaling are increased in aortic SMC from aged compared to adult rats and that MR antagonism reverses the pro-inflammatory and pro-fibrotic phenotype of aged SMC back to a “young SMC phenotype” [37]. In elderly SMC MR deficient mice (18-month-old) we observed a trend towards a decrease in aortic collagen content compared to aged matched controls suggesting that SMC MR may play a direct role in vascular fibrosis although further study is needed. As mentioned previously there have also been several studies indicating that SMC MR activation contributes to the process of vascular calcification providing further evidence for the role of SMC MR signaling in age-associated vascular remodeling. Cardiac hypertrophy also occurs with aging, at least in part in response to rising BP, and contributes to diastolic heart failure, another syndrome that is common in the elderly (59% incidence in people over 85 years of age), but for which therapy is limited[33,34]. Aging-associated cardiac hypertrophy in elderly control mice was also prevented in age-matched SMC MR deleted mice. These findings suggest that SMC MR signaling may play a role in the pathogenesis of age-related vascular stiffness and cardiac hypertophy, important contributors to diastolic heart failure, a common condition in the elderly for which MR antagonists are being tested.

Thus many aspects of cardiovascular aging may be attributed to activation of SMC MR, supporting the concept that SMC MR is a global regulator of vascular aging with important clinical implications. The recent evidence implicating SMC-MR-regulation of calcium channel activity in the regulation of blood pressure would support future clinical trials of combination therapy with MR antagonism and calcium channel blockade as a novel strategy in patients with resistant hypertension or isolated systolic hypertension in the elderly. MR antagonism to prevent the progression of hypertension and vascular aging has potential to

reduce mortality associated with cardiovascular diseases and to extend lifespan. The development of new therapies targeting SMC MR signaling would have other potential beneficial effects on the vasculature, including attenuation of age-associated increases in blood pressure, atherosclerosis, vascular contraction, vascular fibrosis and calcification, and oxidative stress. Further exploration of the molecular mechanisms of SMC MR signaling will not only improve our understanding of vascular function but also has tremendous potential to identify novel downstream drug targets to prevent or treat aging-associated cardiovascular diseases.

Perspective

Why is there a mineralocorticoid receptor in vascular SMC that promotes vascular contraction, inflammation, proliferation, and fibrosis specifically in the setting of vascular injury? The MR gene is thought to have evolved by gene duplication of the GR gene during fish evolution and the unique ability of MR to respond to mineralocorticoids evolved at the time when organisms emerged from the sea onto land [1]. Evolution of a hormone to activate this receptor in the setting of hypotension by promoting sodium retention would have tremendous survival advantage in a land environment of relative sodium and water scarcity to prevent death from dehydration. As higher organisms evolved, MR also provided selective advantage in the setting of injury with blood loss, another potential cause of hypotension and death prior to reproductive age. In the setting of such a vascular injury, expression of a MR in SMC that would mediate vascular contraction to angiotensin II would contribute to maintenance of blood pressure to vital organs until the kidney could increase blood volume. In this setting of acute endothelial damage from injury, recruitment of leukocytes to the area of injury and localized vascular SMC proliferation and fibrosis would promote wound healing increasing the likelihood of surviving the event.

In Western society today, dehydration and acute untreated blood loss is rare. Instead, we live in an environment of chronic high sodium intake and diffuse endothelial damage caused by obesity, diabetes, smoking and other risk factors. In this setting of diffuse vascular endothelial dysfunction or damage, SMC-MR-activation promotes vascular inflammation contributing to plaque instability and atherosclerotic complications, vascular fibrosis that contributes to vascular stiffness with aging, SMC proliferation that contributes to adverse vascular remodeling after stent placement, and enhanced vascular contraction that contributes to resistant hypertension and aging-associated blood pressure elevation. With our current lifestyle, the formerly protective SMC MR becomes a driver of cardiovascular disease in growing populations. Inappropriately elevated Aldo levels are present in 6% of hypertensives and over 20% of people with resistant hypertension. Obesity is associated with elevated Aldo levels due to production of Aldo-releasing factors from adipocytes and the hemodynamics of congestive heart failure promote hyperaldosteronism. Until we can dramatically change the risk factor profile in developed societies, inhibition of SMC MR and the downstream pathways it regulates in the vasculature might provide protection from vascular ischemic events including heart attack and stroke, hypertension, and vascular aging.

