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Mineralocorticoid Receptors in Vascular Function and Disease

Amy McCurley[1] and **Iris Z. Jaffe**[1,2]

[(1)]Molecular Cardiology Research Institute, Tufts Medical Center, Boston, Massachusetts, USA

[(2)]Department of Medicine, Division of Cardiology, Tufts Medical Center, Boston, Massachusetts, USA.

Abstract

The mineralocorticoid receptor (MR), a member of the steroid receptor family, regulates blood pressure by mediating the effects of the hormone aldosterone (Aldo) on renal sodium handling. Over the past decade, it has become clear that MR is expressed in the cardiovascular system and interest has grown in understanding the direct role of the MR in regulating vascular function and contributing to cardiovascular disease. This interest stems from multiple clinical studies in which drugs that decrease MR activation also reduce the incidence of heart attacks, strokes, and mortality out of proportion to modest changes in systemic blood pressure. The presence of functional mineralocorticoid receptors in vascular smooth muscle and endothelial cells is now well established and, while still controversial, data supports the vasculature as an Aldo-responsive tissue. This review summarizes recent advances in our understanding of the role of vascular MR in regulating normal vascular function and in promoting vascular disease. *In vitro* data, *in vivo* animal studies, and human data are reviewed suggesting a role for MR-activation in promoting vascular oxidative stress, inhibiting vascular relaxation, 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, vascular MR activation may promote vascular aging and atherosclerosis thereby contributing to the pathophysiology of heart attack, stroke and possibly even hypertension. Further exploration of the molecular mechanisms for the detrimental vascular effects of MR activation has the potential to identify novel therapeutic targets to prevent or treat common cardiovascular disorders.

Keywords

mineralocorticoid receptor; aldosterone; endothelial function; vascular smooth muscle cells; atherosclerosis; oxidative stress

[*]Correspondence to: Iris Z. Jaffe, M.D./Ph.D. Tufts Medical Center, Molecular Cardiology Research Institute 800 Washington St, Box 80, Boston, MA 02111. Phone 617 636-0620, Fax 617 636-1444, ijaffe@tuftsmedicalcenter.org..

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Introduction: A Role for Mineralocorticoid Receptors in Clinical Vascular Outcomes

The mineralocorticoid receptor (MR), a member of the steroid receptor family, was identified 25 years ago as a critical regulator of blood pressure (BP) by mediating the effects of the hormone aldosterone (Aldo) on renal sodium handling (Rogerson and Fuller, 2000; Arriza et al., 1987). It has since become clear that MR is expressed in non-epithelial cells and interest has grown in understanding the direct role of MR in regulating vascular function and in contributing to cardiovascular diseases. This interest stems from multiple clinical studies demonstrating that inhibition of the renin-angiotensin-aldosterone system (RAAS) prevents vascular ischemic events (heart attacks and strokes) and cardiovascular mortality in diverse patient populations (The SOLVD Investigators., 1991; The SOLVD Investigators., 1992; Dagenais et al., 2001; Dahlof et al., 2002; Pitt et al., 1999; Pitt et al., 2003; Zannad et al., 2011). These benefits were initially attributed to the BP-lowering and potential cardiac remodeling effects of RAAS antagonism with secondary vascular consequences. However, the vascular benefits of MR antagonism in these clinical trials are significantly greater than that expected from the modest decrease in systemic BP with inconsistent effects on cardiac remodeling (Udelson et al., 2010), supporting a direct role for vascular MR-activation in vascular pathology. Additional studies directly support that RAAS antagonists have BP-independent effects on vascular remodeling and cardiovascular events (Dahlof et al., 2002; Lonn et al., 2001). Although the physiologic ligand for vascular MR is still controversial and will be discussed in this review, the importance of understanding the direct vascular effects of Aldo-activated vascular MR has been recently highlighted by clinical data supporting that autonomous Aldo elevation may contribute to a larger fraction of essential hypertension than previously appreciated (Gonzaga and Calhoun, 2008) and by the failure in clinical trials of the HDL-raising CETP-inhibitor torcetrapib, likely due to an off target rise in serum Aldo. In the torcetrapib trials, despite significant improvements in cholesterol profiles (Nissen et al., 2007; Bots et al., 2007; Barter et al., 2007), post hoc analyses support that electrolyte and serum evidence of even mild Aldo excess correlated with increased carotid intimal thickness, coronary atherosclerosis, cardiovascular ischemic events, and mortality in patients with underlying cardiovascular risk factors (Nicholls et al., 2008; Vergeer et al., 2008; Duriez, 2007).

Many studies from the 1970s to the 1990s explored the effects of Aldo on the vasculature and this work has been reviewed previously (Rocha and Funder, 2002). This review specifically focuses on advances in the past decade in our understanding of the role of MR in directly regulating vascular function and in promoting vascular disease. MR activation may alter vascular function via genomic mechanisms, in which MR functions as a ligand-activated transcription factor to modulate vascular gene expression, and by non-genomic, rapid effects of MR that intersect with multiple important vascular signaling pathways including those of epidermal growth factor (EGF), platelet derived growth factor (PDGF), insulin-like growth factor (IGF), and Angiotensin 2 (Ang2). A complete discussion of the non-genomic actions of Aldo and MR is beyond the scope of this review and is summarized elsewhere in this issue. In each section of this review, the most recent data regarding the role of MR will be summarized first in vascular cells *in vitro*, followed by *in vivo* studies in animal models, and finally in human subjects.

