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MECHANISMS OF HYPERTENSION AND TARGET-ORGAN DAMAGE (JE HALL AND ME HALL, SECTION EDITORS)

Mineralocorticoid Receptor and Endothelial Dysfunction in Hypertension

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Abstract

Purpose of Review To review the latest reports of the contributions of the endothelial mineralocorticoid receptor to endothelial dysfunction and hypertension to begin to determine the clinical potential for this pathway for hypertension treatment.

Recent Findings Endothelial mineralocorticoid receptor expression is sex-specifically increased in female mice and humans compared with males. Moreover, the expression of endothelial mineralocorticoid receptors is increased by endothelial progesterone receptor activation and naturally occurring fluctuations in progesterone levels (estrous, pregnancy) predict endothelial mineralocorticoid receptor expression levels in female mice. These data follow many previous reports that have indicated that endothelial mineralocorticoid receptor deletion is protective in the development of obesity- and diabetes-associated endothelial dysfunction in female mouse models. These studies have more recently been followed up by reports indicating that both intact endothelial mineralocorticoid receptor and progesterone receptor expression are required for obesity-associated, leptin-mediated endothelial dysfunction in female mice. In addition, the intra-endothelial signaling pathway for endothelial mineralocorticoid receptors to induce dysfunction requires the intact expression of α-epithelial sodium channels (αENaC) in endothelial cells in females.

Summary Endothelial mineralocorticoid receptors are sex-specifically upregulated in the vasculature of females, a sex difference which is driven by endothelial progesterone receptor activation, and increased activity of these endothelial mineralocorticoid receptors is a crucial mediator of endothelial dysfunction, and potentially hypertension, in obese female experimental models.

Keywords Endothelial MR · Sex differences · Endothelial dysfunction · Hypertension · Obesity · Progesterone

Introduction

The mineralocorticoid receptor (MR) has been utilized as a target for hypertension treatment since the first use of spironolactone as a diuretic in the 1950s. The original pharmacological target of MR antagonists was the sodium-retentive epithelial cells in the distal tubule of the nephron; however, the discovery of extrarenal MR expression has opened the potential of MR antagonists to extend beyond sodium retention in other tissues. Endothelial and smooth muscle cells of both conduit and resistance arteries, including aorta, mesenteric and renal arteries, express the MR [1, 2]. In addition, MR activation in either endothelial or smooth muscle cells of the vasculature has been implicated in the promotion of reactive oxygen species formation, inflammation, vascular stiffness and fibrosis, vascular impairment, and hypertension in various experimental models [3, 4••, 5–7, 8••]. Recent preclinical evidence indicates that the contribution of the endothelial cell-specific MR (ECMR) to endothelial dysfunction is highly dependent on sex [9]. Several seminal studies summarized in this review have led to the current postulation in the field that the ECMR may be a critical pharmacological target for women at risk for endothelial dysfunction and hypertension associated with metabolic diseases.

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Endothelial Mineralocorticoid Receptors Contribute to Endothelial Dysfunction in Female Models of Obesity

Endothelial cells are the primary vascular cell type regulating vascular tone, inflammation, and cell proliferation, and diminished endothelial function predisposes to hypertension and cardiovascular events [10–14]. Emerging clinical and experimental data indicate that females are more sensitive to the development of obesity-associated endothelial dysfunction than men which is further characterized by increased endothelial cell oxidative stress and decreased nitric oxide bioavailability [15–17, 18••, 19••, 20]. Increasing evidence suggests that ECMR activation may underlie this increased risk of endothelial impairment in obese females via acting as an endothelial-specific "switch" that is turned in the direction of increased endothelial cell inflammation by obesity [21].

