Emerging Roles of the Mineralocorticoid Receptor in Pathology: Toward New Paradigms in Clinical Pharmacology

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Abstract—The mineralocorticoid receptor (MR) and its ligand aldosterone are the principal modulators of hormone-regulated renal sodium reabsorption. In addition to the kidney, there are several other cells and organs expressing MR, in which its activation mediates pathologic changes, indicating potential therapeutic applications of pharmacological MR antagonism. Steroidal MR antagonists have been used for decades to fight hypertension and more recently heart failure. New therapeutic indications are now arising, and nonsteroidal MR antagonists are currently under development. This review is focused on nonclassic MR targets in cardiac, vascular, renal, metabolic, ocular, and cutaneous diseases. The MR, associated with other risk factors, is involved in organ fibrosis, inflammation, oxidative stress, and aging; for example, in the kidney and heart MR mediates hormonal tissue-specific ion channel regulation. Genetic and epigenetic modifications of MR expression/activity that have been documented in hypertension may also present significant risk factors in other diseases and be susceptible to MR antagonism. Excess mineralocorticoid signaling, mediated by aldosterone or glucocorticoids binding, now appears deleterious in the progression of pathologies that may lead to end-stage organ failure and could therefore benefit from the repositioning of pharmacological MR antagonists.

I. Introduction

The steroid aldosterone is the main mineralocorticoid hormone; it is synthesized in the glomerular zone of the adrenal cortex in response to hyperkaliemia or sodium depletion as the end-point of activation of the renin-angiotensin system (Rossier et al., 2015). Local production of aldosterone may also occur in peripheral tissue (Bader, 2010; Taves et al., 2011). Aldosterone stimulates renal sodium reabsorption and potassium excretion, therefore playing a major role in the control of blood pressure and extracellular volume homeostasis. We will briefly summarize the main elements of the pathways involved in aldosterone action, because several recent reviews (Pearce et al., 2015; Penton et al., 2015; Rossier et al., 2015) have documented in detail the molecular and cellular events involved in aldosterone effects in the distal tubule, the connecting tubule, and the collecting duct (referred as aldosterone-sensitive distal nephron). Aldosterone binds to the mineralocorticoid receptor (MR), a ligand-dependent transcription factor belonging to the nuclear receptor superfamily (Fuller and Young, 2005; Vienchareun et al., 2007; Zennaro et al., 2009; Fuller et al., 2012). The MR is expressed in a number of tissues beside the aldosterone-sensitive distal nephron. Figure 1 illustrates MR expression in a variety of human tissues.

The aldosterone ligand-MR receptor complex binds to glucocorticoid response elements within the promotor region of aldosterone-induced (or repressed) genes to modulate their transcription and resulting in the expression and activation of sodium transporters or channels. In a typical mineralocorticoid target cell, such as the renal collecting duct principal cell, sodium ions enter the cell through the amiloride-sensitive apical sodium channel (ENaC, for epithelial sodium channel), and are extruded into the peritubular space by the sodium pump (Na-K-ATPase) located in the basolateral membrane (Pearce et al., 2015; Rossier et al., 2015). The apical Na+ channels and basolateral Na+ pumps and K+ channels are coordinately activated and upregulated by membrane "cross-talk" in the presence of aldosterone (Harvey, 1995), leading to a sustained increase in transepithelial sodium reabsorption (Pearce et al., 2015; Penton et al., 2015; Rossier et al., 2015). The MR binds both aldosterone and glucocorticoid hormones with similar high affinity (in the nanomolar range); because glucocorticoids are 100- to 1000-fold more abundant than aldosterone in the plasma, permanent occupancy of the MR by glucocorticoid hormones may occur. In the kidney, this should induce permanent maximal sodium retention, independent of plasma aldosterone levels. The main mechanism ensuring in vivo mineralocorticoid selectivity involves coexpression of MR and the enzyme 11β hydroxysteroid dehydrogenase type 2 (11HSD2), that metabolizes circulating glucocorticoid hormones (cortisol in humans, corticosterone in rodents) into inactive 11-dehydro-derivatives (cortisone, 11-dehydrocorticosterone) with very low affinity for the MR (Farman and Rafestin-Oblin, 2001; Huyet et al., 2012; Odermatt and Kratschmar, 2012). Other mechanisms are important for mineralocorticoid selectivity (i.e., the events that explain the higher