MR Expression and Function in the Vasculature

The blood vessel is a layered structure with an inner intima, composed of a single layer of endothelial cells (EC) that line the lumen and contact circulating blood, a medial layer, composed of vascular smooth muscle cells (VSMC), and an outer adventitial layer, containing fibroblasts and extracellular matrix (ECM, see model in Figure 2, top). In the late

1980s and early 1990s, studies demonstrated Aldo binding and MR expression in vascular cells and in whole vessels from animals and humans (reviewed in (Lombes et al., 2000)). More recently, it has been confirmed that endogenous MR in human vascular cells and vessels can directly respond to ligand to regulate vascular-specific gene expression programs that can modulate vascular cell functions involved in cardiovascular pathophysiology (Jaffe and Mendelsohn, 2005; Jaffe et al., 2007; Jaffe et al., 2010; Caprio.M. et al., 2008; Newfell et al., 2011).

Two endogenous ligands, Aldo and cortisol, bind to human MR with equal affinity (Arriza et al., 1987). Although plasma glucocorticoid concentrations are higher than those of Aldo, Aldo-responsive tissues, such as the kidney, maintain Aldo responsiveness by expressing the cortisol-inactivating enzyme 11-beta-hydroxysteroid dehydrogenase type 2 (11ßHSD2, (Funder et al., 1988)). Although still debated, the expression and function of 11ßHSD2 in VSMC and EC has now been demonstrated in multiple studies from many groups supporting the idea that the vasculature is an Aldo-responsive tissue (Kornel, 1994; Brem et al., 1998; Alzamora et al., 2000; Hatakeyama et al., 2000; Kayes-Wandover and White, 2000; Christy et al., 2003; Jaffe and Mendelsohn, 2005; Caprio.M. et al., 2008). Indeed clinical studies demonstrate that even modest increases in serum Aldo levels, as in patients with heart failure, hypertension, or those treated with torcetrapib, produce BP-independent alterations in vascular function lending support for a role for Aldo in directly regulating vascular function *in vivo* in humans(Farquharson and Struthers, 2000; Gonzaga and Calhoun, 2008; Nissen et al., 2007; Bots et al., 2007; Barter et al., 2007; Nicholls et al., 2008; Vergeer et al., 2008; Duriez, 2007). However, the possibility still exists that under specific conditions or in specific subsets of vascular cells, cortisol could activate vascular MR. For example, in a subset of cultured aortic SMC prone to calcification, 11ßHSD2 expression and function are decreased compared to unselected aortic SMC(Jaffe et al., 2007). Whether subsets of vascular cells respond to cortisol *in vivo* is not known, and if cortisol does act as a vascular MR-ligand under specific conditions, the differential vascular effects of Aldo- versus cortisol-bound MR remain to be explored. In addition, as with other steroid receptors, MR can be activated by ligand-independent mechanisms. MR-mediated gene transcription in human vascular cells has been shown to be activated by direct action of Ang2 (and other factors in serum) via hormone-independent mechanisms that remain to be clarified (Caprio.M. et al., 2008; Jaffe and Mendelsohn, 2005).

Aldo is produced in the zona glomerulosa of the adrenal gland. Extra-adrenal synthesis of Aldo in tissues including the heart has also been reported (Silvestre et al., 1998; Slight et al., 1999). Expression of Aldo sythase (CYP11B2) and production of Aldo, was originally reported in vascular cells and vessels (Hatakeyama et al., 1994; Takeda et al., 1995a; Takeda et al., 1995b; Takeda et al., 1996; Kayes-Wandover and White, 2000), however, subsequent studies have failed to demonstrate Aldo biosynthesis in the vasculature (Ahmad et al., 2004; Gomez-Sanchez et al., 2004; Jaffe and Mendelsohn, 2005) and the physiological relevance of vascular Aldo production remains controversial. Regardless of the Aldo source, it is now generally accepted that vascular cells contain functional MR capable of responding directly to Aldo through genomic and non-genomic mechanisms to regulate normal vascular function and contribute to cardiovascular disease.

MR, Aldosterone, and Vascular Oxidative Stress

The production of reactive oxygen species (ROS) by the vasculature, termed vascular oxidative stress, is a critical determinant of vascular function and a significant contributor to vascular pathology. Vascular oxidative stress is determined by the balance between vascular damaging ROS and vascular protective nitric oxide (NO). ROS are produced by vascular oxidases including NADPH oxidase, xanthine oxidase, mitochondrial oxidases, and by

uncoupling of nitric oxide synthases (NOS) to produce ROS instead of NO. The interaction of ROS with NO also decreases the bioavailability of NO thus further increasing oxidative stress and resulting in impaired EC-dependent vasorelaxation, a marker of endothelial dysfunction. In addition, peroxinitrite, formed by the interaction between ROS and NO, can directly alter many vascular cell functions. Recent reviews address the mechanisms of oxidative stress generation in the vasculature and the role of oxidative stress in cardiovascular disease(Touyz and Briones, 2011; Forstermann, 2010). This section will focus on the relationship between MR signaling and oxidative stress and how this interplay contributes to vascular damage and disease.