The concept of an overactive aldosterone-mineralocorticoid receptor interaction in obese females is not particularly new. It was first published several decades ago that plasma aldosterone levels increase more so with obesity in women compared with men [22]. However, the underlying endocrine mechanisms whereby obesity leads to increases in aldosterone were only recently explored. Our group demonstrated that a hormone classically associated with obesity, the adipocyte-derived hormone leptin, is a direct stimulator of adrenal aldosterone secretion [18••]. In this report we demonstrated that hyperleptinemia was the crucial molecular mediator of increased aldosterone levels in obese female mouse models, which are independent of plasma renin activity, potassium, or adrenocorticotropin levels. Our group's follow-up report to these data further demonstrated that obese female mice (Agouti yellow obese mice) developed both endothelial dysfunction and elevations in blood pressure dependent on both intact leptin and MR signaling [19••]. It is important to note that the use of Agouti obese mice in this report may have an effect on the data obtained as the Agouti peptide is highly expressed in adrenal cells and may have an effect on intracellular calcium levels in these cells, which may alter aldosterone production; however, these notions are as yet untested [23, 24]. These data outlined a mechanistic pathway via which leptin leads to endothelial dysfunction in obese female models through an aldosterone-MR activation axis which has been discussed in detail in the literature elsewhere [25••, 26•].

In parallel with the emergence of our reports on the contribution of leptin and aldosterone to obesity-associated hypertension, work by others has begun to outline the sex-specific contribution of ECMR activation in the pathology of endothelial impairment in obese females. In two seminal reports, Sowers and colleagues demonstrated that specific deletion of ECMR in female mice rendered them protected from aortic stiffness and diastolic dysfunction brought on by a western high-fat, high-sucrose diet in association with an improvement in endothelial nitric oxide synthase (eNOS) and protein kinase B (AKT) phosphorylation and in cardiac inflammatory markers [6, 27]. Although these studies uncovered ECMR-induced mechanisms associated with endothelial dysfunction, the sex-specific endothelial phenotype of ECMR-knockout mice was recently published by Davel et al. In these data her group demonstrated that obesity-associated endothelial dysfunction (relaxation to acetylcholine) in resistance arteries was prevented by ECMR deletion in females [4••]. In accordance with previous reports [19••], these data showed that obesity alone did not induce a parallel endothelial dysfunction in male mice; however, ECMR deletion failed to restore endothelial function in male mice with proprotein convertase subtilisin/kexin type 9 (PCSK9) mutation–induced hyperlipidemia, in contrast to females. Collectively, these data have provided a functional body of evidence that the ECMR is a mediator of endothelial health in the presence of obesity in females; however, the question remained of the mechanism via which females were more sensitive to ECMR-mediated impairment.

Progesterone Drives Sex Differences in Endothelial Mineralocorticoid Receptor Expression

A recent report by our group provides evidence that a predisposition to ECMR-mediated endothelial impairment in females is due to an endogenous sex difference in the endothelial cells of males and females. We demonstrate novel data that female mice and humans endogenously express higher levels of ECMR compared with males in the absence of any metabolic or otherwise pathologic stressor [8••]. We further begin to expand on the sex-specific mechanism in this report by demonstrating that (1) ovariectomy and sex hormone ablation in female mice eliminates the sex difference in ECMR expression between males and females and (2) progesterone supplementation both in vivo and in vitro effectively increases ECMR expression and promotes a sex-specific higher expression of ECMR in females. This report is the first to link endothelial progesterone receptor activation to an increase in ECMR transcription. In addition, we demonstrated in this manuscript that progesterone receptor deletion protects female mice from obesity-associated, leptin-mediated endothelial dysfunction. We also showed a correlation between endogenous fluctuations in progesterone levels in females, as observed in estrous cycling and pregnancy, and concurrent changes in ECMR expression in female mice. These results indicated a pronounced increase in ECMR expression in pregnant mice, a state of highly upregulated circulating progesterone. Therefore, high progesterone levels associated with pregnancy may promote the development of endothelial dysfunction in pregnant obese women, a notion that needs further exploration.

Importantly, this report demonstrated that sex differences in MR expression in the vasculature are restricted to the endothelial cells, as other vascular cells remaining from endothelial extraction showed no sex difference in MR expression. These data are in concurrence with previous data by our group and others which demonstrate that vascular constriction and non-endothelial-dependent relaxation responses are not altered by MR antagonism or ECMR deletion in obese females [4••, 19••, 28•]. Other reports have additionally demonstrated that progesterone either increases [29, 30] or has no effect [31] on MR transcription in non-vascular cell types, indicating both that the intracellular machinery for progesterone-induced MR transcription is likely cell-specific and may not be restricted to endothelial cells.