ABBREVIATIONS: AF, atrial fibrillation; AngII, angiotensin II; CIN, cyclosporine-induced nephrotoxicity; CKD, chronic kidney disease; CSCR, central serous chorioretinitis; CV, cardiovascular; DAMP, danger-associated molecular pattern molecule; EDHF, endothelium-derived hyperpolarizing factor; ENaC, epithelial sodium channel; eNOS, endothelial NO synthase; ESRD, end-stage renal disease; ET1, endothelin 1; ET1RB, endothelin 1 receptor type B; GaI3, galectin 3; HCN, hyperpolarization-activated cyclic nucleotide; HF, heart failure; HFD, high-fat diet; 11HSD2, 11β hydroxysteroid dehydrogenase type 2; IL, interleukin; Igf, transient outward K+ current; KO, knockout; MDR, multidrug resistance; MetS, metabolic syndrome; MI, myocardial infarction; MR, mineralocorticoid receptor; MRA, mineralocorticoid receptor antagonist; NGAL, neutrophil gelatinase-activated lipocline; NO, nitric oxide; BGC, retinal ganglion cells; ROS, reactive oxygen species; SHR, spontaneously hypertensive Kyoto-Okamoto strain; TNF, tumor necrosis factor; VSMC, vascular smooth muscle cells.
efficacy of MR-aldosterone versus MR-glucocorticoid complexes) (Farman and Rafestin-Oblin, 2001; Viengchareun et al., 2007; Fuller et al., 2012). They include distinct nuclear translocation kinetics and transactivation activities related to the stability of the MR depending on its ligand (Hellal-Levy et al., 1999).

To avoid redundancy with previous reviews (Farman and Rafestin-Oblin, 2001; Fuller and Young, 2005; Viengchareun et al., 2007; Fuller et al., 2012). They include distinct nuclear translocation kinetics and transactivation activities related to the stability of the MR depending on its ligand (Hellal-Levy et al., 1999).

MR antagonists (MRA) can be divided into steroidal and nonsteroidal compounds (Table 1).

II. Mineralocorticoid Receptor Antagonists

MR antagonists (MRA) can be divided into steroidal and nonsteroidal compounds (Table 1).

A. Steroidal Compounds

Spironolactone was the first steroidal MR antagonist developed by Searle Laboratories (Skokie, Ill) in 1959 (Menard, 2004), about 30 years before the molecular characterization of the mineralocorticoid receptor by Arriza et al. (1987). Spironolactone was approved as a diuretic and natriuretic drug for the management of hypertension and primary aldosteronism and later on to treat heart failure (Menard, 2004). Spironolactone is a potent competitive MR antagonist but is poorly selective because it also inhibits the androgen and progesterone receptors, leading to side effects such as gynecomastia, impotence, and menstrual irregularities (Kolkhof and Borden, 2012). At high concentrations it may also interfere with the glucocorticoid receptor (GR) (Kolkhof and Borden, 2012). Canrenone is an active metabolite of spironolactone used in some countries (Armanini et al., 2014). An injectable preparation (potassium canrenenate) is more widely available. Of note, progesterone is a natural MRA (Huyet et al., 2012). Eplerenone (9-11a-epoxymexrenone), a second generation MRA, was developed by Ciba-Geigy (Basel, Switzerland) and launched by Pfizer (New York, NY) in 2002 for the treatment of hypertension and heart failure.
TABLE 1
Characteristics of the mineralocorticoid receptor antagonists