Studies exploring the role of MR in promoting vascular oxidative stress are summarized in Table 1. MR activation in VSMC and EC, increases ROS production by increasing the expression and activity of NADPH oxidases (Caprio.M. et al., 2008; Nagata et al., 2006a; Iwashima et al., 2008a; Fiebeler and Luft, 2005; Callera et al., 2005a; Callera et al., 2005b). In EC, Aldo also reduces expression of glucose-6-phosphate dehydrogenase (G6PD), a critical regulator of the intracellular redox state, thereby increasing oxidative stress (Leopold et al., 2007). Although there is some conflicting data in this area, most studies support that MR activation in EC decreases eNOS activity and NO production, further contributing to oxidative stress ((Liu et al., 2003) (Mutoh et al., 2008; Nagata et al., 2006a)and reviewed in (Leopold, 2009)). MR-enhanced vascular oxidative stress has important consequences for vascular cell function. In addition to inhibiting endothelial vasodilatory function, oxidative stress stimulates pro-inflammatory and pro-thrombotic pathways in endothelial cells, important contributors to vascular disease progression and morbidity. In SMC, oxidative stress promotes cell proliferation and a recent study supports a role for oxidative stress in MR-stimulated VSMC migration and proliferation *in vitro* (Montezano et al., 2008). Oxidative stress may also modulate MR-mediated vascular gene expression with important implications for vascular disease. We recently characterized the Aldo-regulated vascular transcriptome in mouse vessels and identified a subset of pro-atherogenic genes with enhanced Aldo-stimulated, oxidative stress-dependent expression in the setting of vascular injury and in areas predisposed to atherosclerosis (Newfell et al., 2011).

Multiple *in vivo* studies in diverse animal models support a role for MR-induced vascular oxidative stress in the mechanisms of hypertension and of Aldo-mediated vascular damage (Table 1). In animal models of mineralocorticoid-induced hypertension, the rise in BP was accompanied by increased oxidative stress and in addition, vascular ROS production and hypertension were inhibited by NADPH-oxidase inhibitors(Nishiyama et al., 2004; Iglarz et al., 2004; Beswick et al., 2001; Hirono et al., 2007). MR antagonist treatment decreased NADPH-oxidase activity, increased eNOS activity, and decreased BP in models of both hypertension and atherosclerosis (Keidar et al., 2003; Keidar et al., 2004; Sanz-Rosa et al., 2005b; Thai et al., 2006; Suzuki et al., 2006). Together, these studies support that MR activation *in vivo* enhances vascular oxidative stress via NADPH oxidase thereby contributing to increased BP and atherosclerosis.

There have been few studies addressing the interaction between MR signaling and oxidative stress in patients with cardiovascular disease. In patients with metabolic syndrome, a population with increased vascular oxidative stress and at high risk for cardiovascular disease, treatment with the ACE inhibitor quinapril reduced markers of vascular oxidative stress(Khan et al., 2004). A recent clinical trial in patients with chronic kidney disease demonstrated that treatment with an MR antagonist decreased oxidative stress, as measured by urinary isoprostanes, supporting a potential role for MR antagonists as clinically effective antioxidants(Renke et al., 2008).

The Role of MR in Vascular Constriction and Relaxation

While still controversial, it has recently been postulated that hypertension could arise from changes in vascular tone, independent of alterations in renal function (Mendelsohn, 2005). The presence of functional MR in blood vessels supports the possibility that MR activation could directly modulate vascular reactivity, and potentially BP, via vascular mechanisms in addition to regulation of renal sodium homeostasis. Vascular relaxation is mediated by dephosphorylation of myosin light-chain in SMC, a process that is regulated by NO activation of guanylyl cyclase (GC) and by calcium signaling (reviewed in (Hofmann, 2005)). As summarized in Table 1, MR activation in vascular SMC and EC increases 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 may directly promote SMC contraction by post-translational modification of soluble GC making it unresponsive to NO or by directly promoting calcium mobilization ((Maron et al., 2009; Leopold, 2009; Michea et al., 2005), see Table 2).

Our understanding of the immediate response of the vessel to acute MR activation had been limited by seemingly contradictory results of *ex vivo* vessel studies (reviewed in (Leopold, 2009)). Although the different outcomes may be due to differences in study design (vascular bed, species, duration and dose of Aldo exposure (Table 2, columns 1 and 2)), in virtually all studies, the effects of Aldo are MR-dependent, implicating vascular MR in direct regulation of vascular tone (Liu et al., 2003; Mutoh et al., 2008; Uhrenholt et al., 2003). Interestingly, when Aldo was infused into vessels intraluminally to target the endothelium and compared to Aldo added to the water bath to target VSMC, a vasodilator response was found with intraluminal administration that required the presence of the endothelium, MR, and NO generation via NOS (Heylen et al., 2009). Denudation of the endothelium or co-incubation with NOS inhibitors resulted in a loss of vasodilation and/or enhanced contraction, again implicating endothelial MR in vasodilation and SMC MR in vasoconstriction (Heylen et al., 2009; Schmidt et al., 2003). Studies in the renal vasculature also vary with one study demonstrating Aldo-induced vasoconstriction(Arima et al., 2003) and another supporting relaxation(Uhrenholt et al., 2003). Importantly, the demonstration of MR expression and responsivness of the renal vasculature to Aldo supports the possibility that Aldo and MR could also modulate renal function via a vascular mechanism.

