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The endothelial mineralocorticoid receptor: Contributions to sex differences in cardiovascular disease

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ABSTRACT

Cardiovascular disease remains the leading cause of death for both men and women. The observation that premenopausal women are protected from cardiovascular disease relative to age-matched men, and that this protection is lost with menopause, has led to extensive study of the role of sex steroid hormones in the pathogenesis of cardiovascular disease. However, the molecular basis for sex differences in cardiovascular disease is still not fully understood, limiting the ability to tailor therapies to male and female patients. Therefore, there is a growing need to investigate molecular pathways outside of traditional sex hormone signaling to fully understand sex differences in cardiovascular disease. Emerging evidence points to the mineralocorticoid receptor (MR), a steroid hormone receptor activated by the adrenal hormone aldosterone, as one such mediator of cardiovascular disease risk, potentially serving as a sex-dependent link between cardiovascular risk factors and disease. Enhanced activation of the MR by aldosterone is associated with increased risk of cardiovascular disease. Emerging evidence implicates the MR specifically within the endothelial cells lining the blood vessels in mediating some of the sex differences observed in cardiovascular pathology. This review summarizes the available clinical and preclinical literature concerning the role of the MR in the pathophysiology of endothelial dysfunction, hypertension, atherosclerosis, and heart failure, with a special emphasis on sex differences in the role of endothelial-specific MR in these pathologies. The available data regarding the molecular mechanisms by which endothelial-specific MR may contribute to sex differences in cardiovascular disease is also summarized. A paradigm emerges from synthesis of the literature in which endothelial-specific MR regulates vascular function in a sex-dependent manner in response to cardiovascular risk factors to contribute to disease. Limitations in this field include the relative paucity of women in clinical trials and, until recently, the nearly exclusive use of male animals in preclinical investigations. Enhanced understanding of the sex-specific roles of endothelial MR could lead to novel mechanistic insights underlying sex differences in cardiovascular disease incidence and outcomes and could identify additional therapeutic targets to effectively treat cardiovascular disease in men and women.

Contents

Abbreviations: 11βHSD2, 11β-Hydroxysteroid dehydrogenase 2; Aldo, Aldosterone; AR, Androgen receptor; EC, Endothelial cell; EnNaC, Endothelial epithelial sodium channel; eNOS, Endothelial nitric oxide synthase; ER, Estrogen receptor; ERK, Extracellular signal-related kinase; GPER, G protein-coupled estrogen receptor; HFpEF, Heart failure with preserved ejection fraction; HFrEF, Heart failure with reduced ejection fraction; ICAM-1, Intracellular adhesion molecule-1; MR, Mineralocorticoid receptor; MI, Myocardial infarction; NO, Nitric oxide; PR, Progesterone receptor; ROS, Reactive oxygen species; SMC, Smooth muscle cell; VCAM-1, Vascular cell adhesion molecule-1; ZG, Zona glomerulosa.

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1. Introduction

1.1. Gaps in knowledge of the mechanisms underlying sex differences in cardiovascular disease

Cardiovascular pathologies such as hypertension, atherosclerosis, and heart failure lead to substantial morbidity, and heart disease remains the leading cause of death in both men and women (Xu, Murphy, Kochanek, Bastian, & Arias, 2018). While premenopausal women are protected from cardiovascular disease relative to age-matched men, this protection is lost with menopause, implicating sex hormones in the pathogenesis of cardiovascular disease. As such, the role of sex hormones in the cardiovascular system, particularly signaling through estrogen receptor (ER) isoforms α and β, has been extensively studied (Arnold, Cassis, Eghbali, Reue, & Sandberg, 2017). However, due to the complex nature of sex steroid signaling pathways, the molecular basis for sex differences in cardiovascular disease is still not fully understood, limiting the ability to tailor therapies to male and female patients.

Additionally, common cardiovascular risk factors such as metabolic syndrome and obesity abolish the protection from cardiovascular disease in women even prior to menopause (Barrett-Connor, Cohn, Wingard, & Edelstein, 1991; Sowers, 1998; Wilson, D'Agostino, Sullivan, Parise, & Kannel, 2002), highlighting the need to investigate molecular pathways outside of traditional sex hormone signaling to fully understand sex differences in cardiovascular disease. However, this area is currently understudied in both the clinical and preclinical literature. Generally, the patient cohorts in cardiovascular disease clinical trials are heavily weighted towards male patients, with women constituting only a minority of study participants. Further, most preclinical studies in the cardiovascular field focus on male animals, with very few directly comparing the sexes.

1.2. The mineralocorticoid receptor: Regulator of blood pressure, mediator of cardiovascular disease

The mineralocorticoid receptor (MR) was first described to contribute to blood pressure control by regulating the transcription and expression of sodium transport proteins in the distal nephron (Arriza et al., 1987). Emerging evidence now points to the MR as a broader mediator of cardiovascular disease risk, potentially serving as a sex-dependent link between cardiovascular risk factors and disease (Davel, Jaffe, Tostes, Jaisser, & Belin de Chantemele, 2018). The MR is a transcription factor that can be activated either by glucocorticoids such as cortisol (corticosterone in rodents), which circulate at high levels, or by its more specific but less abundant ligand aldosterone (Aldo) (Funder, 2010). Individual tissues maintain specificity of the MR for Aldo by expression of the 11β-hydroxysteroid dehydrogenase 2 (11βHSD2) enzyme, which converts MR-binding glucocorticoids to metabolites that cannot bind to the MR and thus affords Aldo specificity to the MR (Naray-Fejes-Toth, Colombowala, & Fejes-Toth, 1998).

Independently of the relationship between the MR and blood pressure, elevated serum Aldo levels are associated with a substantially increased risk of stroke, myocardial infarction (MI), and sudden cardiac death (Ivanes et al., 2012; Milliez et al., 2005). Conversely, inhibition of the MR in large randomized clinical trials such as the RALES, EPHESUS, and EMPHASIS-HF results in significant reductions in mortality in heart failure patients. This decrease in mortality is associated with only modest changes in blood pressure along with trends towards decreased MI risk when secondary endpoints are examined (Pitt et al., 1999; Pitt, Remme, et al., 2003; Zannad et al., 2011). As such, substantial investigation in the preclinical literature has focused on understanding the role of MR signaling in non-renal cells in the development of cardiovascular disease, which has the potential to nominate additional therapeutic targets related to MR signaling.

1.3. Vascular cell-specific mineralocorticoid receptors contribute to cardiovascular disease

The vascular wall is made up of three parts: an inner layer of endothelial cells (ECs) that forms the interface between circulating blood and underlying tissues; a medial layer of smooth muscle cells (SMCs) which contract or relax to control vessel diameter thereby regulating blood flow to downstream organs; and an outer layer of adventitial fibroblasts and adipose cells that provide structural support and regulatory mediators to the inner two layers. The innermost EC layer contributes to vasodilation by activating ion channels and releasing paracrine factors to stimulate dilation of the underlying SMCs, including the anti-inflammatory, antioxidant gas nitric oxide (NO) (Vanhoutte, Zhao, Xu, & Leung, 2016). The endothelium also regulates inflammatory cell recruitment by modulating expression of endothelial-leukocyte adhesion molecules and by the generation of reactive oxygen species (ROS) to produce oxidative stress.

The MR is expressed in vascular SMCs and ECs. In its genomic role as a transcription factor, the MR within ECs (EC-MR) regulates genes that contribute to critical EC functions, including expression of inflammatory mediators and regulators of endothelial sodium handling and junctional integrity (Kirsch et al., 2013; Kusche-Vihrog, Callies, Fels, & Oberleithner, 2010; Moss & Jaffe, 2015). EC-MR also contributes to NO bioavailability and oxidative stress via rapid, "non-genomic" signaling outside of its traditional, gene-transcription role (Wehling, 2018). Multiple studies have demonstrated that ECs express 11βHSD2 that is capable of inactivating cortisol (Caprio et al., 2008; Christy et al., 2003; Liu, Mladinov, Pietrusz, Usa, & Liang, 2009), thus it is likely that Aldo is the relevant ligand for EC-MR. However, some studies show low or variable 11βHSD2 expression in ECs that may depend on cell conditions (Gong, Morris, & Brem, 2008), raising the possibility that glucocorticoids may activate EC-MR under certain conditions. Regardless of the ligand, however, studies in mice with EC-specific MR deletion reveal that EC-MR contributes to the cardiovascular pathology that develops in the setting of risk factors such as obesity, diabetes, and hyperlipidemia (Davel, Anwar, & Jaffe, 2017).

In addition to ECs, functional MR is expressed in human vascular SMCs (Jaffe & Mendelsohn, 2005), where it has been shown to contribute to vasoconstriction and blood pressure regulation (Amador et al., 2016; DuPont et al., 2016; Galmiche et al., 2014; McCurley et al., 2012) and to vascular remodeling in response to injury, aging, and hypertension (Galmiche et al., 2014; Kim et al., 2018; Pruthi et al., 2014) *in vivo* in males. *In vitro*, SMC-MR may also contribute to SMC calcification (Jaffe, Tintut, Newfell, Demer, & Mendelsohn, 2007) and cytokine production (McGraw et al., 2013), although it was recently shown not to contribute to the pathogenesis of atherosclerosis in male mice (Moss, DuPont, Iyer, McGraw, & Jaffe, 2018). The MR also contributes to inflammatory phenotypes in a number of leukocyte cell types, such as T cells, neutrophils, and monocytes (Bene, Alcaide, Wortis, & Jaffe, 2014). *In vitro*, macrophage MR contributes to the production of ROS and inflammatory cytokines and promotes pro-inflammatory "M1-like" macrophage polarization (Bene et al., 2014; Usher et al., 2010) and contributes to plaque development in atherosclerosis models

(Shen et al., 2017). Recent *in vivo* studies further implicate T cell MR in the pathogenesis of hypertension (Sun et al., 2017) and pressure overload-induced cardiac dysfunction (Li, Sun, et al., 2017). Although this review focuses on the role of the MR specifically within ECs in cardiovascular disease, additional investigations of the role of the MR in other cell types will certainly provide substantial insight into the mechanisms driving cardiovascular disease.

1.4. Endothelial cell mineralocorticoid receptors in cardiovascular disease: Is there effect modification by sex?

Substantial recent exploration reveals a role for EC-specific MR in endothelial dysfunction, hypertension, atherosclerosis, and heart failure. However, the vast majority of preclinical investigations into the function of EC-MR have been conducted only in male animals, and those that do use female animals do not typically compare them to male counterparts to examine sex differences. However, rare publications in the existing literature that do directly compare the role of EC-MR between males and females reveal striking sex differences in the role of this receptor in the vascular endothelium. Further, critical analysis of studies performed in each sex separately may yield insight into potential sex-specific mechanisms of EC-MR function in the cardiovascular system.

Here we review the recent literature exploring the role of the MR in mediating sex differences in 1) endothelial dysfunction, 2) hypertension, 3) atherosclerosis, and 4) heart failure, with a focus on the MR in the vascular endothelium. The first part of the review focuses on the clinical literature supporting a sex-specific role for the MR in each cardiovascular disorder. The second part examines the preclinical literature specifically assessing the role of EC-MR in animal models of each disease, commenting on effect modification by sex where there are available data. Finally, the third part of this review summarizes the data regarding the molecular mechanisms that may mediate a sex-specific role for EC-MR in cardiovascular disease. The available data supports that EC-MR may be a key player in determining sex differences in cardiovascular disease and reveals many areas warranting further study.

2. Clinical data: Contribution of the MR to cardiovascular disease in men and women

Activation of the MR in the setting of cardiovascular stress or risk factors appears to contribute to the development of cardiovascular diseases. However, whether there is a difference in this role by sex that might contribute to sex differences in cardiovascular disease risk and outcomes is just beginning to be elucidated. In this section, we review the existing clinical literature on the contribution of the MR to 1) endothelial dysfunction, 2) hypertension, 3) atherosclerosis, and 4) heart failure, with a focus on differentiating the role of the MR between men and women. A summary of the clinical studies using MR antagonists cited in this section can be found in Table 1.

