Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Pharmacology

Vascular mineralocorticoid receptor and blood pressure regulation

Antoine Tarjus[1], Cristian Amador[1], Luis Michea[2] and Frédéric Jaisser[1]

The mineralocorticoid receptor (MR) is a major regulator of blood pressure by modulating sodium balance and blood volume in the distal nephron. The discovery of MR expression in both endothelial and vascular smooth muscle cells a decade ago raised questions about its role in the vascular wall and its involvement in blood pressure regulation. *In vitro* and *in vivo* pharmacological studies have shown that vascular MR is involved in several vascular properties such as extracellular matrix remodeling, inflammation and vascular reactivity. In this review, we focus on recent advances obtained using transgenic model with cell-specific modulation of the expression MR in endothelium or smooth muscle and their impact on blood pressure.

Addresses
[1] INSERM UMR 1138 Team 1, Centre de Recherche des Cordeliers, Université Pierre et Marie Curie, Paris, France
[2] Facultad de Medicina, Universidad de Chile, Independencia 1027, Santiago, Chile

Corresponding author: Jaisser, Frédéric (frederic.jaisser@inserm.fr)

Current Opinion in Pharmacology 2015, **21**:138–144

This review comes from a themed issue on **Cardiovascular and renal**

Edited by **Pernille BL Hansen** and **Boye L Jensen**

For a complete overview see the Issue and the Editorial

Available online 28th February 2015

http://dx.doi.org/10.1016/j.coph.2015.02.004

Introduction

High blood pressure, or hypertension, represents a major risk factor of developing cardiovascular diseases [1] and is predicted to develop in 25% of the worldwide population during the next decade [2]. Thus, understanding the mechanisms leading to hypertension represents an important public health issue. Blood pressure levels are mainly determined by blood flow and vascular resistance. Blood flow depends on cardiac output and circulating volume, whereas vascular resistance is determined by vascular wall stiffness, which is the sum of an active stiffness (the contractile status of small arteries throughout the body) and passive stiffness (components of the vascular wall and extracellular matrix (ECM)) [3]. These determinants of blood pressure are subjected to a range of regulatory influences where the kidney has a pivotal role, by regulation of salt and water balance.

Among hormonal regulators for blood pressure, the role of the mineralocorticoid receptor (MR), a steroidal nuclear receptor has been highlighted for decades. Fifty years ago, the use of spironolactone, a mineralocorticoid receptor antagonist (MRA), was shown to decrease blood pressure in hypertensive patients [4] and then in several forms of hypertension [5]. Until the early 2000, it was commonly admitted that most of the pro-hypertensive effects of aldosterone and/or MR activation where related to their effects in epithelial cells of the aldosterone-sensitive distal nephron (ASDN), through the regulation of the renal sodium reabsorption and extracellular fluid volume. Since the discovery that MR is also expressed in non-epithelial tissues, and particularly in neurons and vessels [6–8], the question arise whether these non-classical targets are also involved in blood pressure regulation by aldosterone/MR. In this review, we focus on the vascular MR and its potential role in blood pressure control.

Vascular consequences of mineralocorticoid receptor antagonism

Pharmacological antagonists of MR (spironolactone, eplerenone) have beneficial effects on vascular tone and remodeling of vascular wall [9]. In experimental models, several studies have shown the beneficial effects of MRA on endothelial dysfunction induced by diabetes [10,11••], high-fat diet [12••] or after myocardial infarction [13]. In diabetic patients, eplerenone improved coronary circulatory function [14]. In human hypertensive patients, MR inhibition improved flow-mediated dilation, a mechanism contributing to vascular tone in hypertension [15•]. Eplerenone has been shown to prevent the potentiation of phenylephrine-induced contraction by aldosterone [16]. In an experimental model of rats treated with aldosterone, Lacolley *et al.* showed that eplerenone prevented the increase in blood pressure as well as pulse pressure and elastic modulus of large vessels [17], two markers of vascular stiffness, involved in hypertension [18]. In patients with end-stage renal disease, MRA also had favorable effects on intima-media remodeling [19]. More recently, beneficial effects of spironolactone have been described in experimental models of pulmonary arterial hypertension (PAH), improving pulmonary vascular remodeling, right ventricular systolic pressure [20]

and pulmonary artery systolic pressure [21]. In patients with PAH, spironolactone, associated with classical therapeutics, also present benefits with an amelioration of exercise tolerance and a decrease of B-type natriuretic peptide plasma concentration [22]. A recent study showed beneficial effect of spironolactone on coronary flow reserve in patients with type 2 diabetes mellitus [23]. Pharmacological inhibition of MR has also demonstrated beneficial effects such as increased lumen and outer diameters of the middle cerebral artery of spontaneously hypertensive stroke-prone rats [24,25] without modifying blood pressure. A recent study performed in rats showed that spironolactone administration completely prevented renal dysfunction characterized by a fall in renal blood flow and glomerular filtration rate associated to renal ischemia, without modifying mean arterial pressure [26].

The specific role of vascular MR into blood pressure regulation and the potential underlying mechanisms are difficult to assess using pharmacological approaches, due to two major limitations: i) MR blockade *in vivo* does not allow to dissociate the impact on renal epithelial cells from a direct effect on vascular function, and ii) the molecular and functional studies on isolated vessels do not permit to discriminate between endothelial and vascular smooth muscle cell effects. The use of transgenic mouse models allowing cell-specific modulation of MR expression recently helped to understand the specific role of MR in the vascular wall and its potential implication in blood pressure regulation.