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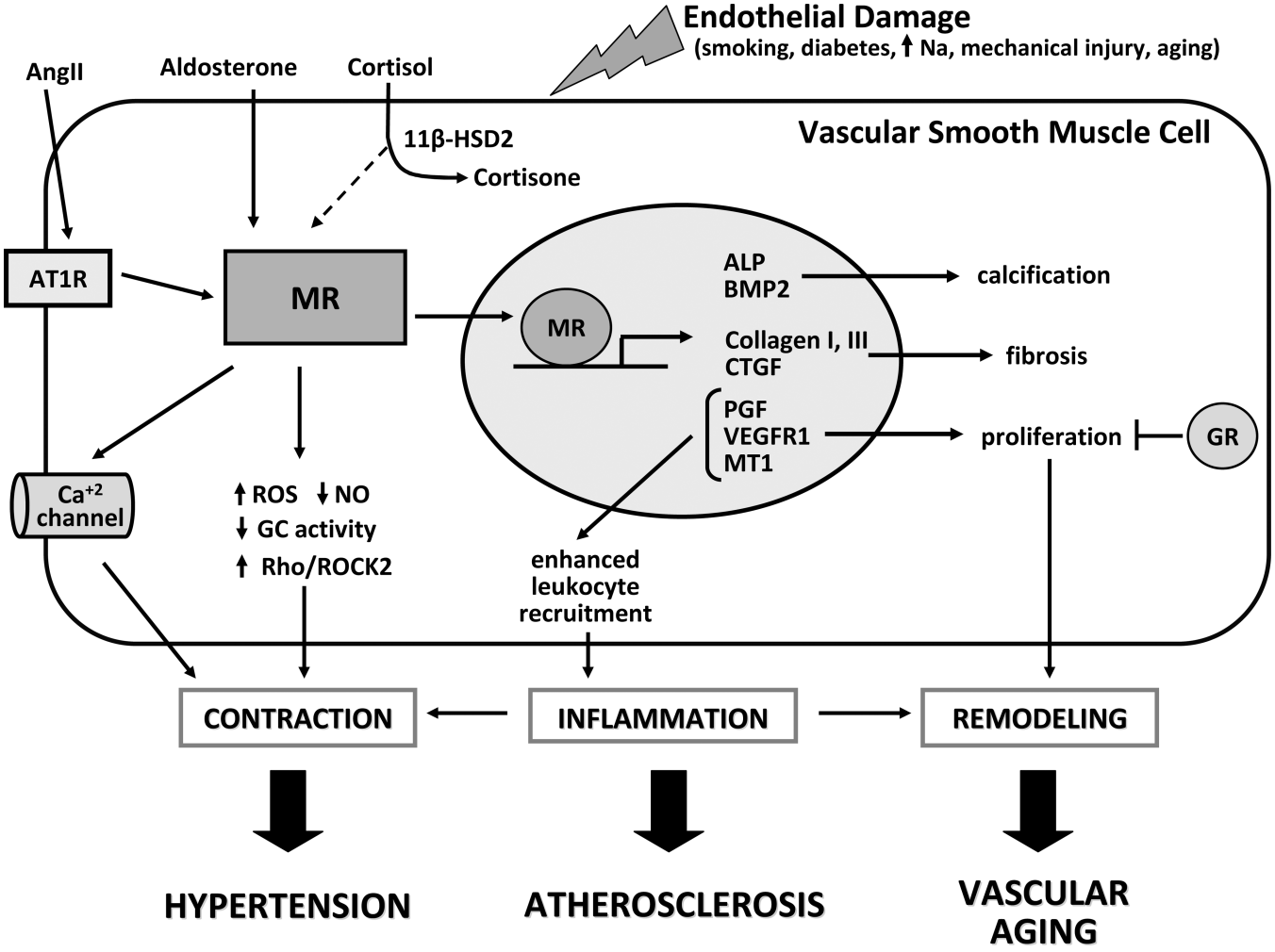

Figure. 1.

Diagram represents signaling of the mineralocorticoid receptor (MR) in vascular smooth muscle cells. MR activation is stimulated by endothelial damage, AngII signaling and aldosterone binding leading to signaling through genomic and non-genomic mechanisms. This signaling causes enhanced vascular contraction, inflammation and remodeling resulting in hypertension, atherosclerosis and vascular aging. ROS, reactive oxygen species; NO, nitric oxide; GC, guanylyl cyclase; GR, glucocorticoid receptor; ALP, alkaline phosphate; BMP2, bone morphogenic protein 2; CTGF, connective tissue growth factor; PGF, placental growth factor; VEGFR, vascular endothelial growth factor receptor; MT1, metalothionine 1