2.1. Endothelial dysfunction

2.1.1. Epidemiology

Endothelial dysfunction is marked by impaired endothelium-dependent vasodilation, reduced NO biosynthesis, and increased vascular inflammation and is the earliest measurable defect in the pathogenesis of vascular diseases. A sub-analysis of the offspring of Framingham Heart Study participants found that female sex significantly correlated with defects in endothelial-dependent dilation, while male sex did not (Hamburg et al., 2011). These epidemiologic data suggest sex-specific mechanisms of endothelial dysfunction and support a potential a role for EC-MR, as mineralocorticoid signaling in the endothelium appears to play a substantial role in the development of endothelial dysfunction in the setting of cardiometabolic risk factors (Davel et al., 2017).

2.1.2. The MR may contribute to endothelial dysfunction in the setting of cardiovascular risk factors

Data supports that under baseline conditions without cardiovascular risk factors, the MR does not play a substantial role in vascular dysfunction (reviewed in Biwer, Wallingford, & Jaffe, 2019). Indeed, chronic MR antagonism had no beneficial effect on endothelial function in a group of younger (age 40's) obese subjects without associated diabetes or other cardiac risk factors (Garg, Kneen, Williams, & Adler, 2014), in older (age 60's) otherwise healthy adults acutely administered eplerenone (Hwang et al., 2016), or in a study of 8 older adults with metabolic syndrome (Hwang et al., 2015).

However, several clinical studies do support a role for the MR in the development of endothelial dysfunction when multiple or severe cardiovascular risk factors are present. This is illustrated by one study which found that MR inhibition had no effect on endothelial function in lean older adults but improved endothelial function in older adults with obesity and/or impaired glucose tolerance (Hwang et al., 2013b). Spironolactone improved NO bioactivity and brachial artery endothelial function in two studies of patients with heart failure (Farquharson & Struthers, 2000; Macdonald, Kennedy, & Struthers, 2004). MR inhibition likewise improved coronary flow reserve, a measure of coronary vessel endothelial function, in type 2 diabetics (Garg et al., 2015) and improved brachial artery endothelial function in patients with hypertension (Fujimura et al., 2012). In another study, acute Aldo administration triggered microvascular endothelial dysfunction in normotensive African Americans; conversely, MR inhibition with spironolactone improved resistance vessel endothelial function in *ex vivo* vessels from hypertensive African Americans regardless of gender (Mohandas et al., 2015).

While it seems that premenopausal women are protected from a wide variety of cardiovascular pathologies relative to age-matched men (Benjamin et al., 2018), studies point to a role for the MR in endothelial dysfunction even prior to menopause in women with enhanced cardiovascular risk. For example, young women with polycystic ovarian syndrome, which is characterized by increased androgen synthesis along with other cardiometabolic risk factors such as obesity, diabetes, and hypertension (Marciniak, Nawrocka Rutkowska, Brodowska, Wisniewska, & Starczewski, 2016), have an increased risk of cardiovascular disease. These women also develop endothelial dysfunction (Paradisi et al., 2001), to which the MR may contribute. Aldo levels are elevated in women with polycystic ovarian syndrome compared to weight-matched controls (Cascella et al., 2006), and prolonged treatment with spironolactone was shown to improve endothelial function in a cohort of polycystic ovarian syndrome patients (Studen, Sebestjen, Pfeifer, & Prezelj, 2011). It is important to note that spironolactone also inhibits the androgen receptor (AR) (Yang & Young, 2016), thus it is difficult to distinguish whether the protective effects of spironolactone in this latter study are due to is anti-MR or its anti-androgen effects. However, in the context of rheumatoid arthritis, an autoimmune condition that also confers greater cardiovascular disease risk to women even prior to menopause, spironolactone treatment also significantly improved endothelial function and reduced inflammatory indicators in this predominantly-female cohort (Syngle, Vohra, Kaur, & Sharma, 2009). These data suggest that the MR may contribute to endothelial dysfunction even in premenopausal women if additional cardiovascular risk factors are present (see Table 1).

2.2. Hypertension

2.2.1. Epidemiology

High blood pressure affects 30% of American adults (Fryar, Ostchega, Hales, Zhang, & Kruszon-Moran, 2017). Hypertension increases the risk of MI and stroke, and prolonged exposure to hypertension can lead to heart and kidney failure. Many pharmacotherapies exist to combat hypertension. This includes MR inhibitors, which have been demonstrated to be effective antihypertensive medications in clinical trials (Pitt et al.,

Table 1
Clinical trials of MR antagonism in cardiovascular pathology cited in this review.

Study population	MRA Used	MRA dosing (duration)	Study type	N: Control/MRA	Sex: men/women	Outcome	MRA improved, worsened, or no effect on outcome	Reference
Endothelial dysfunction								
Healthy older adults	Epl	100 mg/day (2 days)	RCT, double-blind, crossover	22/22	8/14	Endothelial function	Worsened	Hwang et al., 2016
Obesity	Epl	100 mg/day (1 month)	RCT, double-blind, crossover	22/22	10/12	Endothelial function	Improved[d]	Hwang et al., 2013b
Obesity	Spiro	50 mg/day (6 weeks)	RCT, double-blind	16/16	10/22	Endothelial function	No Effect	Garg et al., 2014
Metabolic Syndrome	Epl	100 mg/day (1 month)	RCT, double-blind, crossover	8/8	4/4	Endothelial function	No Effect	Hwang et al., 2015
Type 2 diabetes	Spiro	25 mg/day (6 months)	RCT, double-blind	23/17	27/13	Endothelial function	Improved	Garg et al., 2015
Hypertension	Epl	50mg/day (11 months)	Randomized, double-blind, pre-post	40/20[a]	45/15	Endothelial function	Improved	Fujimura et al., 2012
HFrEF	Spiro	50 mg/day (1 month)	RCT, double-blind, crossover	10/10	10/0	Endothelial function	Improved	Farquharson & Struthers, 2000
HFrEF	Spiro	12.5–50 mg/day (3 months)	RCT, double-blind, crossover	43/43	35/8	Endothelial function	Improved	Macdonald et al., 2004
Polycystic ovary syndrome	Spiro	100 mg/day[c] (6 months)	Pre-post	0/30	0/30	Endothelial function	Improved	Studen et al., 2011
Rheumatoid arthritis	Spiro	2 mg/kg/day (12 weeks)	Pre-post	0/24	2/22	Endothelial function	Improved	Syngle et al., 2009
Hypertension								
Class I and II hypertension	Epl	50–200 mg/day (12 months)	Randomized, double-blind, pre-post	246/253[b]	276/223	Blood pressure	Improved	Williams et al., 2004
Hypertension and LV hypertrophy	Epl	200 mg/day (9 months)	Randomized, double-blind, pre-post	54/50[b]	95/58	Blood pressure	Improved	Pitt, Reichek, et al., 2003 (4E)
Resistant hypertension	Spiro or Epl	25 mg/day Spiro or 50 mg/day Epl (3 months)	Retrospective cohort	0/46	30/16	Blood pressure	Improved[e]	Khosla et al., 2009
Atherosclerosis								
Hemodialysis	Spiro	50 mg 3x/week (2 years)	RCT, double-blind	23/30	34/19	Intima-media thickness	Improved	Vukusich et al., 2010
Primary aldosteronism	Epl	50 mg/day (12 months)	Pre-post	0/14	2/12	Intima-media thickness	Improved	Matsuda et al., 2016
Heart failure								
LV dysfunction after MI	Epl	25–50 mg/day (2 years)	RCT, double-blind	3319/3313	4714/1918	Mortality, hospitalization	Improved	Pitt, Remme, et al., 2003 (EPHESUS)
HFrEF	Spiro	25–50 mg/day (2 years)	RCT, double-blind	841/822	1217/446	Mortality, hospitalization	Improved	Pitt et al., 1999 (RALES)
HFrEF	Epl	25–50 mg/day (2 years)	RCT, double-blind	1373/1364	2127/610	Mortality, hospitalization	Improved	Zannad et al., 2011 (EMPHASIS-HF)
HFpEF	Spiro	25 mg/day (4 months)	Open-label, uncontrolled	0/11	0/11	Exercise capacity	Improved	Daniel et al., 2009
HFpEF	Spiro	25 mg/day (12 months)	RCT, double-blind	209/213	201/221	LV structure and function	Improved	Edelmann et al., 2013 (Aldo-DHF)
HFpEF	Spiro	15–45 mg/day (3 years)	RCT, double-blind	1723/1722	1670/1775	Hospitalization	Improved	Pitt et al., 2014 (TOPCAT)
HFpEF	Spiro	15–45 mg/day (3 years)	RCT, double-blind	1723/1722	1670/1775	Mortality	No Effect	Pitt et al., 2014 (TOPCAT)
HFpEF	Spiro	25 mg/day (6 months)	RCT, double-blind	67/64	21/110	Exercise capacity	Improved	Kosmala et al., 2016
HFpEF	Spiro	25 mg/day (9 months)	RCT, double-blind	38/42	16/64	Exercise capacity, quality of life	No Effect	Upadhya, Hundley, et al., 2017
Vascular stiffness								
Healthy subjects aged 55–79	Epl	100 mg/day (1 month)	RCT, double-blind, crossover	23/23	10/13	Aortic stiffness	Improved	Hwang et al., 2013a
Dilated cardiomyopathy	Spiro	25–100 mg/day (6 months)	Randomized, open-label, blinded endpoint	51/51	28/74	Aortic stiffness	Improved	Vizzardi et al., 2015
HFpEF	Spiro	25 mg/day (9 months)	RCT, double-blind	38/42	16/64	Aortic stiffness	No Effect	Upadhya, Hundley, et al., 2017

MR = mineralocorticoid receptor; MRA = MR antagonist; Spiro = Spironolactone; Epl = Eplerenone; RCT = randomized controlled trial; HFrEF = heart failure with reduced ejection fraction; HFpEF = heart failure with preserved ejection fraction; LV = left ventricle; MI = myocardial infarction.

[a] Controls were 40 mg/day nifedipine (n = 20) or 100 mg/day losartan (n = 20).

[b] Control was 10–40 mg/day enalapril.

[c] Treatment paradigm: 21 days on, 7 days off.

[d] Effect in obese > lean, improvement also correlated with fasting glucose levels.

[e] Effect in women > men, obese > lean.

2003; Williams et al., 2004). Despite this, nearly half of hypertensive patients are inadequately controlled with current antihypertensive drugs (Fryar et al., 2017). Emerging data support that therapy-resistant hypertension is more likely to be dependent on MR signaling than therapy-responsive subtypes (Dudenbostel & Calhoun, 2017; Yugar-Toledo, Modolo, de Faria, & Moreno, 2017). Indeed, MR antagonism with the competitive inhibitors spironolactone or eplerenone effectively reduces blood pressure in patients with therapy-resistant hypertension (Fernet et al., 2018; Glicklich & Frishman, 2015; Rossignol et al., 2018).

The prevalence of hypertension in premenopausal women is lower than that of age-matched men, although hypertension still affects nearly 28% of American women. However, after menopause this is reversed, with women 60 years of age and older experiencing significantly higher rates of hypertension than age-matched men. Further, an increase in therapy-resistant hypertension in postmenopausal women (Fryar et al., 2017) suggests that the mechanisms driving hypertension in women may change with age and estrogen status, potentially becoming more dependent on MR signaling after menopause.