The effects of MR activation on vascular reactivity in healthy humans also remains somewhat controversial due to conflicting results from clinical studies with many demonstrating a constrictive response (Farquharson and Struthers, 2002; Schmidt et al., 1999; Romagni et al., 2003) and some showing vascular relaxation(Nietlispach et al., 2007). The discrepancies may be due to differences in the vascular health of the study participants in addition to differences in dose and duration of Aldo infusion (Table 2). However, when patients with underlying cardiovascular diseases are studied, including patients with atherosclerosis, heart failure, and hypertension, the data are quite consistent with MR-activation promoting increased systemic vascular resistance and reduced forearm blood flow(Wehling et al., 1998; Gunaruwan et al., 2005) and MR antagonism producing improved endothelium-dependent vasodilatation, independent of changes in BP(Farquharson and Struthers, 2000; Macdonald et al., 2004; Nishizaka et al., 2004b). The aggregate of the data supports that in healthy vessels, acute MR activation may evoke endothelium-dependent, NO-mediated vasodilatation while, in the presence of endothelial dysfunction, vascular injury, or high vascular oxidative stress (as in patients with cardiovascular risk factors), MR activation promotes vasoconstriction (reviewed in(Skott et al., 2006)).

Whether the direct effects of MR activation on vascular reactivity translate into alterations in systemic BP has yet to be clearly established. A meta-analysis of two trials studying

eplerenone treatment in uncomplicated essential hypertension demonstrated that some of the effects of MR antagonism on BP can be distinguished from those on urinary electrolyte excretion supporting a vascular contribution of MR to BP regulation(Funder and Mihailidou, 2009; Levy et al., 2004). Additional support comes from a recent study of a mouse model with inducible over-expression of human MR in only endothelial cells resulting in increased BP, independent of changes in renal sodium transport (Nguyen Dinh et al., 2010). Further studies in vascular-specific MR deficient mice will help to clarify the potential role of endogenous vascular MR in the regulation of blood pressure.

MR and Vascular Inflammation

Vascular inflammation plays a critical role in the pathogenesis of cardiovascular diseases including atherosclerosis and hypertensive vasculopathy. Direct activation of MR in human SMC and EC *in vitro* has been shown to promote inflammatory gene expression. Specifically, MR activation in human EC promotes expression of intracellular and vascular cell adhesion molecules (ICAM1 and VCAM1) resulting in enhanced leukocyte adhesion to human coronary EC (Caprio.M. et al., 2008; Deuchar et al., 2011). Exposure of human coronary artery SMC to Aldo stimulates expression of interleukin-16 and cytotoxic T-lymphocyte–associated protein 4(Jaffe and Mendelsohn, 2005) and aged rat aortic SMC demonstrate enhanced MR activation associated with increased inflammatory marker expression(Krug et al., 2010).

A pro-inflammatory role for MR in animal models of atherosclerosis has been demonstrated in mice, rabbits, and non-human primates(Keidar et al., 2004; Rajagopalan et al., 2002; Suzuki et al., 2006; Takai et al., 2005; Keidar et al., 2003). In these models, Aldo administration increased atherosclerotic burden and MR antagonists decreased atherosclerotic lesion size, oxidative stress and inflammation marker expression. More recently, the role of MR in atherosclerosis was explored in an apolipoprotein E deficient mouse model with total body genetic deletion of 11βHSD2, allowing for MR activation by glucocorticoids. In this model, MR activation was associated with atherosclerotic plaques enriched in macrophages and lipids with a relative decrease in collagen content (Deuchar et al., 2011). This pro-inflammatory plaque phenotype is associated with plaque rupture in humans, the cause of most heart attacks and strokes. Since this mouse model also had significant hypertension, likely due to renal MR activation by glucocorticoids, further investigation in alternative models is warranted to determine the direct role of vascular MR activation, in the absence of hypertension, in modulating atherosclerotic plaque phenotype with potentially important clinical implications.

MR signaling also contributes to vascular inflammation in animal models of hypertension. In mineralocorticoid-induced hypertension models, MR activation has been associated with perivascular inflammatory cell infiltration, increased expression of pro-inflammatory factors including ICAM1, monocyte chemoattractant protein (MCP-1), cytokines, and COX-2 in cardiac tissue(Rocha et al., 2002b),(Sun et al., 2002) and increased expression of osteopontin, MCP-1, IL-6, and IL-1β in the kidney(Blasi et al., 2003). In these and other studies, MR inhibition reduced the vascular inflammation and ameliorated cardiac and renal injury even without changes in BP, supporting that MR activation participates in vascular inflammation and damage through a BP-independent, direct vascular process(Joffe and Adler, 2005).