Role of the endothelial mineralocorticoid receptor

MR expression has been evidenced in the endothelial cells of rabbit in the early 90 [27] and then confirmed few years later in human arteries [6], raising the question of MR function in the physiology and pathology of the endothelium. Of note, endothelial MR expression is increased in the microvasculature of spontaneously hypertensive SHR rats [28]. In the last four years, transgenic mouse models allowing cell-specific modulation of MR expression in the endothelium have been characterized.

Endothelial mineralocorticoid receptor overexpression

By using a mouse model with conditional and inducible endothelium-specific MR overexpression (VE-cadherin promoter), our group was the first to highlight the participation of endothelial MR in blood pressure regulation [29••]. These mice presented a mild (two fold) overexpression of the human MR (hMR) mRNA abundance in aorta and mesenteric arteries. The induction of endothelial hMR expression during embryonic development led to modest increase in systolic blood pressure (15 mmHg) in awake and anesthetized mice without modification of heart rate. Interestingly, the suppression of endothelial hMR overexpression in adult mice resulted in normalization of systolic blood pressure, mimicking the effect observed with a MRA (canrenoate) [29••]. Renal epithelial MR does not mediate the rise in blood pressure since renal sodium handling, or extracellular volume in mice overexpressing endothelial MR, were unchanged [29••].

Endothelial hMR overexpression enhanced the blood pressure response to the acute infusion of two vasoconstrictors; angiotensin II (AngII) and endothelin 1 (ET1), and increased the contractility of mesenteric arteries to AngII and ET1 [29••]. Contractile responses to myogenic tone by phenylephrine, or U46619, a thromboxane receptor agonist, were also increased [29••]. Endothelial MR overexpression has no effects on endothelium-dependent or independent relaxation [29••]. These results suggest that endothelial MR overexpression participates to the increase of blood pressure through regulation of vascular reactivity in resistant vessel, without affecting vessel remodeling and renal sodium handling.

Endothelial mineralocorticoid receptor inactivation

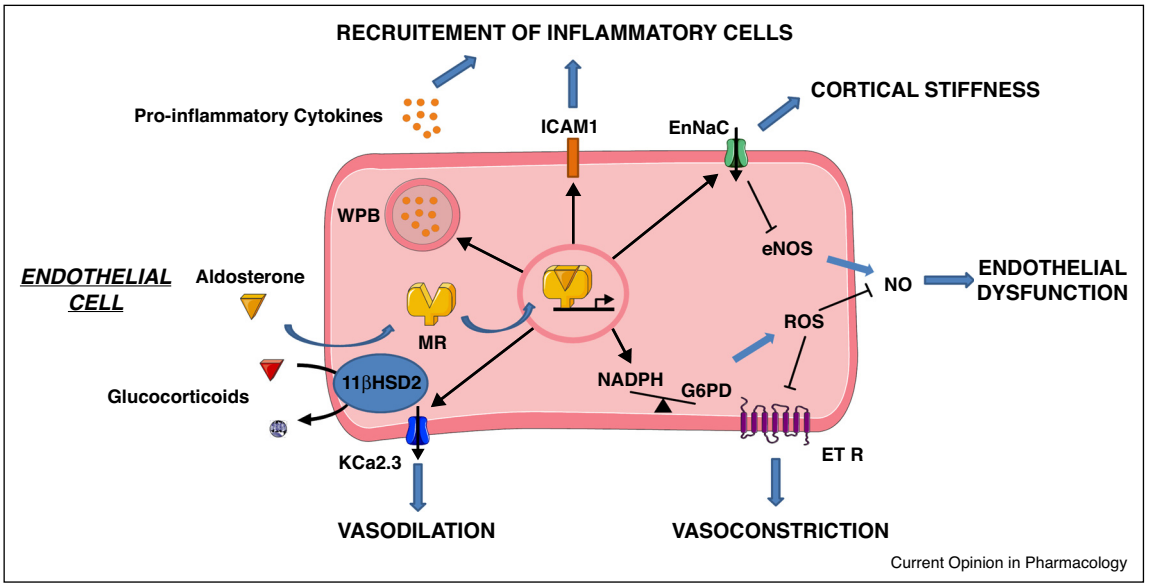
Two recent studies focused on the cell-specific inactivation of MR in the endothelium. Rickard *et al.* [30•] reported the absence of implication of the endothelial MR (Tie2 promoter) in the increased systolic blood pressure induced by deoxycorticosterone–salt (DOCA–salt) treatment. Using a similar mouse model with endothelial MR inactivation (Tie2 promoter), Schafer *et al.* [12••] studied the consequence of endothelial MR inactivation after aldosterone infusion or diet-induced obesity. The authors showed that endothelial MR deletion blunted obesity-induced endothelial dysfunction. Unfortunately, blood pressure was not assessed in this study. Both studies therefore reported beneficial effects of endothelial MR inactivation on endothelial dysfunction (estimated as an altered dilatory response to acetylcholine) induced by high-fat diet or DOCA–salt challenge [12••,30•]. Interestingly, endothelial MR deletion prevented DOCA–salt induced endothelial dysfunction in aorta but not in mesenteric arteries, suggesting a distinct role in different vascular beds [30•].