2.2.2. The MR contributes to hypertension in women

The MR has long been known to regulate blood pressure via its role in controlling renal sodium balance, and data from human studies supports a role for the MR in blood pressure regulation specifically in women. In a recent study of over 1,500 individuals from the HyperPATH consortium, women had a significantly greater rise in blood pressure compared to men in response to stimuli that increase Aldo secretion including salt restriction (Jurgens & Graudal, 2004) and angiotensin-II infusion (Shukri et al., 2018). Similarly, in a study of obese patients with chronic kidney disease, female subjects experienced a greater decrease in blood pressure with MR antagonism than males (Khosla, Kalaitzidis, & Bakris, 2009). A gene variant of ERβ was also shown to associate with salt sensitivity of blood pressure specifically in premenopausal women (Manosroi et al., 2017), suggesting the potential for cross-regulation of blood pressure by MR and estrogen signaling.

2.3. Atherosclerosis

2.3.1. Epidemiology

Atherosclerosis is increasingly common, and downstream consequences of atherosclerosis, including MI and ischemic stroke, account for a majority of deaths worldwide (Barquera et al., 2015). Atherosclerosis is a diffuse vascular pathology in which inflamed, lipid-laden plaques accumulate in the vascular wall. Under conditions of excess inflammation, these plaques can rupture and thrombose, occluding the vessel and preventing blood flow to downstream tissues. The clinical consequence of this occlusion is ischemia resulting in damage to the brain in stroke, to the heart in MI, and to the skeletal muscle in critical limb ischemia.

It is quite clear that sex differences exist in the incidence of cardiovascular ischemic events in humans, with premenopausal women experiencing significantly fewer of these events than age-matched men (Benjamin et al., 2018). The actions of both estrogen and testosterone have been demonstrated to be beneficial in atherosclerosis in clinical and preclinical models (Boese, Kim, Yin, Lee, & Hamblin, 2017). However, it is not clear whether women's protection from cardiovascular ischemic events prior to menopause is due to less plaque burden or fewer plaque rupture events. One study found carotid intima-media thickness, a clinical index of plaque size, to be only slightly higher in young men than women, while coronary calcium score, which correlates with plaque inflammation and susceptibility to rupture, is substantially higher in men than in women (Benjamin et al., 2018).

2.3.2. MR activation contributes to atherosclerosis in men and women

Clinical studies of the role of the MR in atherosclerosis in human patients tend to combine men and women. However, the available clinical data suggests that in both men and women, Aldo and MR activation contribute to atherosclerosis progression and complications. de Rita, Hackam, and Spence (2012) reported that elevated plasma Aldo concentration significantly correlated with plaque progression while sex and age did not. One study of end-stage renal disease patients, a population at high risk for atherosclerotic ischemic events, analyzed plaques in men and women separately and showed that spironolactone treatment prevented increases in intima-media thickness in both sexes (Vukusich et al., 2010). Finally, Matsuda et al. (2016) demonstrated that eplerenone reduced intima-media thickness in a small cohort of 12 primary aldosteronism patients, 10 of whom were women.

While atherosclerotic plaque progression can contribute to chronic angina and symptoms that reduce quality of life, plaque rupture is more dependent on inflammation and contributes most to the morbidity and mortality associated with atherosclerosis (Libby, Lichtman, & Hansson, 2013). In atherosclerosis patients, Aldo levels correlated with serum inflammatory factors regardless of gender, suggesting a pro-inflammatory role of Aldo in both men and women (Tomaschitz et al., 2011). Further, multiple studies correlate MR activation with increased risk of ischemic events downstream of plaque rupture (Ivanes et al., 2012; Milliez et al., 2005). These studies include both men and women but do not separate the data by sex. Thus, the available data in human observational studies and clinical trials suggests that MR inhibitors could be a useful tool to reduce atherosclerotic plaque progression and complications in both men and women and warrant further clinical study and exploration of the mechanisms by which the MR contributes to vascular disease in both sexes.

2.4. Heart failure

2.4.1. Epidemiology

When the cardiac systolic pump function becomes impaired, this is known as heart failure with reduced ejection fraction (HFrEF). When the heart is unable to fully relax during diastole to allow blood to fill the ventricles, but is still able to contract, this is known as heart failure with preserved ejection fraction (HFpEF). HFrEF and HFpEF each account for about half of the total burden of heart failure (Dunlay, Roger, & Redfield, 2017). Men are somewhat more likely to develop HFrEF, likely due to higher rates of hypertension and MI in younger men, both of which are common causes of HFrEF (Benjamin et al., 2018; Dunlay et al., 2017). By contrast, HFpEF is more common in women and is the most common type of heart failure in the growing population over 65 years of age (Upadhya, Pisani, & Kitzman, 2017). Risk factors for HFpEF include advanced age and obesity, both of which are increasingly common in women (Flegal, Kruszon-Moran, Carroll, Fryar, & Ogden, 2016; Owan et al., 2006; Tsujimoto & Kajio, 2017). Although women with HFpEF generally have improved survival compared to men, this protection is lost in women with diabetes (Martinez-Selles et al., 2012), and women with HFpEF reported reduced quality of life relative to men in a recent study (Faxen et al., 2018). Thus, HFpEF is a growing clinical problem, especially in the rapidly growing elderly population and in women with cardiovascular risk factors.

2.4.2. Clear benefits of MR antagonism in heart failure with reduced ejection fraction

Inhibition of the MR is well known to prevent mortality and improve outcomes in patients with HFrEF (Pitt et al., 1999; Pitt, Remme, et al., 2003; Zannad et al., 2011). The benefits of MR antagonism on HFrEF may apply to both men and women, but the data supporting this is scarce (see Tables 1 and 2). A sub-analysis of the Framingham Heart Study showed that serum Aldo levels correlate with cardiac remodeling in women but not men, suggesting that there may indeed be differences between the sexes in the way the MR signaling pathway contributes to heart failure (Vasan et al., 2004). Despite these data, clinical trials investigating the role of the MR in HFrEF continue to recruit predominantly male subjects. For example, the landmark RALES, EPHESUS, and EMPHASIS-HF trials that showed a clear mortality benefit of MR

Table 2
Clinical and preclinical evidence for a sex-specific role of the MR and EC-MR in cardiovascular disease.

Model	Sex	Intervention	Results	References
Endothelial dysfunction				
MR in humans	Men	Spiro, Epl	MR antagonists improve endothelial function in heart failure, diabetes, hypertension (mostly male cohorts).	Farquharson & Struthers, 2000; Macdonald et al., 2004; Fujimura et al., 2012; Garg et al., 2015
	Women	Spiro	MR antagonists improve endothelial function in women with polycystic ovarian syndrome.	Studen et al., 2011
		Spiro	MR antagonists improve endothelial function in women with Rheumatoid Arthritis.	Syngle et al., 2009
	Both	Spiro	MR antagonists improve endothelial function in hypertensive African American men and women (analyzed separately).	Mohandas et al., 2015
MR in animals	Male	Epl, Finerenone	MR antagonists improve endothelial function.	Rajagopalan et al., 2002; Gonzalez-Blazquez et al., 2018
	Female	Spiro	MR antagonism improves endothelial function in leptin-sensitized obese females.	Huby et al., 2016
EC-MR-KO mice	Male	Tie2 Cre	EC-MR-KO improves endothelial function of the aorta in obesity.	Schafer et al., 2013
		VE-Cadherin Cre	EC-MR-KO improves endothelial function of the mesenteric arterioles in hypertension.	Mueller et al., 2015
		VE-Cadherin Cre	EC-MR-KO does not alter endothelial function of the mesenteric arterioles in obesity or hyperlipidemia.	Davel, Lu, et al., 2018
	Female	VE-Cadherin Cre	EC-MR-KO corrects the endothelial dysfunction with obesity and hyperlipidemia.	Davel, Lu, et al., 2018
Hypertension				
MR in humans	Men	Epl	MR antagonism reduces blood pressure (mostly male cohort).	Pitt, Reichek, et al., 2003
	Women	Salt restriction, angiotensin-II	RAAS stimulation increases blood pressure more in women than in men.	Jurgens & Graudal, 2004; Shukri et al., 2018
		Spiro, Epl	Women with Resistant Hypertension experienced greater blood pressure decrease with MR antagonism than men.	Khosla et al., 2009
	Both	Epl	MR antagonism reduces blood pressure (roughly equal male/female cohort).	Williams et al., 2004
MR in animals	Male	Spiro, Epl	MR antagonism reduces blood pressure in gonad-intact and castrated males.	Michaelis et al., 2012; reviewed in DuPont & Jaffe, 2017
	Female	Spiro	MR antagonism reduces blood pressure in obese females.	Huby et al., 2016
		Spiro	MR antagonism does not reduce blood pressure in ovariectomized females.	Michaelis et al., 2012
		Epl	MR antagonism prevents endothelial tight junction remodeling in the cerebral arteries of hypertensive females.	Tada et al., 2010
EC-MR-KO mice	Male	VE-Cadherin tetOFF overexpression	EC-MR overexpression increases blood pressure.	Nguyen Dinh Cat et al., 2010
		Tie2 Cre, VE-Cadherin Cre	EC-MR-KO does not affect blood pressure at baseline or in disease models.	Rickard et al., 2014; Mueller et al., 2015; Dinh et al., 2016; Lother et al., 2016; Salvador et al., 2017; Laursen et al., 2018
		Tie2 Cre, VE-Cadherin Cre	EC-MR-KO attenuates the pathologic remodeling that occurs with hypertension.	Rickard et al., 2014; Lother et al., 2016; Diaz-Otero et al., 2017; Diaz-Otero et al., 2018
	Female	VE-Cadherin Cre	EC-MR-KO likely does not affect blood pressure at baseline or in disease models.	Jia, Habibi, et al., 2015; Davel, Lu, et al., 2018
Atherosclerosis				
MR in humans	Men	Aldo (observational)	Aldo levels correlate with cardiovascular ischemia (mostly male cohorts).	Milliez et al., 2005; Ivanes et al., 2012
	Women	Epl	MR antagonism reduces IMT in primary hyperaldosteronism (mostly female cohort).	Matsuda et al., 2016
	Both	Aldo (observational)	Aldo levels correlate with larger plaques independently of sex.	de Rita, Hackam, and Spence, 2012
		Spiro	MR antagonism reduces plaque volume in men and women with end-stage renal disease.	Vukusich et al., 2010
		Aldo (observational)	Aldo levels correlate with soluble inflammatory markers independently of sex.	Tomaschitz et al., 2011
MR in animals	Male	Aldo, 11βHSD2-KO	MR activation increases plaque size, inflammation.	Deuchar et al., 2011; McGraw et al., 2013; Marzolla et al., 2017
		Spiro, Epl	MR antagonism reduce plaque size, inflammation.	Rajagopalan et al., 2002; Keidar et al., 2003; Suzuki et al., 2006; Raz-Pasteur et al., 2012; Raz-Pasteur et al., 2014; Kratz et al., 2016; Li, Chen, et al., 2017; Moss et al., 2019
	Female	Spiro	MR antagonism does not reduce plaque inflammation in female mice	Moss et al., 2019
EC-MR-KO mice	Male	VE-Cadherin Cre	EC-MR-KO attenuates atherosclerotic plaque inflammation and inflammatory cell recruitment without changing plaque size.	Moss et al., 2019
	Female	VE-Cadherin Cre	Females have less plaque inflammation and inflammatory cell recruitment than males, and EC-MR-KO does not confer further protection in females.	Moss et al., 2019
HFrEF				
MR in humans	Men	Epl	MR antagonists reduce mortality (mostly male cohorts). Significant effect remains in male sub-group of meta-analysis.	Pitt et al., 1999; Pitt, Remme, et al., 2003; Zannad et al., 2011; Rossello et al., 2019
	Women	Epl	MR antagonism may reduce mortality in women (trend but not significant in meta-analysis).	Rossello et al., 2019
		Aldo (observational)	Aldo levels correlate with cardiac remodeling in women but not men.	Vasan et al., 2004