The molecular mechanisms for the pro-inflammatory effects of MR are incompletely understood. MR may directly activate inflammatory gene expression in vascular cells. In mouse and human vessels, vascular MR has recently been shown to upregulate expression of the placental growth factor (PGF, a VEGF family member known to promote monocyte

chemotaxis), via an MR-responsive element in the upstream PGF gene region(Jaffe et al., 2010) and ICAM1 transcription is regulated by Aldo in EC via the ICAM1 proximal promoter(Caprio.M. et al., 2008). The inflammatory effects of Aldo and MR in hypertensive rat models have been shown to involve cross talk with the pro-inflammatory transcription factor NF-κB(Kobayashi et al., 2005; Sanz-Rosa et al., 2005a; Rocha et al., 2002a; Sun et al., 2002). MR and NF-κB signaling also both interact with other inflammatory signaling pathways such as protein kinase Cε, ERK and Rho kinase (Kobayashi et al., 2005). Our understanding of how these multiple inflammatory pathways interact with MR signaling to contribute to vascular inflammation and disease is incomplete and requires further investigation. Finally, the recent demonstration of functional MR expression in cells of the immune system supports that immune cell MR may also contribute directly to vascular inflammation although the mechanisms and resulting outcomes may depend on the specific immune cells involved. For example, deletion of MR from macrophages in a mouse reduces cerebral infarct size and inflammation as well as mineralocorticoid-induced cardiovascular inflammation and fibrosis (Rickard et al., 2009; Frieler et al., 2011; Usher et al., 2010) while a recent study of Aldo-activated neutrophils support an anti-inflammatory role for MR in these cells(Bergmann et al., 2010). Although complete discussion of the role of MR in immune cell function is beyond the scope of this review, it is clear that activation of MR alters inflammatory cell function and this may be mediated by MR in vascular cells, via direct or paracrine mechanisms, as well as by direct activation of MR in the inflammatory cells.

Studies of the pro-inflammatory effects of MR activation in humans have relied predominantly on the measurement of circulating biomarkers of inflammation, many of which have been associated with the development of hypertension and cardiovascular ischemic events(Pickering, 2007). Infusion of Aldo or Ang2 into healthy subjects increased circulating IL-6 concentrations and the Ang2 effect was blocked by spironolactone, suggesting an MR-dependent mechanism(Luther et al., 2006). Treatment with the MR antagonist spironolactone has been shown to reduce MCP-1 and plasminogen activator inhibitor (PAI-1) levels in subjects with type II diabetes and hypertension, respectively(Takebayashi et al., 2006; Ma et al., 2005). In human aortas from patients with atherosclerosis undergoing coronary artery bypass grafting, spironolactone inhibits vascular expression of the pro-inflammatory growth factors PGF and connective tissue growth factor (CTGF) supporting that direct effects of vascular MR-activation may contribute to vascular inflammation in humans(Jaffe et al., 2010; Newfell et al., 2011). Overall, cellular, animal, and human studies support that vascular MR activation participates in the inflammatory response by up-regulating adhesion molecules, chemokines, cytokines, and growth factors that promote the recruitment and activation of inflammatory cells and the proliferation of vascular cells and may contribute to the progression of and complications associated with atherosclerotic vascular disease.

MR and Vascular Remodeling

Vascular remodeling is the pathologic response of the vessel to vascular damage and contributes to human ischemic vascular disease. Remodeling occurs when the endothelium 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 during percutaneous revascularization. This damage initiates a cascade of events that constitute the vascular injury response, resulting in the stimulation of VSMC to migrate, proliferate, and produce ECM. This section will review recent studies exploring the mechanisms by which MR promotes vascular remodeling in animals and in humans.

Studies in cultured VSMC demonstrate a mitogenic effect of Aldo that is mediated by SMC MR and is synergistic with Ang2, PDGF, and EGF signaling (see Model in Figure 1). The mechanism involves activation of multiple signaling pathways including a rapid, non-genomic phosphorylation of MAPK followed by a slower, genomic effect involving Kiras2a and Dusp1(Min et al., 2005; Xiao et al., 2000; Montezano et al., 2008). Crosstalk between Aldo and Ang2 also plays a role in VSMC migration via a c-Src-regulated, redox-sensitive, RhoA pathway(Miyata et al., 2005; Montezano et al., 2008). Ang2, acting via the AT1R, has been shown to directly activate MR-mediated gene transcription in human VSMC supporting a new mechanism for the synergy between Aldo and Ang2 in vascular remodeling(Jaffe and Mendelsohn, 2005). MR activation in vascular cells also directly promotes expression of genes that influence vascular remodeling. In human VSMC, Aldo potentiated oxidative stress-induced collagen and fibronectin synthesis in an EGF-dependent manner(Grossmann and Gekle, 2007; Grossmann et al., 2007; Gekle et al., 2007) and gene expression profiling studies in human vascular cells and mouse vessels implicate MR activation in enhancing expression of genes involved in vascular proliferation, fibrosis and calcification (Jaffe and Mendelsohn, 2005; Jaffe et al., 2007; Jaffe et al., 2010; Newfell et al., 2011).