Unlike endothelial overexpression, MR deletion in endothelium had no consequences on systolic blood pressure in basal conditions. However, the mechanisms whereby endothelial MR contributes to vascular reactivity need to be clarified.

Underlying mechanisms

At the molecular level, MR is involved in several functions of the endothelium, such as endothelial cell exocytosis of Weibel–Palade bodies containing pro-inflammatory cytokines [31], adhesion of inflammatory cells [6] and reactive oxygen species (ROS) production (Figure 1). MR activation alters the balance of ROS production through an increased activity of the NADPH oxidase [21] and a reduced degradation of ROS through decreased expression

Figure 1



MR-modulated pathways in endothelial cell. MR activation in the endothelial cells leads to pro-inflammatory status via expression of ICAM1 at the membrane and secretion of Weibel–Palade bodies (WPB) containing pro-inflammatory cytokines and Von Willebrand Factor. Endothelial MR also regulates the expression of several ion channels such KCa2.3, which participates to the Endothelial-Derived Hyperpolarizating Factor pathway, and the Na^+ channel EnNaC, responsible for endothelial cortical stiffening and eNOS pathway inhibition. MR is also contributing to ROS production via the alteration of the balance between pro (NAPDH-mediated) and anti-oxidative (G6PD-mediated) pathways. The increase of ROS is responsible for the decrease of NO bioavailability and ETB receptor activity via its sulfenylation. The selectivity of aldosterone is maintained due to the presence of the 11βHSD2, which catalyze glucocorticoids into non-active metabolites. 11βHSD2, 11-β-hydroxysteroid dehydrogenase 2; ET R, Endothelin-1 Receptor; EnENaC, Endothelial Sodium Channel; eNOS, endothelial Nitric Oxide Synthase; G6PD, glucose-6-phosphate dehydrogenase; ICAM1, InterCellular Adhesion Molecule-1; MR, Mineralocorticoid Receptor; NADPH, nicotinamide adenine dinucleotide phosphate-oxidase; NO, Nitric oxide; ROS, Reactive Oxygen Species; WPB, Weibel–Palade Bodies.

and activity of the anti-oxidant enzyme glucose-6-phosphate dehydrogenase [32] (Figure 1). This then alters nitric oxide (NO) bioavailability, an important second messenger in endothelial cells. MR antagonism prevents the effects on oxidative stress and their consequences on vascular reactivity [32]. In pulmonary artery endothelial cells, the high production of hydrogen peroxide induced by MR activation is responsible for a sulfenic posttranslational modification of the Endothelin Receptor type-B leading to a reduced NO bioavailability in the pulmonary vasculature [21] (Figure 1). MR-dependent ROS production also impaired the differentiation and migration of bone marrow-derived Endothelial Progenitor Cells, which are crucial for endothelial repair and vascular homeostasis [33]. Ion channels may be direct MR targets involved in the functional consequences of MR activation in the endothelium. The Ca^{2+}-activated K^+ channel (KCa2.3) is upregulated in Human Umbilical Vein Endothelial Cells (HUVEC) by aldosterone, an effect blunted by spironolactone (Jaisser unpublished data). Indeed aldosterone induced KCa2.3 protein expression in the choroid vessels, an effect blunted by canrenoate, a MR antagonist [34] (Figure 1). We recently reported that the activity of KCa2.3 is mandatory to the vasodilatory effect of aldosterone in this vascular bed [34]. This may also occur in other vascular beds. Of note, it has been proposed that the Epithelial Sodium Channel (ENaC), a classical MR target in the ASDN, could also be regulated by aldosterone via MR in the endothelium [35,36]. Endothelial ENaC (EnNaC) contributes to the stiffening of endothelial cell [37] and to the activity of eNOS [38] (Figure 1). Whether MR-modulated pathways in the endothelium affect blood pressure required further studies.

Three studies using transgenic mice model of endothelial MR expression give some clues about the role of MR in blood pressure regulation. At basal state, the MR absence in endothelium has no consequences on blood pressure regulation. On the contrary, overexpression of endothelial MR leads to a moderate increase of blood pressure (15 mmHg) [29••]. This observation is interesting regarding pathological conditions where the MR can be overactivated. Since the participation of endothelium MR in blood pressure levels is moderate, others mechanisms can contribute to hypertension. One of the main information is that endothelial MR seems to participate to blood pressure regulation mainly through vascular reactivity. Overexpression increases vasoconstrictive pathways while inactivation

prevents endothelial dysfunction by the amelioration of vasodilation pathway. Endothelial MR modulation does not seem to act on vascular wall stiffness and remodeling but only via vascular reactivity. It is important to note that these transgenic models use different promoters to achieve endothelial specificity for MR expression/inactivation. Moreover, the Tie2-promoter has been described also to be active in hematopoietic cells [39], where a role of MR has already been described (like macrophages for example).

Role of the vascular smooth muscle mineralocorticoid receptor

The vascular smooth muscle cells (VSMC) are the major component of the vascular wall and play a crucial role in the regulation of vascular tone. MR is expressed in VSMC in physiological condition [7] and its expression is increased in aging rats [40]. Transgenic models with targeted MR overexpression in the VSCM have not been reported yet and we will only discuss the consequences of MR inactivation in VSCM.