Table 2 (*continued*)

Model	Sex	Intervention	Results	References
MR in animals	Male	Epl, Aldosterone synthase inhibitor	MR antagonists improve systolic function and reduce mortality.	Fraccarollo et al., 2003; Wang et al., 2004; Munoz-Pacheco et al., 2013; Furuzono et al., 2017
		pH1tet-inducible anti-MR shRNA expression	Inducible genetic MR knockdown improves systolic function and reduces mortality.	Montes-Cobos et al., 2015
	Female	Epl	Female rats had larger improvements in systolic function and cardiac remodeling than males.	Kanashiro-Takeuchi et al., 2009
EC-MR-KO mice	Male	Tie2 Cre, VE-Cadherin Cre	EC-MR-KO improves systolic function and prevents cardiac remodeling.	Rickard et al., 2014; Lother et al., 2016; Salvador et al., 2016; Salvador et al., 2017
	Female	–	Not studied	–
HFpEF				
MR in humans	Men	Spiro	MR antagonism does not improve mortality in men (no trend or significant effect in TOPCAT meta-analysis).	Merrill et al., 2019
	Women	Spiro	MR antagonism improves diastolic dysfunction (mostly-female cohorts).	Pandey et al., 2015; Fukuta et al., 2019
		Spiro	MR antagonism may or may not improve exercise capacity (mostly-female cohorts).	Daniel et al., 2009; Upadhya, Hundley, et al., 2017
		Spiro	MR antagonism improves mortality in women (significant effect in TOPCAT meta-analysis).	Merrill et al., 2019
	Both	Spiro	MR antagonism improves left ventricular function and structure and reduces hospitalization rates, with no overall effect on mortality (roughly equal male/female cohorts).	Edelmann et al., 2013; Pitt et al., 2014
MR in animals	Male	–	Male mice do not develop diastolic dysfunction with Western diet.	Manrique et al., 2013
	Female	Spiro	MR antagonism prevents diastolic dysfunction and cardiac inflammation with Western diet.	Bostick et al., 2015
EC-MR-KO mice	Male	–	Not studied	–
	Female	VE-Cadherin Cre	EC-MR-KO prevents diastolic dysfunction and cardiac inflammation with Western diet.	Jia, Habibi, et al., 2015

MR = mineralocorticoid receptor; EC-MR = endothelial-specific MR; KO = knockout; Aldo = aldosterone; Spiro = spironolactone; Epl = eplerenone; RAAS = renin-angiotensin-aldosterone system; 11βHSD2 = 11β-hydroxysteroid dehydrogenase 2; shRNA = short hairpin RNA.

antagonism in HFrEF patients were heavily weighted towards male participants, with women making up only 27% of study subjects. While this resulted in the individual studies being under-powered to assess sex differences, combination of the data in a recent meta-analysis did enable sub-analysis of the data by sex. In this combined data, the male sub-group retained the significant mortality benefit of MR antagonism, while the female sub-group also tended towards a decline in sudden cardiac death with MR antagonism that was not statistically significant (Rossello et al., 2019). Thus, it is unclear whether MR antagonism is beneficial in female HFrEF patients, as has been shown definitively for men. It will be critical to include more women in future trials of MR antagonists in heart failure in order to fully understand the role of this receptor in HFrEF in women.

2.4.3. MR antagonism may specifically benefit women with heart failure with preserved ejection fraction

In contrast to the clear benefit observed with MR antagonism in HFrEF, investigations into the role of the MR in HFpEF in humans have produced variable results. However, the growing body of literature does suggest a role for the MR in this disease. In an open-label trial in which 11 women with HFpEF were administered spironolactone, the authors observed an improvement in peak exercise capacity from baseline and a reduction in the median heart failure score (Daniel, Wells, Stewart, Moore, & Kitzman, 2009). Subsequent studies and meta-analyses in mostly-female cohorts have largely found that spironolactone improves diastolic function in HFpEF patients (Fukuta, Goto, Wakami, Kamiya, & Ohte, 2019; Pandey et al., 2015). Results vary as to whether spironolactone improves exercise tolerance in HFpEF patients, with some studies showing increased exercise capacity with MR antagonism (Daniel et al., 2009; Kosmala et al., 2016) and others showing no benefit (Fukuta et al., 2019; Pandey et al., 2015; Upadhya et al., 2017).

Larger randomized trials of MR antagonism in HFpEF have produced extensive controversy in recent years. The Aldo-DHF trial randomized over 400 patients and demonstrated improved left ventricular functional and structural parameters in HFpEF patients randomized to spironolactone (Edelmann et al., 2013). Subsequently, however, the TOPCAT trial randomized over 3,000 HFpEF patients in 6 countries to either placebo or spironolactone, and the results revealed that MR antagonism reduced the rate of hospitalization for heart failure but did not significantly affect mortality (Pitt et al., 2014). Subsequent sub-analysis of this otherwise negative trial revealed heterogeneity in the data that may have masked the beneficial effects of MR antagonism in certain patient subgroups. For example, subjects who qualified for the study based on natriuretic peptide levels (the majority of patients enrolled in the Americas) had a significant mortality benefit with spironolactone, while patients who qualified based solely on clinical criteria (the majority of patients enrolled in Russia and Georgia) did not (Bristow et al., 2016; Pfeffer et al., 2015). Another sub-analysis, which included 1,767 of the randomized patients and was equally comprised of men and women, demonstrated that women with HFpEF had a significant reduction in cardiovascular and all-cause mortality with spironolactone, while men did not (Merrill, Sweitzer, Lindenfeld, & Kao, 2019). While such post-hoc analyses are hypothesis-generating, the results may help to contextualize the sex differences observed in mortality in HFpEF patients and provide opportunities for further study to identify sex-specific therapies for this disease, for which there are currently no available pharmacotherapeutic options.

2.5. Summary of the clinical data

In summary, observational studies and clinical trials support that the MR contributes to the pathogenesis endothelial dysfunction, hypertension, atherosclerosis, and heart failure, as MR antagonist therapy has been shown to improve outcomes in patients with these conditions (see Tables 1 and 2). In some cases, there appear to be differences between men and women in the role of the MR in disease, as evidenced by the female predominance of salt-sensitivity of blood pressure and

sex differences in the efficacy of MR therapy in heart failure. Importantly, many clinical trials use spironolactone as an MR antagonist, while others use the less potent but more selective eplerenone. Thus, it is possible that off-target effects of spironolactone on the progesterone or androgen receptors could contribute to sex differences observed in the effect of MR inhibition in various cardiovascular pathologies. Further careful study will be needed to fully understand the sex-specific contributions of the MR to cardiovascular disease in humans, with a greater focus on the inclusion of female patients in clinical trials and the use of selective MR inhibitors.

3. Preclinical data: Sex-specific roles for the endothelial mineralocorticoid receptor in cardiovascular disease

The clinical literature points to a role for the MR in cardiovascular disease, in some cases with sex-specific effects. However, studies in humans by necessity rely on the use of systemic MR inhibitors, thus precluding examination of the role of the MR in particular cell types. By contrast, genetic animal models of cell-specific MR deletion have enabled investigations into the contribution of the MR specifically within the vascular endothelium to cardiovascular disease. In this section, the preclinical literature implicating EC-MR in 1) endothelial dysfunction, 2) hypertension, 3) atherosclerosis, and 4) heart failure is discussed, particularly in light of new evidence supporting a sex-specific role for EC-MR in cardiovascular disease. See Table 2 for a summary of the studies in this section describing the sex-specific role of the MR in cardiovascular disease and potential correlations to human clinical data.

3.1. A sex-specific role for EC-MR in endothelial dysfunction

3.1.1. MR inhibition in animals improves endothelial function, particularly in the context of obesity

As has been demonstrated in human studies (Table 1), MR inhibition in animal models improves indices of endothelial function, particularly in the context of cardiovascular risk factors. Specifically, MR antagonist treatment improved aortic endothelial function and peak relaxations and reduced ROS generation in male hyperlipidemic rabbits (Rajagopalan, Duquaine, King, Pitt, & Patel, 2002) and in rats with chronic kidney disease (Gonzalez-Blazquez et al., 2018).

Obesity in particular may represent a state of enhanced MR activation, as higher body mass index correlates with higher Aldo levels in patients administered a high-salt diet (Bentley-Lewis et al., 2007). This is likely due to adipocyte-derived factors that increase Aldo release either directly from the fat (Briones et al., 2012) or from the adrenal gland (Huby, Otvos Jr., & Belin de Chantemele, 2016). Specifically, the adipokine leptin, which circulates at significantly higher levels in obese females than males (Deng & Scherer, 2010), may mediate the role of the MR in endothelial dysfunction in obesity in females. Leptin was shown in preclinical models to increase Aldo secretion from the adrenal gland and to induce endothelial dysfunction in females in an MR-dependent manner (Faulkner & Belin de Chantemele, 2019; Huby et al., 2016). This may be especially important for the pathogenesis of endothelial dysfunction in females, as leptin levels and rates of obesity are both higher in women than in men (Deng & Scherer, 2010; Flegal et al., 2016).

3.1.2. EC-MR contributes to endothelial dysfunction, with sex- and vascular bed-dependent effects

Preclinical animal studies reveal that in the setting of cardiovascular risk factors, EC-MR is a mediator of endothelial dysfunction and its specific deletion from ECs has a positive impact on vascular function (Davel et al., 2017). Either global MR inhibition with eplerenone or EC-specific MR deletion improved dilation of aortic rings in obese male mice and in lean mice with Aldo infusion (Schafer et al., 2013). Similarly, EC-MR deletion improved resistance vessel endothelial function in a model of male mice exposed to angiotensin-II-induced hypertension (Mueller et al., 2015). In female mice, EC-MR deletion also prevented Western diet-induced aortic endothelial dysfunction (Jia et al., 2016).

One recent study directly compared the role of EC-MR in mesenteric microvessel dysfunction in the setting of obesity and hyperlipidemia in male and female littermates. In this study, obese male mice were able to compensate for endothelial dysfunction, with no role for EC-MR in endothelial dysfunction of the mesenteric arteries. By contrast, diet-induced obesity did result in endothelial dysfunction in female mice, and genetic deletion of EC-MR restored endothelial-dependent microvessel relaxation by increasing NO bioavailability. Notably, there was no role for EC-MR in endothelial function in healthy male or female mice; the EC-MR-dependent endothelial dysfunction was observed in females only with the addition of obesity and/or hyperlipidemia (Davel, Lu, et al., 2018).

The study by Davel, Lu, et al. (2018) was the first to directly compare the role of the MR in endothelial function between males and females, revealing significant sex differences in the role of EC-MR in vasodilatory pathways and microvascular endothelial dysfunction in response to cardiometabolic risk factors. Comparison to the prior literature in males suggest that the role of EC-MR in modulating endothelial function depends on the vascular bed and cardiovascular risk factor interrogated. Whereas Davel, Lu, et al. (2018) found no role for EC-MR in mesenteric microvessel dysfunction that occurs with obesity in males, Schafer et al. (2013) previously demonstrated a role for EC-MR in aortic endothelial dysfunction in obese males. Further, Mueller et al. (2015) showed improvement in microvessel function with EC-MR deletion in male mice subjected to angiotensin-II hypertension.

Thus, the role of EC-MR in endothelial function may depend on sex, vascular bed, and clinical context. Further studies comparing the role of the MR in endothelial dysfunction between men and women and in the setting of a variety of cardiovascular risk factors could translate these preclinical results into actionable sex-specific therapies to reverse endothelial dysfunction and prevent further cardiovascular disease.