Multiple animal models support that Aldo exacerbates vascular remodeling in association with endothelial damage *in vivo* and that these effects are reversed by Aldo antagonists, implicating MR in the mechanism(Wakabayashi et al., 2006; Pu et al., 2003; Sanz-Rosa et al., 2005b; Virdis et al., 2002; Nagata et al., 2006b). Specifically, eplerenone has been shown to attenuate constrictive remodeling and collagen accumulation in pig coronary arteries after angioplasty(Ward et al., 2001; Van Belle et al., 1995). An increase in vascular fibronectin content has been reported after Aldo administration and eplerenone treatment has been shown to decrease aortic arterial stiffness, a significant cardiovascular risk factor(Pu et al., 2003; Lacolley et al., 2002). *In vivo* studies have implicated diverse mediators of MR-induced remodeling including endothelin, Ang2, PAI-1, growth factors, and oxidative stress signaling pathways (Figure 1 and reviewed in (Epstein, 2001; Stowasser, 2001)). Several studies have reported that blockade of the endothelin system prevented vascular remodeling in Aldo- and Ang2-induced hypertension(Pu et al., 2003; Virdis et al., 2002; Rajagopalan et al., 1997). More recently, our lab has identified the vascular endothelial growth factor family member, placental growth factor (PGF), as a mediator of Aldo-induced vascular remodeling. Aldo infusion, at doses that did not elevate blood pressure, enhanced vascular SMC proliferation and fibrosis in mouse carotid arteries following wire injury, an effect that was lost in mice deficient in PGF. We further demonstrated that PGF gene expression was directly and transcriptionally regulated by MR in mouse and in human vessels suggesting the PGF/VEGF pathway as a potential renal-independent mechanism for the vascular protective effects of MR antagonists in animal models and in humans(Jaffe et al., 2010).

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 medial thickness and narrowed vessel lumens compared to patients with similar degrees of essential hypertension and other forms of secondary hypertension(Rizzoni et al., 1996; Holaj et al., 2007; Bernini et al., 2008). Excess Aldo is also responsible for arterial stiffness and carotid artery fibrosis in these patients. In a randomized trial of spironolactone in non-diabetic hemodialysis patients, MR antagonism reduced carotid intima-media thickness in treated patients (Vukusich et al., 2010). Thus, MR activation contributes to vascular remodeling by acting synergistically with endothelial damage, Ang2, and growth factor signaling to promote VSMC proliferation, migration, and extracellular matrix deposition. Blockade of the mineralocorticoid receptors may exert beneficial effects by abrogating the MR-induced pathophysiological remodeling. As the details of the molecular mechanisms by

which MR promotes vascular remodeling are clarified, novel therapeutic targets may be elucidated to prevent negative vascular remodeling in humans.

Summary and Conclusions

The presence of functional mineralocorticoid receptors in the vasculature is now well established and, while still controversial, data supports the vasculature as an Aldo-responsive tissue. In vitro, animal, and human data support a role for MR-activation in promoting vascular cell oxidative stress, inflammation, proliferation, migration and ECM production thereby promoting vasoconstriction, atherosclerosis, vascular remodeling and fibrosis (see Model in Figure 2). The detrimental vascular effects of MR activation appear to be independent of changes in blood pressure and are synergistic with the presence of endothelial dysfunction and/or vascular oxidative stress. Thus, vascular MR may contribute to the progression of vascular dysfunction in humans with cardiovascular risk factors that promote endothelial dysfunction. MR activation in these patients may contribute to atherosclerosis and plaque instability, resulting in heart attacks and strokes, to vascular aging with associated vascular stiffness, and possibly even to hypertension. Further investigation of the molecular mechanisms for the detrimental vascular effects of MR activation has the potential to identify novel therapeutic targets to prevent or treat common cardiovascular disorders.

Abbreviations

11ßHSD2	11-beta-hydroxysteroid dehydrogenase type 2
Aldo	Aldosterone
Ang2	Angiotensin 2
AT1R	Angiotensin type1 receptor
BP	blood pressure
CETP	cholesterol-ester-transfer-protein
CTGF	connective tissue growth factor
EC	endothelial cells
ECM	extracellular matrix
EGF	epidermal growth factor
ERK	extracellular signal-regulated kinase
GC	guanylyl cyclase
HDL	high density lipoprotein
ICAM1	intercellular adhesion molecule 1
IGF	insulin-like growth factor
MCP-1	monocyte chemoattractant protein
MR	Mineralocorticoid Receptor
NO	nitric oxide
NOS	nitric oxide synthase
PAI-1	plasminogen activator inhibitor 1
PDGF	platelet derived growth factor

PGF	placental growth factor
RAAS	renin-angiotensin-aldosterone system
ROS	reactive oxygen species
VCAM1	vascular cell adhesion molecule 1
VEGF	vascular endothelial growth factor
VSMC	vascular smooth muscle cells

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MCE 7898 Research Highlights

Mineralocorticoid receptors are expressed in vascular smooth muscle and endothelial cells.

MR-activation promotes vascular oxidative stress vascular contraction.

MR-activation contributes to vessel inflammation, fibrosis, and remodeling.

In humans with cardiovascular risk factors, vascular MR-activation may promote vascular aging and atherosclerosis.