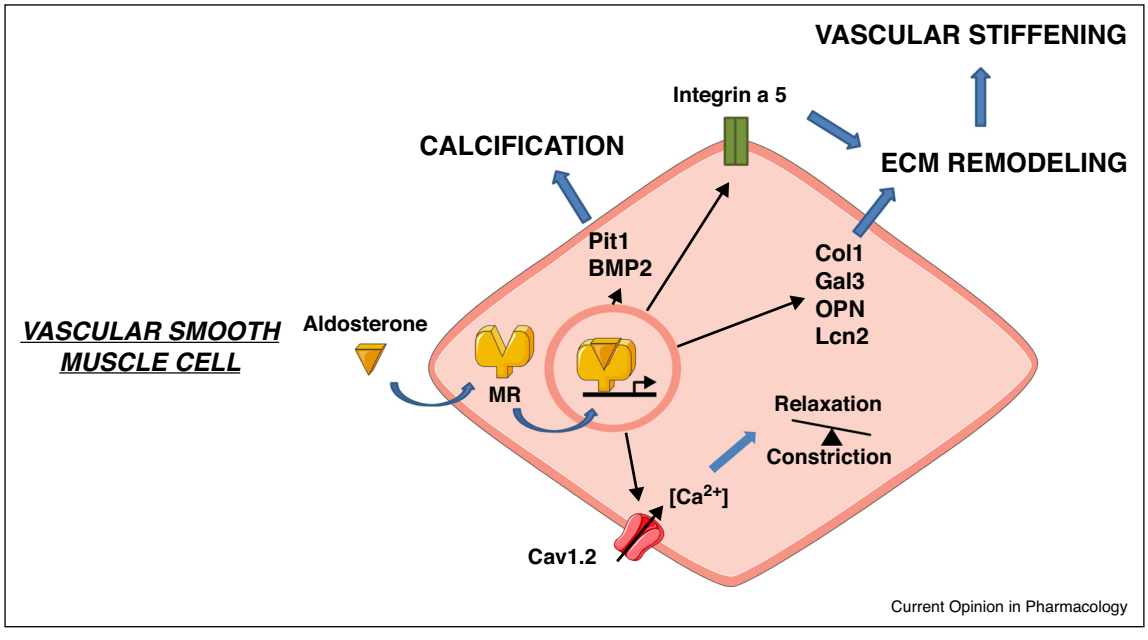
Vascular smooth muscle mineralocorticoid receptor inactivation

The role of VSMC-MR was studied by using two different models of VSMC-specific MR gene inactivation [41••,42•]. In both cases, it has been reported that VSMC-MR is involved in blood pressure regulation without any modification in renal sodium handling. McCurley and collaborators [41••] used a mouse model allowing temporal and VSMC-specific inactivation of MR (smooth muscle actin promoter). The genetic inactivation of MR in adult (two months of age) mice prevented the increase in blood pressure induced by aging: five months later, mice presented lower (15 mmHg) systolic blood pressure. In a constitutive model of VSMC-specific MR inactivation (SM22 promoter), Galmiche *et al.* [42•] reported a similar basal blood pressure decrease in five-month-old MR KO mice. Interestingly, VSMC-MR inactivation prevented the *in vivo* increase in blood pressure induced by AngII infusion [41••] but not by aldosterone-salt challenge [41••,42•]. Relaxation was not altered. Inactivation of VSMC-MR blunted the contractile response to pressure (myogenic tone), phenylephrine, AngII and to the thromboxane receptor agonist U46619, in aged mice only [41••]. Inactivation of VSMC-MR was also shown to decrease the contractile response to KCl and extracellular calcium (Ca^{2+}) [43].

Underlying mechanisms

The role of VSMC-MR in the contractile response could be explained by a decreased expression of L-type Ca^{2+} channel (Cav1.2) in aortas from VSMC-MR KO aged mice [41••] which may result in an altered Ca^{2+} signaling pathway in VSMC-MR KO cells (Figure 2). Indeed, Cav1.2 channel agonist BayK8644 had lower vasoconstrictive effects in mesenteric arteries from aged VSMC-MR

Figure 2



MR-modulated pathways in vascular smooth muscle cells. MR activation in VSMC induces the expression of profibrotic molecules such as Collagen 1 or Galectin-3 involved in Extracellular Matrix Remodeling (ECM). MR also participates to the regulation of Integrin α5 or Pit1, two proteins involved in vascular stiffening through cell adhesion tightening or calcification, respectively. MR also modulates the activity of the Cav1.2 calcium channel responsible for VSMC contraction. BMP2, Bone Morphogenetic Protein 2; Col1, Collagen 1; Gal3, Galectin-3; Lcn2, Lipocalin 2; MR, Mineralocorticoid Receptor; OPN, Osteopontin; Pit1, POU domain, class 1, transcription factor 1.

KO mice [41••]. The decreased Ca^{2+} signaling could in turn affect the contractile machinery activity. VSMC-MR KO exhibited reduced phosphorylation of Myosin Phosphatase-Targeting Subunit 1 (MYPT1), Myosin Light Chain Kinase (MLCK) and Myosin Light Chain 2 (MLC2) [43]. ENaC channel is also expressed in the VSMC and participates to myogenic tone [44]. Whether ENaC is regulated by MR in the VSMC like in the endothelium is unknown. *In vitro* MR activation in VSMC induced the expression of various pro-fibrotic markers [7,45–47]. *In vivo* studies showed that VSMC-MR plays a role in the remodeling of the extracellular matrix of the vascular wall as demonstrated using VSMC-MR KO mice with aldosterone-salt challenge [42•]. Molecular targeted modulated by MR in VSMC and involved in ECM remodeling include Galectin-3 (Gal3) [47], Osteopontin (OPN) [45] and Lipocalin 2 (Lcn2) [48] (Figure 2).