3.2. A nuanced role for EC-MR in hypertension

3.2.1. The MR may regulate blood pressure in females with cardiometabolic risk factors, as has been shown for males

As in humans, ample data demonstrates that the MR influences blood pressure in male animal models (reviewed in DuPont & Jaffe, 2017). As most mechanistic studies exploring the role of the MR in blood pressure changes have used only male animals, less is known regarding females. One notable exception is a study by Huby et al. (2016) in which MR inhibition with spironolactone substantially reduced blood pressure in female agouti yellow obese mice. Another study directly compared the blood pressure-lowering effect of spironolactone between gonadectomized male and female rats, revealing that while high salt diet increased blood pressure in both sexes, MR inhibition with spironolactone reduced blood pressure only in males (Michaelis et al., 2012). These studies highlight the need for further detailed exploration into MR-mediated mechanisms of hypertension in females and for direct comparisons between the sexes.

3.2.2. EC-MR may not directly control blood pressure but modulates the response to hypertension

A focus on the specific role of endothelial MR reveals that genetic deletion of EC-MR in male mice does not affect blood pressure at baseline (Mueller et al., 2015; Salvador et al., 2017) or in models of experimentally-induced hypertension (Dinh et al., 2016; Laursen et al., 2018; Lother et al., 2016; Mueller et al., 2015; Rickard et al., 2014). By contrast, male mice overexpressing the MR specifically in ECs have higher systolic blood pressure and exaggerated vasoconstrictor responses, suggesting that under conditions where EC-MR is upregulated, it may contribute to elevated blood pressure potentially via crosstalk between vascular ECs and SMCs (Nguyen Dinh Cat et al., 2010).

Data concerning the contribution of EC-MR to blood pressure regulation or hypertension in females is scarce but suggests that EC-MR may not play a role in blood pressure regulation in females, as has been shown rigorously for males. In their recent study of sex differences in endothelial function, Davel, Lu, et al. (2018) measured blood pressure by tail cuff plethysmography in a subset of animals and reported no effect of EC-MR deletion in either sex under any of the dietary conditions studied. Likewise, measurement of blood pressure in anesthetized female animals after Western diet feeding revealed no difference in blood pressure with EC-MR deletion (Jia et al., 2015). However, studies directly comparing males and females and using sensitive blood pressure measurement techniques such as radiotelemetry in conscious mice are needed to confirm this lack of a role for EC-MR in blood pressure regulation in females.

Although EC-MR may not contribute to blood pressure regulation per se, studies indicate that it may be critical for the pathologic arterial and myocardial remodeling observed as a consequence of hypertension. In a study of male mice with angiotensin-II-induced hypertension, EC-MR deletion completely prevented the decreases in cerebral vessel outer diameter, lumen diameter, and cross-sectional area observed in MR-intact littermates with the same degree of hypertension. This indicates that EC-MR is necessary for the pathologic cerebral arterial remodeling observed in hypertension (Diaz-Otero et al., 2017), and further studies suggest a role for the MR in cognitive dysfunction induced by hypertension (Diaz-Otero et al., 2018). EC-MR deletion also prevented pathologic cardiac remodeling without modulating blood pressure in two studies of male mice in models of experimental hypertension-induced cardiac dysfunction (Lother et al., 2016; Rickard et al., 2014).

Thus, the role of EC-MR in hypertension and its downstream consequences is well characterized in male animal models, but less is known regarding its role in females. As hypertension affects over a quarter of all women in America, and two-thirds of women over the age of 60, understanding the sex-specific mechanisms and downstream consequences of this pathology in females will be critical to crafting appropriate sex-specific therapies and preventative strategies.

3.3. EC-MR differentially contributes to atherosclerosis in males and females

3.3.1. The MR promotes atherosclerosis in male animals

Despite human data suggesting a role for the MR in atherosclerosis in both men and women, preclinical investigations exploring mechanisms in animal models have almost exclusively focused on the pathology in males. In the apolipoprotein-E-knockout atherogenic mouse model, Aldo administration along with high fat diet increases plaque size and inflammation in males in as little as 4 weeks (Marzolla et al., 2017; McGraw et al., 2013). Similarly, deletion of 11βHSD2, which leads to constitutive activation of the MR by corticosterone, accelerates plaque formation and inflammation in male apolipoprotein-E-knockout mice (Deuchar et al., 2011). Conversely, MR inhibition with eplerenone or spironolactone has repeatedly been shown to decrease plaque size and inflammation in male mice (Keidar et al., 2003; Kratz, Schirmer, Baumhakel, & Bohm, 2016; Moss et al., 2019; Raz-Pasteur, Gamliel-Lazarovich, Coleman, & Keidar, 2012; Raz-Pasteur, Gamliel-Lazarovich, Gantman, Coleman, & Keidar, 2014; Suzuki et al., 2006), rabbits (Rajagopalan et al., 2002), and pigs (Li, Chen, et al., 2017).

3.3.2. EC-MR contributes to atherosclerotic plaque inflammation, with sex-specific effects

Preclinical studies indicate that EC-MR plays a critical role in the inflammation of the atherosclerotic plaque. In male apolipoprotein-E-knockout mice, intracellular adhesion molecule (ICAM)-1, a surface protein expressed on endothelial cells that mediates leukocyte-endothelial interactions, was found to be necessary for Aldo to increase plaque formation and inflammation (Marzolla et al., 2017). In male mice, activation of the MR by genetic 11βHSD2 ablation also increased endothelial expression of vascular cell adhesion molecule (VCAM)-1, another mediator of leukocyte-endothelial adhesion (Deuchar et al., 2011).

A recent study further explored the possibility that EC-MR regulates inflammation of the atherosclerotic plaque, this time directly comparing male and female mice. In this study, EC-MR deletion in males significantly reduced atherosclerotic plaque inflammation and leukocyte rolling and adhesion to the vasculature *in vivo*. By contrast, gonad-intact female littermates exhibited less atherosclerotic plaque inflammation and fewer leukocyte-endothelial interactions than males, even with intact MR. Moreover, in females, EC-MR deletion did not provide additional protection against atherosclerotic vascular inflammation, in contrast to the observed benefit of EC-MR deletion in males (Moss et al., 2019). These data reveal a significant sex difference not only in atherosclerotic vascular inflammation overall, but in the role of EC-MR in regulating inflammation in the context of atherosclerosis. The results of this study suggest new mechanisms for the contribution of Aldo and the MR to cardiovascular ischemia in humans and for the protection from atherosclerotic plaque rupture observed in premenopausal women. Further investigations into the relationship between female sex and EC-MR function, such as studies using ovariectomized versus gonad-intact females with intact MR or EC-MR deletion, could identify mechanisms by which MR and female sex hormone signaling interact *in vivo* in the context of atherosclerosis.

3.4. EC-MR contributes to the pathogenesis of heart failure with both reduced and preserved ejection fraction

3.4.1. MR inhibitors improve function and survival in animals with heart failure

Inhibition of the MR has been demonstrated to improve clinically relevant features of HFrEF in animal models, especially when combined with other standard heart failure therapies. Addition of eplerenone to a standard regimen consisting of an angiotensin-converting enzyme inhibitor, a thiazide diuretic, and a β-adrenergic blocker prevented left ventricular hypertrophy and echocardiographic anomalies in male spontaneously hypertensive heart failure rats beyond the effect of standard therapy alone (Munoz-Pacheco et al., 2013). Combination of MR inhibition with angiotensin-converting enzyme blockade appears to be especially effective at attenuating cardiac contraction defects and fibrosis in male rats (Fraccarollo et al., 2003) and mice (Wang et al., 2004) subjected to MI to induce heart failure. In the transverse aortic constriction model of pressure overload-induced heart failure, MR inhibition either by inducible whole-body genetic knockdown (Montes-Cobos et al., 2015) or by inhibition of the Aldo synthase enzyme (Furuzono et al., 2017) reduced mortality and improved cardiac function in male mice, even without additional therapies.

Only one study has compared male and female animals side-by-side to assess sex-specific roles of MR signaling in experimental heart failure. Kanashiro-Takeuchi, Heidecker, Lamirault, Dharamsi, and Hare (2009) found that after MI, female rats benefited more from eplerenone therapy than males. Specifically, ejection fraction, infarct size, cardiac fibrosis, and contraction anomalies were all improved in female rats, while males experienced smaller changes in these parameters that did not reach statistical significance in this study.

3.4.2. In animal models, EC-MR contributes to HFrEF in males and to HFpEF in females, but sex differences have not been studied

EC-MR has also been shown to contribute to the pathophysiology of HFrEF, at least in male animals. EC-MR deletion reduced ventricle weight and prevented an increase in cardiac fibrosis in male hypertensive mice (Lother et al., 2016; Rickard et al., 2014) and improved ejection fraction in male mice in the transverse aortic constriction model (Salvador et al., 2017), independent of effects on inflammation (Salvador et al., 2016). As no study has investigated the role of EC-MR in HFrEF in female animals, further studies are needed to understand whether EC-MR contributes to this pathology in females.

Much of the preclinical literature in HFpEF focuses on female animal models, opposite of the trends in the other cardiovascular outcomes described in this review. This is largely due to the activities of the Sowers research group, which uses a model of female mice fed a Western diet (containing high fat and high sucrose) resulting in obesity-induced cardiac diastolic dysfunction. This group found that only female mice, not male mice, develop diastolic dysfunction in this treatment paradigm, suggesting sex differences in the mechanisms driving HFpEF (Manrique et al., 2013). In this model, MR antagonism improved diastolic function and reduced cardiac fibrosis, inflammation, and other markers of adverse myocardial remodeling (Bostick et al., 2015). This correlates with clinical studies in human HFpEF patients demonstrating beneficial effects of MR antagonists on diastolic function (Fukuta et al., 2019; Pandey et al., 2015) (see Table 2). EC-MR deletion in female mice recapitulates most of these benefits of pharmacologic MR blockade, implying that the MR within ECs plays a critical role in the development of diastolic dysfunction in this model (Jia, Habibi, et al., 2015). Future work is needed to explore potential sex differences in the role of EC-MR in HFpEF in different model systems in which both sexes develop dysfunction. Such investigations could shed light on the mechanisms driving the sex differences in outcomes and quality of life in patients with HFpEF (Faxen et al., 2018; Martinez-Selles et al., 2012) and would support future clinical trials of MR inhibition in HFpEF, particularly in the context of obesity.

3.5. Summary of the data from animal models

In animal models, the MR specifically within the vascular endothelium promotes endothelial dysfunction, mediates inflammation in atherosclerosis, and contributes to cardiac remodeling in heart failure. In many cases, these preclinical data are consistent with the effects of MR inhibition that have been observed in human clinical cohorts (Table 2). While sex differences in these diseases have been directly investigated in a few cases, for the most part our understanding of the mechanisms driving cardiovascular disease comes from studies in male model systems or comparisons of males and females studied separately. Notable exceptions, such as studies of the role of the MR in endothelial-dependent relaxation (Davel, Lu, et al., 2018), atherosclerotic inflammation (Moss et al., 2019), and diastolic dysfunction in diet-induced obesity (Jia, Habibi, et al., 2015; Manrique et al., 2013) point to intriguing sex differences in the function of the MR in the vascular endothelium. Additional studies directly comparing male and female animals are needed to provide critical insight into the mechanisms mediating sex differences in cardiovascular disease in humans.

4. Molecular mechanisms for the sex-specific roles of endothelial mineralocorticoid receptors in cardiovascular disease

In this section, we review the literature describing the contribution of EC-MR to: 1) inflammation; 2) vascular stiffness; and 3) oxidative stress as potential mechanisms for the sex-dependent role of EC-MR in various cardiovascular pathologies. We further discuss the relevant mechanistic insight gleaned from studies exploring 4) crosstalk between the MR and sex hormone signaling. Fig. 1 provides a model summarizing these data.