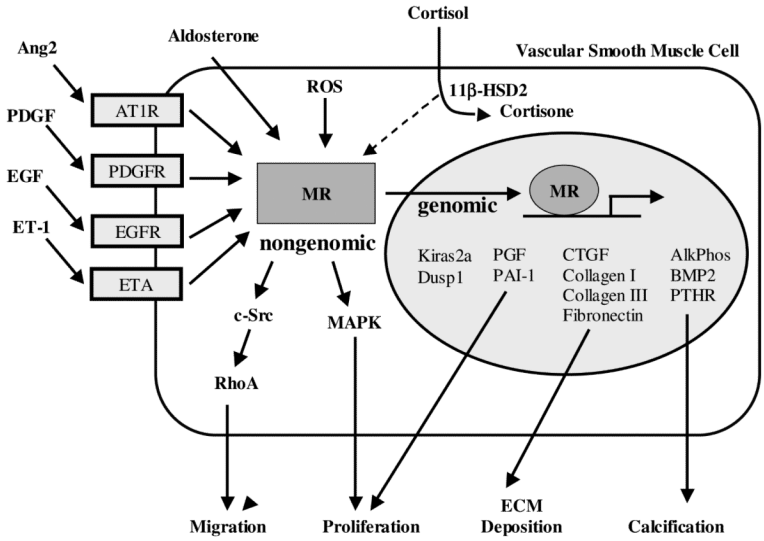

Figure 1.
MR in Vascular Remodeling

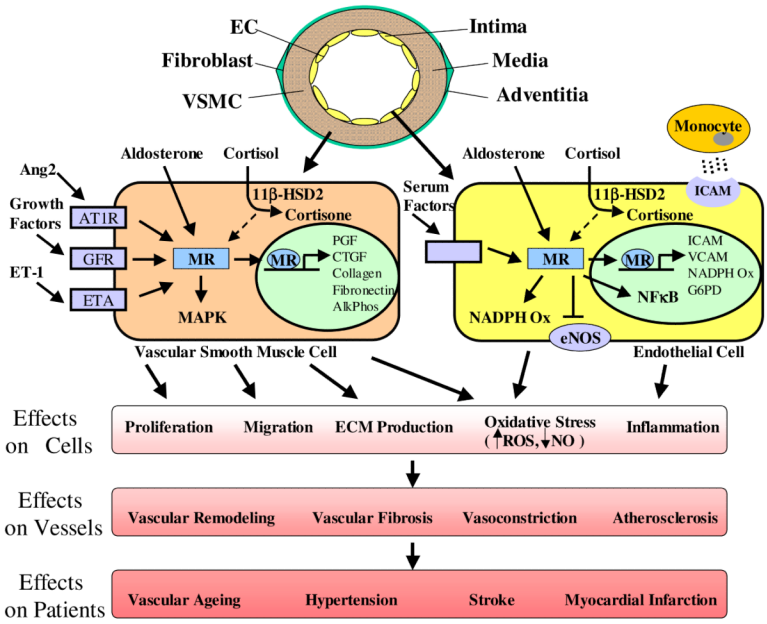

Figure 2.
Mineralocorticoid Receptors in Vascular Dysfunction and Disease

Table 1

Studies of the Role of MR in Vascular Oxidative Stress

Model	Treatment (dose; duration)	Findings	Overall effect of MR activation	References
Endothelial cells	Aldo 0.01-100nM; 10 min	increased NO production/eNOS activity	↑ NO/eNOS	Mutoh et al, 2008; Liu et al, 2003
Endothelial cells	Aldo 100nM; 16 hrs	decreased NO production/eNOS activity	↓ NO/eNOS ↑ROS	Nagata et al, 2006a
Endothelial cells	Aldo 10nM; 3 hrs	increased expression of NADPH oxidase subinit Nox4	↑ ROS	Caprio et al., 2008
Endothelial cells	Aldo 1-100nM; 24 hrs	decreased G6PD expression/activity with increased ROS and reduced bioavailable NO; inhibited by NADPH oxidase or Rac1 inhibitor	↓ NO ↑ROS	Leopold et al, 2007; Iwashima et al., 2008
Vascular smooth muscle cells	Aldo 0.1-100nM; 30-60min	increased ROS production via c-Src, MAPK pathways	↑ ROS	Callera et al, 2005a&b
Vascular smooth muscle cells	Aldo 1-100nM; 24 hrs	increased ROS production	↑ ROS	Maron et al., 2009
Vascular smooth muscle cells	AngII and Aldo (0.1nM), 5-30min	Co-stimulation induced c-Src, ERK1/2, JNK & NADPH oxidase activation, superoxide formation & cell migration	↑ ROS	Fiebeler & Luft, 2005; Montezano et al, 2008
Aldo-salt hypertensive rat model	Aldo 0.75 μg/hr ± 1% NaCl; 3-6 wks	Aldo treatment increased BP and vascular & systemic ROS; abrogated by eplerenone, losartan or tempol (ROS scavenger)	↑ ROS	Nishiyama et al, 2004; Iglarz et al., 2004; Hirono et al 2007
DOCA-salt hypertensive rat model	Apocynin 0.1mM & 1.5mM; acute or 28 days	NADPH-oxidase inhibitor blocked superoxide formation in aortic rings and in whole body and reduced systolic BP	↑ ROS	Beswick et al, 2001
APOE-deficient mouse model	Aldo 2ug/mouse/day; 4 weeks; Eplerenone 200 mg/kg/day; 12 wks	Aldo increased plaque area and oxidative stress; MR inhibition reduced systolic BP, lipid peroxidation, and plaque area	↑ ROS	Keidar et al, 2004; Keidar et al, 2003
APOE-deficient mouse model	Eplerenone 1.67 g/kg/day or Valsartan 0.5 mg/kg/day; 6 wks	Reduced plaque area & expression of oxidative stress-related markers	↑ ROS	Suzuki et al, 2006
Spontaneously hypertensive rat model	Eplerenone 30-100 mg/kg/day; 10 weeks	Reduced systolic BP, aortic media/lumen ratio, NO-dependent vascular relaxation, and systemic oxidative stress	↑ ROS	Sanz-Rosa et al, 2005b
Rat model of heart failure	Spironolactone 7mg/kg/day	Prevented decrease in eNOS in the LV & aorta and improved NO-dependent vasorelaxation	↓ eNOS	Thai et al, 2006
Patients with metabolic syndrome	Quinapril 20mg/day; 4 weeks	ACE inhibition reduced isoprostanes & inreased erythrocyte superoxide dismutase activity	↑ ROS	Khan et al, 2004
Patients with chronic kidney disease	Spironolactone 25 mg/day; 8 weeks	Levels of urinary isoprostanes reduced	↑ ROS	Renke et al, 2008