In pulmonary artery smooth muscle cells, MR activation induced the proliferation of smooth muscle cells, an effect prevented by spironolactone [20]. Smooth muscle MR is also involved in vascular calcification by regulating the expression of the phosphate transporter Pit1, which has an osteogenic function in the smooth muscle [49] (Figure 2). In human coronary artery SMC, MR activation by aldosterone upregulates the expression of genes implicated in vascular calcification, including Bone Morphogenetic Protein-2, Alkaline Phosphatase and Osteoprotegerin [7]. Indeed *in vivo* administration of spironolactone blunted the vascular calcification observed in the Klotho deficient mouse model [49]. The MR-mediated effects in VSMC may therefore contribute to long-term consequences of aging, vascular remodeling and stiffness on blood pressure.

Conclusion and perspectives

The use of transgenic models has shed a new light on the physiological and pathophysiological role of the vascular MR. Endothelial MR, as VSMC-MR, contributes to blood pressure. These observations suggest that vascular MR could contribute to blood pressure regulation, in addition to the epithelial MR in kidneys. How endothelial and VSMC-MR may act synergistically to affect blood pressure remains unknown. The underlying mechanisms by which vascular MR modulates blood pressure remains to be fully explored. Endothelial-MR and VSMC-MR participate into active stiffness of the vascular wall through modulation of vasoconstrictive and vasodilatory pathways. The participation of MR into vasoactive responses could be explained through the regulation of ion channels and ROS production observed in cellular models. VSMC-MR is also involved in the regulation of passive stiffness through the remodeling of the vascular wall such as VSMC proliferation, ECM remodeling or media calcification which could secondarily affect blood pressure levels.

Transgenic models with modulation of vascular MR expression will allow addressing some of the pending questions. One of them concerns the ligand that activates MR in the vascular wall (e.g., glucocorticoids and/or aldosterone, Figure 1). The enzyme 11βHSD2, responsible for the selectivity of aldosterone versus glucocorticoids for MR binding [50] is expressed in the endothelial cells while its expression in VSCM is questioned. Indeed, there are evidences that glucocorticoids can activate vascular MR in pathological conditions such as central serous chorioretinopathy [34]. Whether gender modulates the effects of vascular MR on blood pressure remains to be analyze. Of note Barrett Mueller *et al.* [51] recently reported that endothelial estrogen receptor modulates MR regulation of the pro-inflammatory gene ICAM-1. Whether other functions of MR in the vasculature are also modulated by ER signaling remains to be explored

The role of the vascular MR could also depend on the vascular bed that is considered. While the implication of vascular MR expressed in resistant vessels has been addressed in recent studies, the role of MR in coronary vessels, cerebral and renal vasculature remains unknown. Whether MR activation in the renal vasculature (either in VSMC or endothelial cells) is involved in endothelial or renal dysfunction and its relation with the development of hypertension, remain unknown. The use of transgenic models will allow us to decipher the contribution of endothelial-MR and VSMC-MR in the different vascular bed and the possible implication in blood pressure regulation. Moreover, there is growing evidences of the participation of MR in the immune cells [52•,53]. Since endothelial MR activation promotes leukocyte-endothelial adhesion [6], possible interactions between immune and vascular MR activation could be involved into blood pressure regulation. Exciting developments in the field are expected, which will certainly deeply explore whether vascular MR has specific role in particular pathophysiological and clinical settings.

Conflict of interest statement

Nothing declared.

Acknowledgements

The authors thank Nicolette Farman for her meaningful suggestions. This work was supported by the FP7-funded COST-ADMIRE BM1301 network, the C09S01 ECOS-CONICYT exchange program and the Leducq Foundation Transatlantic Network on Hypertension. A.T. was recipient of PhD grant from the Ministère de la Recherche et de l'Enseignement Supérieur. CA was supported by CONICYT, Beca de Postdoctorado en el Extranjero, Becas-Chile, No. 74130051.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R, Prospective Studies C: **Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one**

million adults in 61 prospective studies. *Lancet* 2002, **360**: 1903-1913.

2. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J: **Global burden of hypertension: analysis of worldwide data**. *Lancet* 2005, **365**:217-223.

3. Coffman TM: **Under pressure: the search for the essential mechanisms of hypertension**. *Nat Med* 2011, **17**:1402-1409.

4. Wolf RL, Mendlowitz M, Roboz J, Styan GP, Kornfeld P, Weigl A: **Treatment of hypertension with spironolactone. Double-blind study**. *JAMA* 1966, **198**:1143-1149.

5. Colussi G, Catena C, Sechi LA: **Spironolactone, eplerenone and the new aldosterone blockers in endocrine and primary hypertension**. *J Hypertens* 2013, **31**:3-15.

6. Caprio M, Newfell BG, Ia Sala A, Baur W, Fabbri A, Rosano G, Mendelsohn ME, Jaffe IZ: **Functional mineralocorticoid receptors in human vascular endothelial cells regulate intercellular adhesion molecule-1 expression and promote leukocyte adhesion**. *Circ Res* 2008, **102**:1359-1367.