4.1. Inflammation

Inflammation plays a critical role in the pathophysiology of a number of cardiovascular diseases, including hypertension, atherosclerosis, and heart failure (Ruparelia, Chai, Fisher, & Choudhury, 2017). EC-MR has been demonstrated to contribute to a number of inflammatory processes, in some cases sex-dependently. This may represent a molecular mechanism by which EC-MR contributes to a wide variety of cardiovascular diseases.

4.1.1. EC-MR regulates inflammatory endothelial adhesion molecules

The first description of the MR gene regulatory function within ECs was in human coronary artery ECs, where EC-MR was shown to transcriptionally regulate ICAM-1, a key endothelial mediator of leukocyte adhesion (Caprio et al., 2008). Later studies in apolipoprotein-E-knockout male mice demonstrated that ICAM-1 is necessary for Aldo to enhance atherosclerosis (Marzolla et al., 2017), and MR inhibition with eplerenone in Dahl salt-sensitive rats decreased renal ICAM-1 expression (Kobayashi et al., 2005), further implicating MR regulation of ICAM-1 in tissue inflammation (Fig. 1A-i). Additional *in vitro* studies using human ECs demonstrated that estrogen, via ERα, inhibits MR transcription of ICAM-1, suggesting that estrogen signaling diminishes the role of MR-induced ICAM-1 in inflammation (Barrett Mueller et al., 2014). This is in line with a study in which the effect of estrogen on atherosclerosis in females was found to be independent of ICAM-1 (Gourdy et al., 2003), while in male mice ICAM-1 deletion has been shown to reduce lesion size (Bourdillon et al., 2000). Taken together, these data suggest a model in which EC-MR regulates ICAM-1 in males to promote inflammation, while in females ERα blocks this function of EC-MR (Fig. 1B-iv).

The MR has also been linked to regulation of VCAM-1, another endothelial molecule involved in leukocyte adhesion to the vasculature. Deletion of 11βHSD2, which leads to overactivation of the MR by corticosterone, increased endothelial VCAM-1 expression in the aortic roots of male apolipoprotein-E-knockout mice (Deuchar et al., 2011). In another study, VCAM-1 expression was inhibited by eplerenone in the renal tissue of Dahl salt-sensitive rats (Kobayashi et al., 2005). Conversely, VCAM-1 may be negatively regulated by estrogen: in a study of ovariectomized female atherosclerotic mice, addition of estrogen decreased VCAM-1 relative to placebo (Gourdy et al., 2003). Scant data studying VCAM-1 regulation in EC-MR deficient mice points to potential endothelial-specific regulation of this molecule in males that may vary by the model used. In one model of male mice subjected to mineralocorticoid/high-salt hypertension, EC-MR deletion prevented VCAM-1 upregulation in cardiac ECs (Lother et al., 2016). By contrast, EC-MR deletion did not alter whole-heart VCAM-1 expression in males subjected to pressure-overload cardiac hypertrophy (Salvador et al., 2017). No study has yet explored the role of EC-MR in regulating VCAM-1 in females.

4.1.2. EC-MR sex-dependently regulates the selectins, endothelial molecules critical for leukocyte recruitment

The selectins are a family of molecules expressed on the EC surface that mediate leukocyte rolling interactions with the endothelium, the necessary first step for tissue inflammation. P-selectin is involved in leukocyte capture and fast rolling, while E-selectin is necessary for leukocyte slow-rolling interactions, which precede firm adhesion and trans-endothelial migration (Sundd, Pospieszalska, Cheung, Konstantopoulos, & Ley, 2011). EC-MR was recently found to regulate E-selectin *in vivo* in males. When male and female littermates were directly compared, TNFα-induced mesenteric venous expression of E-selectin was lower in females than in males and not further affected by the deletion of EC-MR. This pattern of E-selectin expression correlated with sex-dependent effects on leukocyte slow rolling in the vasculature in the setting of an acute inflammatory stimulus and with the accumulation of inflammatory cells in aortic plaques in a model of hyperlipidemia-induced atherosclerosis (Moss et al., 2019). This recent study is consistent with prior *in vitro* investigations suggesting E-selectin regulation by the MR (Seeger, Wallwiener, & Mueck, 2009; Hashikabe, Suzuki, Jojima, Uchida, & Hattori, 2006) and demonstrating that patients with high Aldo levels have higher circulating levels of soluble E-selectin (Tomaschitz et al., 2011). Further, E-selectin has been demonstrated to be negatively regulated by estrogen signaling (Tyree, Zou, & Allegretto, 2002), consistent with the reduction in E-selectin expression observed in female mice compared to males (Moss et al., 2019).

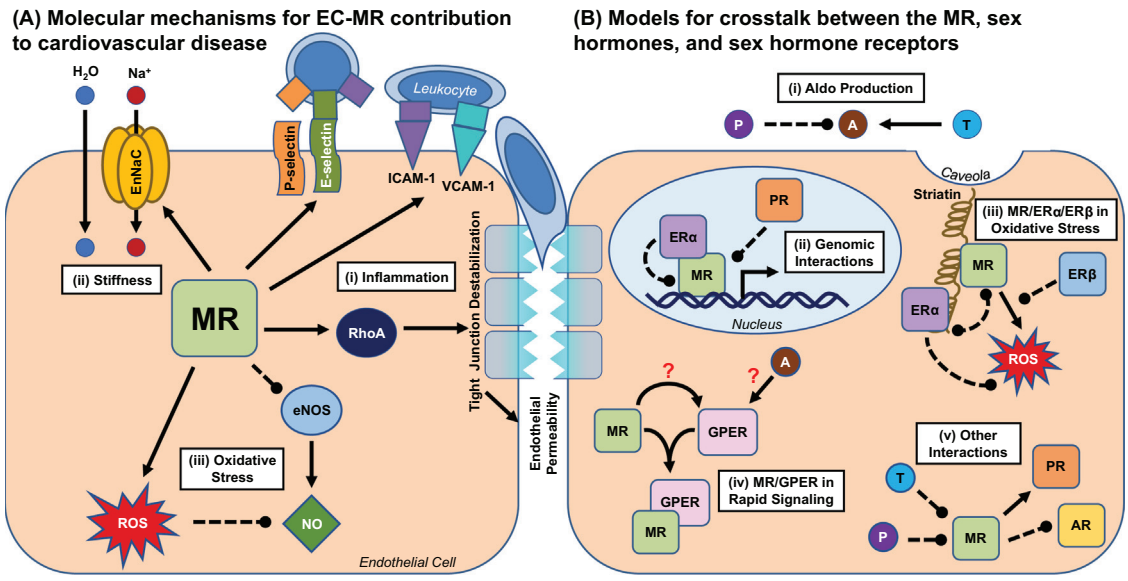

Fig. 1. Molecular Mechanisms for the Sex-Specific Contributions of EC-MR to Cardiovascular Disease. (A) The MR participates in a number of processes in ECs that may contribute to cardiovascular disease in a sex-specific manner. (i) EC-MR promotes the expression of endothelial adhesion molecules such as P- and E-selectin, ICAM-1, and VCAM-1, and this differs by sex for E-selectin and ICAM-1. This results in differential leukocyte recruitment to the vasculature in males and females. EC-MR also promotes endothelial permeability by activating RhoA, which leads to tight junction destabilization and may facilitate leukocyte trans-endothelial migration. (ii) The MR is well known to promote the expression of sodium transport proteins such as EnNaC, which in the endothelium can promote vascular stiffness. Whether this differs by sex is unclear, as all studies of EC-MR in vascular stiffness have been performed in female mice. (iii) EC-MR promotes oxidative stress in both males and females, though the mechanism for this effect may differ by sex. The ROS produced by this effect inactivate NO, thus preventing effective endothelium-dependent dilation of the underlying smooth muscle cells. This effect appears to vary by sex, arterial bed, and disease model. (B) There are several potential nodes for crosstalk between the MR and sex hormone receptors, many of which have yet to be fully explored. (i) Sex hormones may modulate production of the MR ligand Aldo at the level of the adrenal gland: testosterone may increase Aldo production, while progesterone may inhibit it. (ii) Activated ERα can bind to and inhibit the transcriptional function of the MR, which requires nuclear translocation but does not require ERα itself to bind DNA. The PR has also been demonstrated to inhibit MR transcriptional activities. (iii) The MR and ERα may compete for occupancy of striatin at the caveolar membrane, where they mediate non-genomic effects on eNOS and other rapid signaling cascades. (iv) Possible interactions between Aldo, the MR, and GPER are particularly controversial. Activation of either the MR or GPER can activate similar rapid signaling pathways, and many of these effects can be blocked by either MR inhibition or GPER inhibition. Possible models for this crosstalk include activation of GPER by MR, direct binding of Aldo to GPER, and complex formation between the MR and GPER. (v) Progesterone has been shown to bind to and inhibit the MR, and testosterone has been hypothesized to do the same. AR/MR interactions are not well characterized but may include inhibition of the AR by MR. ERβ has also been demonstrated to attenuate Aldo-induced ROS production, through unclear mechanisms. The MR may also promote PR activity. Solid arrow = positive regulation, dotted line = negative regulation; A = Aldo; AR = androgen receptor; EnNaC = endothelial epithelial sodium channel; eNOS = endothelial nitric oxide synthase; ER = estrogen receptor; GPER = G protein-coupled estrogen receptor; ICAM-1 = intracellular adhesion molecule-1; NO = nitric oxide; P = progesterone; PR = progesterone receptor; ROS = reactive oxygen species; T = testosterone; VCAM-1 = vascular cell adhesion molecule-1.

P-selectin may also be regulated by EC-MR, though the data supporting this is less certain than that for E-selectin. P-selectin expression in whole-kidney lysates was increased in Dahl salt-sensitive rats relative to normotensive rats, and this expression was reduced by eplerenone (Kobayashi et al., 2005). *In vitro*, the Aldo-induced increase in leukocyte adhesion to ECs in static culture could be prevented by P-selectin inhibition, implicating P-selectin in this effect of EC-MR (Jeong et al., 2009). P-selectin is critical for leukocyte fast-rolling interactions with the endothelium, and Moss et al. (2019) found that leukocyte fast rolling tended to be reduced by EC-MR deletion in males and females, however this was not statistically significant and P-selectin expression was not assessed in that study. That this tendency was the same in both sexes is consistent with data indicating that P-selectin is not involved in the protective effect of estrogen on atherosclerosis, suggesting that it is not an estrogen target and therefore may not be differentially regulated between the sexes (Gourdy et al., 2003).

4.1.3. A role for EC-MR in endothelial permeability

The integrity of the endothelial tight junction also contributes to inflammation, as endothelial permeability to proteins, lipids, and leukocytes facilitates inflammation of underlying tissues. MR activation by Aldo treatment disrupted the membrane localization of tight junction proteins in human cultured ECs, resulting in permeability of the endothelial monolayer to labeled dextrans (Kirsch et al., 2013). Conversely, in female rats, eplerenone blocked degradation of tight junction proteins in response to hemodynamic instability, thereby preventing cerebral aneurysm formation (Tada et al., 2010). Thus, EC-MR may contribute to endothelial permeability, at least in females. This may be via its regulation of the RhoA signaling pathway, which among other activities promotes EC-EC junction stability via actions on the cytoskeleton (Shimokawa, Sunamura, & Satoh, 2016). Aldo has been found to activate RhoA in various cardiovascular cell types (Kirsch et al., 2013; Lavall et al., 2014; Nguyen Dinh Cat et al., 2018), leading to F-actin stress fiber formation. In cultured human ECs, this promotes disruption of endothelial junction proteins and permeability of the endothelial monolayer (Kirsch et al., 2013). Genomic ER signaling may also activate RhoA in ECs (Oviedo et al., 2011; Simoncini et al., 2006), although non-genomic estrogen signaling may counteract this effect (Li et al., 2016). Thus, in the case of endothelial junction integrity, estrogen signaling may not block MR effects on endothelial junction integrity and may instead work in parallel to promote endothelial permeability.