Table 2

Studies of the Role of MR in Vascular Contraction and Relaxation

Model	Treatment (dose; duration)	Findings	Overall Effect of MR Activation	References
Cultured vascular smooth muscle cells	Aldo 1-100nM; 24 hrs	guanylyl cyclase modified to become NO insensitive	Contraction	Maron et al., 2009
Ex vivo rat aorta	Aldo 100nM; 10 min	enhanced NO-dependent vasorelaxation	Relaxation	Mutoh et al, 2008
Ex vivo rat aorta	Aldo 0.01-100nM; 10 min	EC-dependent attenuation of PE-mediated vasocontriction, blocked by spiro; higher aldo concentrations (100nM) had no effect	Relaxation	Liu et al., 2003
Ex vivo rat mesenteric arterioles	Aldo 0.001-100nM; 30min	NO-dependent increased vasodilation; denuding endothelium elicited a vasoconstrictor response	Relaxation/ Contraction with Injury	Heylen et al, 2009
Ex vivo rabbit aterioles	Aldo 1nM-10μM; 5 min	counteracted KCl-mediated vasoconstriction, blocked by spiro	Relaxation	Uhrenholt et al, 2003
Ex vivo rabbit aterioles	Aldo 1-10nM; 10-60min	MR-independent vasocontriction	Contraction	Arima et al, 2003
Ex vivo rat arterioles	Aldo 10nM; 10-60min	vasocontriction in resistance vessels; blocked by eplereone	Contraction	Michea et al, 2005
Mouse with EC-specific MR overexpression	no treatment	moderate hypertension and increased vasocontriction with PE, AngII and ET1	Contraction	Nguyen Dinh et al, 2010
healthy human brachial artery	Aldo 500ng/min; 8 min	increased forearm blood flow; greater vasoconstriction with L-NMMA & PE	Contraction	Schmidt et al., 2003
healthy human brachial artery	Aldo 12 pM/kg/min; 4 h	attenuated endothelium-dependent vasodilatation; no effect on blood flow	Contraction	Farquharson & Struthers, 2002
healthy human forearm vasculature	Aldo 0.05 or 0.5 mg; 8 hrs	increased systemic vascular resistance	Contraction	Schmidt et al, 1999
healthy human forearm vasculature	Aldo 2.5 pmol/min; 60min	reduced forearm blood flow	Contraction	Romagni et al, 2003
healthy human forearm vasculature	Aldo 3.3 to 55 pM/min; 15min or Fludocortisone 0.3mg/d; 14 d	acute increase in EC-independent vasodilation; chronic increase in EC-dependent vasodilation	Relaxation	Nietlispach et al, 2007
forearm vasculature in men with coronary heart diease	Aldo 1mg; 10min	increased systemic vascular resistance	Contraction	Wehling et al., 1998
brachial artery in men with congestive heart failure	Aldo 10 ng/min; 10 min	reduced forearm blood flow	Contraction	Gunaruwan et al, 2005
forearm vasculature in men with congestive heart failure	Spironolactone 12.5 or 50 mg/d; 1 or 3 months	increased forearm blood flow; greater vasoconstriction with L-NMMA	Contraction	Farquharson & Struthers 2000; Macdonald et al, 2004
brachial artery in subjects with hyperaldosteronism	Spironolactone 12.5 or 25 mg/d; 3 months	increased flow mediated dilation	Contraction	Nishizaka et al., 2004
systemic vasculature of subjects with essential hypertension	Eplerenone 50-200mg/week; 4-12 weeks	80% of patients saw a reduction is systemic blood pressure	Contraction	Levy et al 2004