7. Jaffe IZ, Mendelsohn ME: **Angiotensin II and aldosterone regulate gene transcription via functional mineralocortocoid receptors in human coronary artery smooth muscle cells**. *Circ Res* 2005, **96**:643-650.

8. Kwak SP, Patel PD, Thompson RC, Akil H, Watson SJ: **5′-Heterogeneity of the mineralocorticoid receptor messenger ribonucleic acid: differential expression and regulation of splice variants within the rat hippocampus**. *Endocrinology* 1993, **133**:2344-2350.

9. Virdis A, Neves MF, Amiri F, Viel E, Touyz RM, Schiffrin EL: **Spironolactone improves angiotensin-induced vascular changes and oxidative stress**. *Hypertension* 2002, **40**:504-510.

10. Adel H, Taye A, Khalifa MM: **Spironolactone improves endothelial dysfunction in streptozotocin-induced diabetic rats**. *Naunyn Schmiedebergs Arch Pharmacol* 2014, **387**:1187-1197.

11. •• Schafer A, Vogt C, Fraccarollo D, Widder J, Flierl U, Hildemann SK, Ertl G, Bauersachs J: **Eplerenone improves vascular function and reduces platelet activation in diabetic rats**. *J Physiol Pharmacol* 2010, **61**:45-52.

The authors showed that the MR antagonist eplerenone blunted the endothelial dysfunction induce by High-Fat Diet. Using a mouse model with endothelial-specific MR deletion, they showed that endothelial MR participates to High-Fat Diet-induced endothelial dysfunction.

12. •• Schafer N, Lohmann C, Winnik S, van Tits LJ, Miranda MX, Vergopoulos A, Ruschitzka F, Nussberger J, Berger S, Luscher TF *et al.*: **Endothelial mineralocorticoid receptor activation mediates endothelial dysfunction in diet-induced obesity**. *Eur Heart J* 2013, **34**:3515-3524.

The authors showed that the MR antagonist eplerenone blunted the endothelial dysfunction induce by High-Fat Diet. Using a mouse model with endothelial-specific MR deletion, they showed that endothelial MR participates to High-Fat Diet-induced endothelial dysfunction.

13. Sartorio CL, Fraccarollo D, Galuppo P, Leutke M, Ertl G, Stefanon I, Bauersachs J: **Mineralocorticoid receptor blockade improves vasomotor dysfunction and vascular oxidative stress early after myocardial infarction**. *Hypertension* 2007, **50**:919-925.

14. Joffe HV, Kwong RY, Gerhard-Herman MD, Rice C, Feldman K, Adler GK: **Beneficial effects of eplerenone versus hydrochlorothiazide on coronary circulatory function in patients with diabetes mellitus**. *J Clin Endocrinol Metab* 2007, **92**:2552-2558.

15. • Fujimura N, Noma K, Hata T, Soga J, Hidaka T, Idei N, Fujii Y, Mikami S, Maruhashi T, Iwamoto Y *et al.*: **Mineralocorticoid receptor blocker eplerenone improves endothelial function and inhibits Rho-associated kinase activity in patients with hypertension**. *Clin Pharmacol Ther* 2012, **91**:289-297.

The authors showed the beneficial effects of MR antagonism on vascular reactivity in hypertensive patients.

16. Michea L, Delpiano AM, Hitschfeld C, Lobos L, Lavandero S, Marusic ET: **Eplerenone blocks nongenomic effects of aldosterone on the Na+/H+ exchanger, intracellular Ca2+ levels, and vasoconstriction in mesenteric resistance vessels**. *Endocrinology* 2005, **146**:973-980.

17. Lacolley P, Labat C, Pujol A, Delcayre C, Benetos A, Safar M: **Increased carotid wall elastic modulus and fibronectin in aldosterone-salt-treated rats: effects of eplerenone**. *Circulation* 2002, **106**:2848-2853.

18. Mitchell GF: **Arterial stiffness and hypertension**. *Hypertension* 2014, **64**:13-18.

19. Vukusich A, Kunstmann S, Varela C, Gainza D, Bravo S, Sepulveda D, Cavada G, Michea L, Marusic ET: **A randomized, double-blind, placebo-controlled trial of spironolactone on carotid intima-media thickness in nondiabetic hemodialysis patients**. *Clin J Am Soc Nephrol* 2010, **5**:1380-1387.

20. Preston IR, Sagliani KD, Warburton RR, Hill NS, Fanburg BL, Jaffe IZ: **Mineralocorticoid receptor antagonism attenuates experimental pulmonary hypertension**. *Am J Physiol Lung Cell Mol Physiol* 2013, **304**:L678-L688.

21. Maron BA, Zhang YY, White K, Chan SY, Handy DE, Mahoney CE, Loscalzo J, Leopold JA: **Aldosterone inactivates the endothelin-B receptor via a cysteinyl thiol redox switch to decrease pulmonary endothelial nitric oxide levels and modulate pulmonary arterial hypertension**. *Circulation* 2012, **126**:963-974.

22. Maron BA, Waxman AB, Opotowsky AR, Gillies H, Blair C, Aghamohammadzadeh R, Loscalzo J, Leopold JA: **Effectiveness of spironolactone plus ambrisentan for treatment of pulmonary arterial hypertension (from the [ARIES] study 1 and 2 trials)**. *Am J Cardiol* 2013, **112**:720-725.