4.1.4. EC-MR-mediated inflammation may contribute to cardiovascular fibrosis

Fibrosis is often a consequence of inflammation. While no studies have directly compared the role of EC-MR in cardiovascular fibrosis between males and females, analysis of the existing literature reveals the possibility of sex differences. One study found that EC-MR deletion did not alter cardiac inflammation or fibrosis in male mice subjected to transverse aortic constriction, a model of pressure overload-induced cardiac remodeling (Salvador et al., 2017). By contrast, EC-MR deletion in females attenuated cardiac (Jia, Habibi, et al., 2015) and aortic (Jia et al., 2016) fibrosis in a Western diet-fed model, which corresponded to reductions in inflammatory markers in these mice. Western diet-

fed females also develop renal artery dysfunction, inflammation, and fibrosis, which was recently also shown to be prevented by EC-MR deletion (Aroor et al., 2019). Thus, the limited data so far could be interpreted to suggest that EC-MR may specifically contribute to cardiovascular fibrosis only in females via effects on inflammation. However, since each study was performed in only one sex and in different models of cardiovascular fibrosis, it is not possible to distinguish true sex differences from differences in the models or methods used by different investigators. Direct comparison of males and females in the same model system is needed to definitively interrogate these potential sex differences in the role of EC-MR in fibrosis.

In summary, EC-MR appears to contribute to inflammation by regulating EC adhesion molecule expression and endothelial permeability (Fig. 1A-i). In some circumstances, these processes are differentially regulated in males and females and appear to be subject to opposite regulation by estrogen signaling. Further work, especially studies comparing inflammation in male and female animal models, will be instrumental in elucidating the sex-specific mechanisms by which EC-MR contributes to inflammation to induce cardiovascular pathology.

4.2. Vascular stiffness

Vascular stiffening occurs with aging and in response to chronic cardiometabolic risk factors and precedes and predicts the development of cardiovascular diseases including hypertension and atherosclerosis (Huveneers, Daemen, & Hordijk, 2015). The phenomenon of vascular stiffening involves dysfunction of all parts of the vessel wall, including the vascular SMCs, ECs, and extracellular matrix (Jia, Aroor, et al., 2015). Mineralocorticoid signaling contributes to stiffening of the vascular wall, particularly in the presence of cardiovascular disease or risk factors, as MR blockade reduced aortic stiffness in human subjects with dilated cardiomyopathy (Vizzardi et al., 2015) and attenuated the aortic stiffening observed in female mice fed a Western diet (DeMarco et al., 2015). This role for the MR in vascular stiffness may only emerge in the presence of cardiovascular risk factors, as one study found that in older but otherwise healthy individuals, MR blockade did not change indices of arterial stiffness (Hwang et al., 2013a).

The MR in vascular SMCs contributes to arterial stiffening, as specific deletion of SMC-MR was recently shown to attenuate aortic stiffness in aging male mice (Kim et al., 2018). However, the MR specifically within the vascular endothelium has also been found to contribute to arterial stiffness via regulation of endothelial ion channels. EC stiffness is modulated by changes in intracellular ion concentrations thereby altering intracellular water content. The resulting mechano-signals are then transmitted to neighboring ECs and the SMCs of the vessel wall. In female mice, Aldo administration induced aortic stiffness via endothelial expression of the epithelial sodium channel (EnNaC, Fig. 1A-ii), a well-known gene target of the MR in the renal epithelium (Jia et al., 2018b; Kusche-Vihrog et al., 2010). Conversely, EC-MR deletion attenuated aortic stiffness observed in female mice administered Aldo or fed a Western diet, also via regulation of EnNaC (Jia et al., 2016). Downstream of EC-MR, EnNaC activity in the endothelium also promotes endothelial permeability and inflammation, thus increasing susceptibility to further cardiovascular dysfunction (Jia et al., 2018a).

In addition to EnNaC, the MR also regulates other ion channels, though many of these investigations have been performed in non-ECs (reviewed in DuPont, Hill, Bender, Jaisser, & Jaffe, 2014). Notably, in breast cancer-derived ECs, Aldo has been shown to upregulate expression of the sodium/hydrogen exchanger via a mechanism that involves both the MR and rapid estrogen signaling (Rigiracciolo et al., 2016). Further study is required to determine whether EC-MR may regulate homeostasis of ion channels beyond EnNaC or the sodium/hydrogen exchanger to promote endothelial and vascular stiffness and thus contribute to the pathogenesis of cardiovascular disease.

4.3. Oxidative stress

The role of the MR in oxidative stress and its contribution to endothelial dysfunction has been reviewed elsewhere (Davel et al., 2017; Queisser & Schupp, 2012). The activity of the MR in the endothelium appears to be critical for these activities in both sexes. In male mice, EC-MR deletion prevented Aldo-induced increases in superoxide formation in the cerebral arteries (Dinh et al., 2016). In females fed a Western diet, EC-MR deletion increased eNOS activation and reduced nitrogen free radicals in the aorta (Jia et al., 2016). Thus, it appears that in both male and female animal models, EC-MR contributes to vascular oxidative stress. However, the mechanism by which EC-MR exerts these effects may differ between males and females. Hyperlipidemic male mice were recently found to develop endothelial dysfunction characterized by impaired endothelium-mediated vasodilation that was not ameliorated with genetic EC-MR deletion. Female hyperlipidemic littermates also developed endothelial dysfunction, but in females, EC-MR deletion resulted in a compensatory increase in NO production and NO-mediated dilation (Davel, Lu, et al., 2018) (Fig. 1A-iii). This enhanced role for EC-MR in females may have been possible due to higher Aldo levels or low estrogen levels in these female mice with cardiometabolic risk factors (Davel, Lu, et al., 2018). Indeed, data from human studies suggests that Aldo is increased (Bentley-Lewis et al., 2007) and sex hormones may be dysregulated in the context of obesity (Poddar, Chetty, & Chetty, 2017), which may activate EC-MR to promote vascular oxidative stress in obese females. This is supported by a separate study of ovariectomized female spontaneously hypertensive rats, in which estrogen replacement attenuated oxidative stress in the coronary arteries, while the addition of drospirenone, a progestin with anti-MR activity, had no additional effect in estrogen-replete females (Borgo et al., 2016).

4.4. Crosstalk between the mineralocorticoid receptor and sex hormones

4.4.1. Sex-dependent regulation of Aldo production

Women tend to have higher levels of circulating Aldo than men, both at baseline (Sequeira et al., 1986) and in pathogenic states (Bentley-Lewis et al., 2007; Shukri et al., 2018; Szymanski et al., 2011), a finding that has been recapitulated in rodent models (Tang, 1985; Davel, Lu, et al., 2018; Faulkner & Belin de Chantemele, 2018). Sex-specific effects on Aldo levels may be related to sex differences in adrenal Aldo production in the zona glomerulosa (ZG). Female rat ZG cells produced more Aldo at baseline than cells from male rats (Huang et al., 2019), and Aldo synthase expression was increased in female mice exposed to either leptin sensitization or obesity, resulting in higher circulating Aldo levels and blood pressure (Huby et al., 2016). By contrast, female rats exhibit a higher Aldo clearance rate than males (Morris, Caron, Graham, Silverman, & DeConti, 1975), suggesting sex-specific control of Aldo balance at both the production and excretion levels.

The mechanism for the sex dependence of Aldo homeostasis may also be related to sex hormones other than estrogen. Women in the luteal phase of the menstrual cycle, when progesterone levels are highest, have higher Aldo levels than in the follicular phase, when progesterone levels drop (Szmuilowicz et al., 2006). This study also observed an increase in Aldo production in the ZG cells of female rats when treated with progesterone. It is unclear whether serum levels of glucocorticoids, which can also activate the MR, follow the same pattern, as two small studies show discrepant results: one study of 5 women found that both Aldo and corticosterone were higher in the luteal phase than the follicular (Schwartz & Abraham, 1975), while another study found no difference in cortisol levels between the phases in 4 women (Stewart et al., 1993). The progesterone-related effect on Aldo production may be due to increased secretion of the hormone, rather than synthesis, as a separate study found that progesterone inhibited the Aldo synthase enzyme in transfected cells (Vecchiola et al., 2013).

By contrast, data support that estrogen likely does not to influence Aldo production. Estrogen did not correlate with Aldo levels in the above study of menstrual cycle variation, nor did estrogen alter Aldo production in rat ZG cells (Szmuilowicz et al., 2006) or in a separate study of human adrenocortical cells (Yanes & Romero, 2009). Estrogen also did not affect the activity of the Aldo synthase enzyme in transfected cells (Vecchiola et al., 2013). Consistent with these results, a study of human adrenocortical cells found that estrogen increased Aldo production only when ERβ was inhibited, indicating that ERβ may prevent Aldo secretion that may otherwise occur with estrogen exposure (Caroccia et al., 2014).

Testosterone and AR signaling also appear to influence Aldo production, with opposite effects depending on the timing of exposure. AR signaling during prenatal development promotes Aldo production in male offspring (Martinez-Arguelles, Guichard, Culty, Zirkin, & Papadopoulos, 2011), while in adult male animals AR signaling inhibits Aldo production (Carsia, McIlroy, & John-Alder, 2018; Hofmann et al., 2012; Kau et al., 1999). It is unclear whether AR signaling may regulate Aldo production in females, as one study demonstrated decreased Aldo production in testosterone-treated female geckos (Carsia et al., 2018) while another study observed no effect on Aldo production in ovariectomized female rats treated with an AR inhibitor (Hofmann et al., 2012). Thus, Aldo levels in males and females appear to be regulated by sex steroid hormone signaling. The female sex hormone progesterone generally upregulates Aldo production in women and in rat adrenal cells, while testosterone inhibits Aldo production in adult male animals (Fig. 1B-i).

4.4.2. Interactions between the MR and estrogen receptors

In addition to sex differences in Aldo levels that may result in differential MR activation in males and females, there is also evidence that the MR can interact with sex hormone receptors directly in the effector cells, providing another mechanistic link between MR signaling and sex differences in cardiovascular disease. In particular, the α and β isoforms of the ER have been shown to modulate MR function. In a study of human ECs *in vitro*, ERα activation triggered the formation of a complex containing ERα and the MR that inhibited MR transcriptional function. This repression of the MR required ERα to be able to translocate to the nucleus but did not involve the DNA-binding domain or the rapid non-genomic signaling functions of ERα (Fig. 1B-ii). In functional assays, Aldo treatment of human ECs induced ICAM-1 expression and leukocyte adhesion, effects that were blocked by co-administration of estrogen (Barrett Mueller et al., 2014). Recently, EC-MR was shown to regulate ICAM-1 and E-selectin only in males *in vivo* or in the absence of estrogen *in vitro*, further implicating MR-estrogen crosstalk in the regulation of endothelial inflammatory mediators (Moss et al., 2019). Other data also suggests that the MR can interact with ERs in vascular SMCs: in these cells, both ERα and ERβ attenuated Aldo-induced oxidative stress (Muehlfelder, Arias-Loza, Fritzemeier, & Pelzer, 2012), suggesting that ERβ, like ERα, may antagonize MR-mediated processes in the vasculature, through mechanisms that have not yet been elucidated (Fig. 1B-iii). Thus, interactions between the MR and ERs are likely not limited to ECs but may occur in many cell types throughout the body. Gene expression profiling has been performed in vascular tissue to describe the gene sets activated by estrogen (Schnoes et al., 2008), ERα and ERβ (O'Lone et al., 2007), and Aldo (Newfell et al., 2011). Independent pathway analyses from these studies supports that the MR activates genes in the vasculature related to oxidative stress and inflammation, while estrogen signaling appears to inhibit similar pathways. Direct comparison of the data sets described in these three publications and further studies on the impact of estrogen signaling on MR-mediated vascular gene expression could provide exciting insight into potential genomic crosstalk between the MR and ERs.