Endothelial Epithelial Sodium Channels Are a Potential Mechanism for Endothelial Mineralocorticoid Receptor–Mediated Endothelial Impairment

ECMR activation, similar to MRs expressed in renal tubules, increases expression and activation of endothelial-specific epithelial sodium channels (EnNaC) which has been implicated in reactive oxygen species and inflammation in the endothelium [32]. It has been demonstrated that ECMR deletion in female mice has no endogenous effect on EnNaC expression; however, in the presence of diet-induced obesity, ECMR expression is required for increases in EnNaC expression [6]. This finding complies with the notion that high aldosterone, which is induced by obesity in females, is required for overactivity of the ECMR in the vasculature of females. Three EnNaC have been identified, α, β and λ; however, the β and λ subunits primarily function as activation regulatory subunits while the α subunit is critical to channel function [33, 34]. A recent report indicates that αEnNaC expression is required for the development of endothelial dysfunction in female obese mice [35••]. These obese αEnNaC female mice also demonstrated reduced vascular reactive oxygen species, inflammatory markers and improvement of eNOS phosphorylation indicating that αEnNaC may be the link from ECMR activation to endothelial impairment in females, a notion that continues to be explored in the field.

Endothelial Mineralocorticoid Receptors and Hypertension

In male mice, ECMR deletion does not impact increases in blood pressure induced by in high salt, angiotensin II (ANGII) or ANGII+N(ω)-nitro-L-arginine methyl ester (LNAME) [36]. In females, we have previously demonstrated that MR antagonism in obese [19••] and salt-sensitive [37•] mice ablates increases in blood pressure in association with an improvement in endothelial function in these models. Importantly, we have demonstrated that MR antagonism improved blood pressure independent of decreases in renal sodium retention as assessed by 24-h urine collection, which may indicate an extra-renal effect of MR antagonism in female mice [37•]. However, further study of cumulative sodium balance and changes in extracellular fluid volume is needed to investigate sex-specific renal MR effects on blood pressure in response to MR antagonism. Unfortunately, obese mouse models remain limited with regard to blood pressure investigation as high-fat diets have proven to be inconsistent as a model of hypertension. Our group and others have shown that high-fat diet alone does not induce hypertension [28•, 38, 39] in male or female mice. Therefore, despite that αEnNaC deletion prevented endothelial stiffness, it is difficult to determine a role for blood pressure regulation as a western diet did not alter blood pressure [35••]. Our previous manuscripts have shown that leptin administration induces endothelial dysfunction in female mice and that obese mice require leptin receptor activation for sustained hypertension [8••, 18••, 19••]; therefore, a role for leptin in obesity-associated endothelial impairment and hypertension is implied but a model of leptin-infusion-induced hypertension remains to be developed to further explore this avenue.

An important additional finding in our recent report indicated that obese hyperglycemic female mice not only present with increased ECMR expression compared with obese males but also that the obesity itself served to increase ECMR expression in females [8••]. These studies were performed in Agouti obese female mice, a model which is characteristically hypertensive [19••]. Therefore, glucose handling or hyperlipidemia associated with obesity may prove an important caveat to the development of hypertension in response to obesity and ECMR overactivation in females and remains to be investigated. The uncovering of the mechanisms via which ECMR-mediated endothelial impairment leads to hypertension and cardiovascular risk in obese females is a crucial future direction for the field in order to determine appropriate clinical directions for ECMR inhibition in the treatment of hypertension in obese females.

Conclusion

Clinical data indicates that MR antagonists are more efficacious for hypertension and cardiovascular disease treatment in female patients compared with males [40, 41] and additionally, a predisposition for obesity-associated endothelial dysfunction is apparent in females compared with males [20]. Recent evidence indicates that an endogenous sex-specific upregulation in ECMR expression persists in females, which is driven by endothelial progesterone receptor activation and potentiated by obesity. In addition, functional evidence in female

mouse models indicates that this increased ECMR expression and subsequent EnNaC activation plays a significant role in the development of endothelial impairment in obese females. These preclinical evidences indicate an emerging role for antagonism of the ECMR and/or EnNaC in the treatment of the growing obese female population at risk for hypertension.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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