23. Garg R, Rao AD, Baimas-George M, Hurwitz S, Foster C, Shah RV, Jerosch-Herold M, Kwong RY, Di Carli MF, Adler GK: **Mineralocorticoid receptor blockade improves coronary microvascular function in individuals with type 2 diabetes**. *Diabetes* 2015, **64**:236-242.

24. Rigsby CS, Pollock DM, Dorrance AM: **Spironolactone improves structure and increases tone in the cerebral vasculature of male spontaneously hypertensive stroke-prone rats**. *Microvasc Res* 2007, **73**:198-205.

25. Rigsby CS, Ergul A, Portik Dobos V, Pollock DM, Dorrance AM: **Effects of spironolactone on cerebral vessel structure in rats with sustained hypertension**. *Am J Hypertens* 2011, **24**:708-715.

26. Sanchez-Pozos K, Barrera-Chimal J, Garzon-Muvdi J, Perez-Villalva R, Rodriguez-Romo R, Cruz C, Gamba G, Bobadilla NA: **Recovery from ischemic acute kidney injury by spironolactone administration**. *Nephrol Dial Transplant* 2012, **27**:3160-3169.

27. Lombes M, Oblin ME, Gasc JM, Baulieu EE, Farman N, Bonvalet JP: **Immunohistochemical and biochemical evidence for a cardiovascular mineralocorticoid receptor**. *Circ Res* 1992, **71**:503-510.

28. DeLano FA, Schmid-Schonbein GW: **Enhancement of glucocorticoid and mineralocorticoid receptor density in the microcirculation of the spontaneously hypertensive rat**. *Microcirculation* 2004, **11**:69-78.

29. •• Nguyen Dinh Cat A, Griol-Charhbili V, Loufrani L, Labat C, Benjamin L, Farman N, Lacolley P, Henrion D, Jaisser F: **The endothelial mineralocorticoid receptor regulates vasoconstrictor tone and blood pressure**. *FASEB J* 2010, **24**:2454-2463.

Using a transgenic mice model with inducible endothelial-specific MR over expression, the authors showed for the first time the involvement of endothelial MR in basal blood pressure regulation. Mice do not present endothelial dysfunction but an increased blood pressure response to vasoconstrictors such as ET1 and AngII.

30. • Rickard AJ, Morgan J, Chrissobolis S, Miller AA, Sobey CG, Young MJ: **Endothelial cell mineralocorticoid receptors regulate deoxycorticosterone/salt-mediated cardiac remodeling and vascular reactivity but not blood pressure**. *Hypertension* 2014, **63**:1033-1040.

The authors showed in a mouse model with endothelial-specific MR deletion that endothelial MR does not participates to DOCA–salt induced increase of blood pressure. However, endothelial MR deletion prevents endothelial dysfunction induced by DOCA–salt and prevents cardiac recruitment of leucocytes.

31. Jeong Y, Chaupin DF, Matsushita K, Yamakuchi M, Cameron SJ, Morrell CN, Lowenstein CJ: **Aldosterone activates endothelial exocytosis**. *Proc Natl Acad Sci U S A* 2009, **106**:3782-3787.

32. Leopold JA, Dam A, Maron BA, Scribner AW, Liao R, Handy DE, Stanton RC, Pitt B, Loscalzo J: **Aldosterone impairs vascular reactivity by decreasing glucose-6-phosphate dehydrogenase activity**. *Nat Med* 2007, **13**:189-197.

33. Thum T, Schmitter K, Fleissner F, Wiebking V, Dietrich B, Widder JD, Jazbutyte V, Hahner S, Ertl G, Bauersachs J: **Impairment of endothelial progenitor cell function and vascularization capacity by aldosterone in mice and humans**. *Eur Heart J* 2011, **32**:1275-1286.

34. Zhao M, Celerier I, Bousquet E, Jeanny JC, Jonet L, Savoldelli M, Offret O, Curan A, Farman N, Jaisser F *et al.*: **Mineralocorticoid receptor is involved in rat and human ocular chorioretinopathy**. *J Clin Invest* 2012, **122**:2672-2679.

35. Kusche-Vihrog K, Sobczak K, Bangel N, Wilhelmi M, Nechyporuk-Zloy V, Schwab A, Schillers H, Oberleithner H: **Aldosterone and amiloride alter ENaC abundance in vascular endothelium**. *Pflugers Arch* 2008, **455**:849-857.

36. Warnock DG, Kusche-Vihrog K, Tarjus A, Sheng S, Oberleithner H, Kleyman TR, Jaisser F: **Blood pressure and amiloride-sensitive sodium channels in vascular and renal cells**. *Nat Rev Nephrol* 2014, **10**:146-157.

37. Jeggle P, Callies C, Tarjus A, Fassot C, Fels J, Oberleithner H, Jaisser F, Kusche-Vihrog K: **Epithelial sodium channel stiffens the vascular endothelium in vitro and in Liddle mice**. *Hypertension* 2013, **61**:1053-1059.

38. Perez FR, Venegas F, Gonzalez M, Andres S, Vallejos C, Riquelme G, Sierralta J, Michea L: **Endothelial epithelial sodium channel inhibition activates endothelial nitric oxide synthase via phosphoinositide 3-kinase/Akt in small-diameter mesenteric arteries**. *Hypertension* 2009, **53**:1000-1007.