Current evidence suggests additional non-genomic interactions between estrogen- and Aldo-mediated pathways via the scaffolding protein striatin (Fig. 1B-iii). In a cultured human EC line (EAhy.926), the scaffolding protein striatin recruits ERα to the caveolar membrane, thus facilitating its activation by estradiol and rapid downstream phosphorylation and activation of eNOS (Lu et al., 2004). Striatin protein expression is upregulated by the MR in EAhy.926 cells (Pojoga et al., 2012), and striatin facilitates the non-genomic phosphorylation of ERK1/2 and induction of ROS observed upon MR activation (Coutinho et al., 2014; Grossmann et al., 2005). The striatin pathway appears to confer protection from salt sensitivity of blood pressure, at least in male rodents (Garza et al., 2015). Thus, the binding of both the MR and ERα to the striatin scaffold could be an additional mechanism for interactions between the non-genomic functions of these two receptors. However, as this link is currently only circumstantial, a direct investigation into this mechanism is certainly warranted.

An additional emerging mechanism for nongenomic crosstalk between MR and estrogen signaling is via the G-protein coupled ER (GPER), which was first described as a mediator of rapid estrogen effects (Filardo, Quinn, Bland, & Frackelton Jr., 2000). Since then, various pharmacologic and genetic perturbations of this receptor have shown it to be involved in a number of disease processes from obesity and metabolic syndrome to inflammation, often in a sex-specific manner (Sharma & Prossnitz, 2017). Further studies implicate GPER as a potential mediator of nongenomic Aldo signaling as well, though whether this occurs via direct binding of Aldo to GPER or downstream of traditional binding of Aldo to the MR is still controversial (Fig. 1B-iv). GPER was first suggested as an Aldo-binding receptor by Gros et al. (2011), wherein the authors demonstrated that Aldo can induce ERK1/2 phosphorylation and apoptosis in rat vascular SMCs infected with MR or GPER overexpression vectors, effects which could be inhibited by the GPER antagonist G15. Subsequently, Aldo stimulation of ERK1/2 phosphorylation was shown to be blocked by either G15 or short hairpin RNA-mediated knockdown of GPER in rat vascular ECs (Gros, Ding, Liu, Chorazyczewski, & Feldman, 2013). By contrast, Ferreira et al. (2015) showed that GPER was involved in Aldo-induced vasoconstriction but not vasodilation in the mesenteric resistance arteries of female mice, suggesting GPER effects on SMCs but not ECs. Finally, Aldo induces vasoconstriction of the afferent renal arteriole, an effect that can be blocked by GPER inhibition (Ren, Janic, Kutskill, Peterson, & Carretero, 2016). While these data implicate GPER in the rapid effects of Aldo on vascular cells, it is not clear that GPER is the sole mediator of nongenomic Aldo signaling. In all of the above-mentioned studies, the addition of MR antagonists eplerenone and spironolactone blocked the rapid effects attributed to GPER, as did siRNA knockdown of the MR in a study of breast cancer cell lines (Rigiracciolo et al., 2016). This indicates that the canonical MR is also involved in the rapid, nongenomic effects of Aldo. Further, data from Cheng et al. (2014) suggested that Aldo may not directly bind to GPER, furthering the controversy over whether GPER may be a novel Aldo receptor.

Despite the controversy, growing evidence supports that GPER and the MR mediate rapid Aldo-induced signaling in concert (Fig. 1B-iv). Indeed, GPER and the MR have been shown to colocalize in the presence of Aldo in breast cancer cell lines (Rigiracciolo et al., 2016). Further studies to enhance our understanding of the potential interactions between the MR, Aldo, estrogen, and GPER could provide additional insight into the mechanisms of sex differences observed in Aldo- and MR-mediated cardiovascular disease.

4.4.3. Interactions between the MR and the progesterone receptor

Progesterone and the progesterone receptor (PR) may also interact with the MR, providing yet another link between MR signaling and female sex hormones. Whereas progesterone levels positively correlate with Aldo secretion as described above, progesterone itself can bind to and inhibit the MR in mammalian cells (Mooij et al., 2015; Rupprecht et al., 1993) (Fig. 1B-v). While progesterone negatively regulates the wild-type MR, a point mutation in the ligand-binding domain of the MR has been identified that instead leads to activation of the MR by progesterone, resulting in early-onset hypertension and severe pregnancy-associated hypertension (Geller et al., 2000). The PR has also been

shown to inhibit MR transcriptional activity (McDonnell, Shahbaz, Vegeto, & Goldman, 1994), while the MR may in turn activate the PR. Indeed, Aldo promoted cell spreading and F-actin stress fiber formation in PR-positive breast cancer cells, an effect that was largely absent in PR-negative cells (Leo, Guo, Woon, Aw, & Lin, 2004) (Fig. 1B-v). However, this paradigm of mutual signaling is tentative at best, and specific study of the interactions between the MR and PR will be critical to understanding what role, if any, progestin signaling may have in MR-mediated cardiovascular disease.

4.4.4. Interactions between the MR and the androgen receptor

The majority of data concerning potential interactions between MR and AR signaling comes from the prostate cancer literature. Prostate cancer cell lines have been shown to express 11βHSD2, thereby conferring Aldo specificity to the MR by modifying cortisol to MR-inactive metabolites (Page, Warriar, & Govindan, 1994). In a recent study, Aldo treatment sensitized prostate cancer cells to the AR inhibitor enzalutamide and MR knockdown increased AR expression (Shiota et al., 2018). By contrast, testosterone and its active metabolite dihydrotestosterone have been shown to bind to and inhibit the MR (Takeda et al., 2007). In addition, the AR coactivator XRCC6 can also bind to the MR, inhibiting its transcription of target genes in the H9c2 embryonic cardiac myocyte cell line (Yang et al., 2014). Of note, this latter result is the only evidence to date linking the MR to the AR in a cardiovascular-relevant cell type, and this paradigm has not been studied in ECs. Taken together, these data suggest a feedback model in which MR activation suppresses AR expression and activity, and androgens themselves may in turn inhibit the MR (Fig. 1B-v). Much more study is needed to confirm this hypothesis and to explore the possible physiologic ramifications of MR/AR crosstalk in the cardiovascular system.

4.5. Summary of the molecular mechanistic data

The existing data indicates that the MR within the vascular endothelium contributes to cardiovascular disease via several molecular mechanisms. EC-MR regulates the expression of inflammatory adhesion molecules and promotes endothelial barrier permeability (Fig. 1A-i), which together promote tissue inflammation in cardiovascular disease models such as atherosclerosis and heart failure. By regulating expression of the epithelial sodium channel and other ion channels in ECs, EC-MR promotes vascular stiffness, a precursor to hypertension and risk factor for atherosclerosis and cardiac dysfunction (Fig. 1A-ii). By promoting oxidative stress and inhibiting NO availability, EC-MR contributes to impaired endothelial function in the setting of risk factors including hypertension, obesity and hyperlipidemia (Fig. 1A-iii).

Where it has been studied, the role of EC-MR in promoting tissue inflammation, vascular stiffness, and endothelial dysfunction has often been found to be sex-specific. This is particularly evident in animal models of cardiovascular risk factors such as obesity and hyperlipidemia. This may be due to a variety of interactions between the MR and sex hormone receptors that either promote or inhibit MR activity. Progesterone and testosterone have been shown to regulate adrenal production of Aldo (Fig. 1B-i); estrogen and progesterone receptors inhibit the genomic activity of the MR (Fig. 1B-ii); the MR and various estrogen receptors may cooperate or inhibit one another in the context of rapid, non-genomic signaling (Fig. 1B-iii-iv); and progesterone and testosterone themselves have been shown to directly inhibit the MR (Fig. 1B-v). The MR may in turn regulate the activity of the androgen and progesterone receptors (Fig. 1B-v). In our review of the literature, we found no evidence to suggest that sex hormones can directly activate the wild-type MR.

The current understanding of this complex ecosystem of ligands, receptors, and subcellular process regulation is limited by a scarcity of literature rigorously comparing differences in these mechanisms between the sexes. However, the available data suggests that in general, sex hormones tend to inhibit the harmful effects of the MR in the cardiovascular system. This paradigm could help to explain increases in cardiovascular disease risk in postmenopausal women, when ovarian hormone levels are low (Benjamin et al., 2018), and in men with low testosterone levels (Channer & Jones, 2003; Rovira-Llopis et al., 2017). This may also be consistent with activated MR promoting cardiovascular disease even in premenopausal women who exhibit additional cardiovascular risk factors such as obesity, where hormone production and MR activity are often dysregulated (reviewed in Poddar et al., 2017). Certainly, further study is warranted to fully understand the nature of the interactions between the MR and sex hormones in order to adequately design therapies to combat cardiovascular disease in both sexes.

5. Conclusions and perspectives

In this review, we have explored the evidence for a sex-specific role for EC-MR in cardiovascular disease. In humans, the MR is involved in the pathophysiology of endothelial dysfunction, hypertension, atherosclerosis, and heart failure, and inhibition of the MR has been demonstrated to be beneficial in each of these conditions. In male animal models, EC-MR has been shown to contribute to endothelial dysfunction in response to cardiovascular risk factors, to tissue inflammation, and to the adverse cardiac remodeling that occurs in models of heart failure and hypertension, without contributing to the blood pressure regulation itself. In women, the role of the MR becomes evident after menopause, when preclinical data suggests that the MR may be more active due to low levels of MR-inhibiting sex hormones and increased Aldo levels in obese females. However, a role for the MR in cardiovascular disease can be observed even in premenopausal women if additional cardiovascular risk factors are present that may diminish the beneficial effects of female sex hormones. The specific role of EC-MR in cardiovascular pathology in females is just beginning to be understood, with data supporting a role for this receptor in endothelial dysfunction and cardiac diastolic dysfunction but not inflammation or hypertension. However, substantial further investigation is needed to fully appreciate the nuances of potential sex differences and sex hormone effects in many different models of cardiovascular disease.

An important limitation in this field is the paucity of women in clinical trials and, until recently, the nearly exclusive use of male animals in preclinical investigations. With the recent requirement by the National Institutes of Health that biological sex be addressed as an important variable in basic science and clinical research (McCullough et al., 2014), data is beginning to surface demonstrating sex differences in the role of the MR in cardiovascular disease, with mechanistic insights likely to expand. Further, tens of thousands of men and women have been randomized to MR antagonist therapy in clinical trials. Sub-analysis of this existing wealth of data by sex and equitable inclusion of women in future studies would provide excellent opportunities to understand sex differences in the renin-angiotensin-aldosterone system in human subjects.

It is critical to understand the differences between men and women in the etiology, natural history, and downstream consequences of cardiovascular pathology. Endothelial-specific MR may be a tantalizing factor mediating sex differences in endothelial dysfunction, atherosclerosis, and heart failure. By contrast, EC-MR may not contribute to sex differences in hypertension incidence, instead mediating the adverse consequences of elevated blood pressure. Additionally, the MR has been suggested to contribute to the pathophysiology of MI (Beygui et al., 2006), cardiac arrhythmia (Neefs et al., 2017), and certain pathologies of the heart valves (Liu et al., 2018), but whether there is an effect of sex or a role for EC-MR has not yet been explored. Such an understanding could profoundly impact the clinical management of male and female patients, with current and emerging new MR antagonists as versatile tools in the treatment of cardiovascular disease. Furthermore, understanding the molecular mechanisms driving sex differences in the role of EC-MR in cardiovascular disease has the potential to nominate additional therapeutic targets downstream of EC-MR that could

allow for tailored treatment of cardiovascular disease to improve outcomes in both men and women.

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Declarations of competing interests

The authors declare that there are no conflicts of interest.

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