39. Sickinger S, Maier H, Konig S, Vallant N, Kofler M, Schumpp P, Schwelberger H, Hermann M, Obrist P, Schneeberger S *et al.*: **Lipocalin-2 as mediator of chemokine expression and granulocyte infiltration during ischemia and reperfusion**. *Transpl Int* 2013, **26**:761-769.

40. Krug AW, Allenhofer L, Monticone R, Spinetti G, Gekle M, Wang M, Lakatta EG: **Elevated mineralocorticoid receptor activity in aged rat vascular smooth muscle cells promotes a proinflammatory phenotype via extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase and epidermal growth factor receptor-dependent pathways**. *Hypertension* 2010, **55**:1476-1483.

41. •• McCurley A, Pires PW, Bender SB, Aronovitz M, Zhao MJ, Metzger D, Chambon P, Hill MA, Dorrance AM, Mendelsohn ME *et al.*: **Direct regulation of blood pressure by smooth muscle cell mineralocorticoid receptors**. *Nat Med* 2012, **18**:1429-1433.

Using a mouse model with conditional, inducible VSMC-specific MR deletion, the authors are showed the first time the involvement of VSMC-MR in age-induced increase of blood pressure. They report a decrease blood pressure response to exogenous AngII.

42. • Galmiche G, Pizard A, Gueret A, El Moghrabi S, Ouvrard-Pascaud A, Berger S, Challande P, Jaffe IZ, Labat C, Lacolley P *et al.*: **Smooth muscle cell mineralocorticoid receptors are mandatory for aldosterone-salt to induce vascular stiffness**. *Hypertension* 2014, **63**:520-526.

Using a mouse model with targeted deletion of MR in VSMC, Galmiche *et al*. showed the role of VSMC-MR in Integrin α5 regulation and aortic stiffness induced by aldosterone-salt challenge.

43. Tarjus A, Belozertseva E, Louis H, El Moghrabi S, Labat C, Lacolley P, Jaisser F, Galmiche G: **Role of smooth muscle cell mineralocorticoid receptor in vascular tone**. *Pflugers Arch* 2014.

44. Drummond HA: **betaENaC is a molecular component of a VSMC mechanotransducer that contributes to renal blood flow regulation, protection from renal injury, and hypertension**. *Front Physiol* 2012, **3**:341.

45. Kiyosue A, Nagata D, Myojo M, Sato T, Takahashi M, Satonaka H, Nagai R, Hirata Y: **Aldosterone-induced osteopontin gene transcription in vascular smooth muscle cells involves glucocorticoid response element**. *Hypertens Res* 2011, **34**:1283-1287.

46. Zhu CJ, Wang QQ, Zhou JL, Liu HZ, Hua F, Yang HZ, Hu ZW: **The mineralocorticoid receptor-p38MAPK-NFkappaB or ERK-Sp1 signal pathways mediate aldosterone-stimulated inflammatory and profibrotic responses in rat vascular smooth muscle cells**. *Acta Pharmacol Sin* 2012, **33**:873-878.

47. Calvier L, Miana M, Reboul P, Cachofeiro V, Martinez-Martinez E, de Boer RA, Poirier F, Lacolley P, Zannad F, Rossignol P *et al.*: **Galectin-3 mediates aldosterone-induced vascular fibrosis**. *Arterioscler Thromb Vasc Biol* 2013, **33**:67-75.

48. Latouche C, El Moghrabi S, Messaoudi S, Nguyen Dinh Cat A, Hernandez-Diaz I, Alvarez de la Rosa D, Perret C, Lopez Andres N, Rossignol P, Zannad F *et al.*: **Neutrophil gelatinase-associated lipocalin is a novel mineralocorticoid target in the cardiovascular system**. *Hypertension* 2012, **59**:966-972.

49. Voelkl J, Alesutan I, Leibrock CB, Quintanilla-Martinez L, Kuhn V, Feger M, Mia S, Ahmed MS, Rosenblatt KP, Kuro OM *et al.*: **Spironolactone ameliorates PIT1-dependent vascular osteoinduction in klotho-hypomorphic mice**. *J Clin Invest* 2013, **123**:812-822.

50. Fuller PJ, Yao Y, Yang J, Young MJ: **Mechanisms of ligand specificity of the mineralocorticoid receptor**. *J Endocrinol* 2012, **213**:15-24.

51. Barrett Mueller K, Lu Q, Mohammad NN, Luu V, McCurley A, Williams GH, Adler GK, Karas RH, Jaffe IZ: **Estrogen receptor inhibits mineralocorticoid receptor transcriptional regulatory function**. *Endocrinology* 2014, **155**:4461-4472.

52. • Rickard AJ, Morgan J, Tesch G, Funder JW, Fuller PJ, Young MJ: **Deletion of mineralocorticoid receptors from macrophages protects against deoxycorticosterone/salt-induced cardiac fibrosis and increased blood pressure**. *Hypertension* 2009, **54**:537-543.

The authors showed in a mouse model with endothelial-specific MR deletion that endothelial MR does not participates to DOCA–salt induced increase of blood pressure. However, endothelial MR deletion prevents endothelial dysfunction induced by DOCA–salt and prevents cardiac recruitment of leucocytes.

53. Usher MG, Duan SZ, Ivaschenko CY, Frieler RA, Berger S, Schutz G, Lumeng CN, Mortensen RM: **Myeloid mineralocorticoid receptor controls macrophage polarization and cardiovascular hypertrophy and remodeling in mice**. *J Clin Invest* 2010, **120**:3350-3364.