<table>
<thead>
<tr>
<th>MRA Generation</th>
<th>Structure</th>
<th>Example/Class</th>
<th>Selectivity</th>
<th>Potency</th>
<th>Half-Life</th>
<th>Tissue Distribution</th>
<th>Tissue Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>steroidal</td>
<td>Spironolactone</td>
<td>low</td>
<td>high</td>
<td>long</td>
<td>6 × higher in kidney</td>
<td>no</td>
</tr>
<tr>
<td>II</td>
<td>steroidal</td>
<td>Eplerenone</td>
<td>high</td>
<td>low</td>
<td>Short (4 hours)</td>
<td>3 × higher in kidney</td>
<td>no</td>
</tr>
<tr>
<td>III</td>
<td>nonsteroidal</td>
<td>Finerenone</td>
<td>high</td>
<td>high</td>
<td>?</td>
<td>equal</td>
<td>CV &gt; kidney</td>
</tr>
</tbody>
</table>

(Manerd, 2004; Kolkhof and Borden, 2012). This MRA is much more selective for MR than spironolactone, but less potent (40× less), requiring higher dosage to achieve similar MR antagonism. The different pharmacokinetic and pharmacodynamic properties of spironolactone and eplerenone, however, result in a difference in efficacy in the treatment of hypertension: 100 mg eplerenone is almost 50 to 75% as potent as 100 mg spironolactone in patients with essential hypertension (Weinberger et al., 2002; Parthasarathy et al., 2011). Spironolactone is rapidly metabolized into several active metabolites with a half-life of about 15 hours, whereas eplerenone has no active metabolite and has a shorter half-life of less than 4 hours. The half-life of the steroidal MRA has not been explored in disease subjects (Kolkhof and Borden, 2012), except for eplerenone in chronic kidney disease (Schwenk et al., 2015). This is important to consider when an MRA is selected for clinical trials. The use of MRAs that do not need to be metabolized in active moieties for full efficacy, such as eplerenone, may be preferred when effects are expected relatively rapidly after administration. Short half-life is important to consider for safety concerns to yield a rapid decrease of active moieties when halting therapy in case of toxic side effects. Racial difference in response to MR blockade may exist: African Americans with heart failure are less responsive to the renal effect of spironolactone and develop less hyperkalemia (Cavallari et al., 2004); this is not the case with eplerenone (Flack et al., 2003). It has been reported that spironolactone (at micromolar concentrations) inhibits aldosterone biosynthesis (Netchitalio et al., 1985) and blocks enzymes involved in steroidogenesis (Penhoat et al., 1988; Ye et al., 2009). In contrast, eplerenone (1–30 μM) did not impair basal and angiotensin II-induced cortisol and aldosterone production in human adrenocortical H295R cells (Ye et al., 2009). Thus spironolactone and eplerenone have differential effects on steroidogenesis. Recently, spironolactone was reported to be effective in antitumor therapy, an effect independent of the classic mineralocorticoid pathway, requiring retinoid X receptor coactivation (Leung et al., 2013). This may highlight novel aldosterone and/or MR-independent effects of spironolactone.

The “hormonal related” side effects of spironolactone are due to its inhibitory actions on other steroid receptors such as the androgen and progesterone receptors. Hyperkalemia is a potential life-threatening side effect of MRAs, related to their efficacy to decrease urinary potassium excretion. Indeed these drugs are potassium-sparing diuretics, and their “propensity” to lead to hyperkalemia has been observed in most clinical studies, especially when renal function is impaired. It should be emphasized that clinically relevant hyperkalemia (requiring discontinuation of MRA or administration of potassium chelator) is rare. Close follow up of kaliemia is required, especially at the onset of MRA administration, as well as regular monitoring of renal function. Some of the beneficial effects of MRA may be related to a decreased occurrence of potassium depletion and hypokalemia often observed in patients with heart failure and treated with diuretics. Nevertheless, the risk of hyperkalemia clearly accounts for the underuse of this efficient therapeutic class (Albert et al., 2009; Maron and Leopold, 2010; Jaisser et al., 2011; Rossignol et al., 2012). Despite this risk, even in patients with impaired renal function, the benefit/risk balance is in favor of MRA administration (Rossignol et al., 2014).

Excessive/inappropriate MR activation in pathology is often inferred from the benefits provided by MRA administration. Is it conceivable that spironolactone could have other targets? Some studies suggest competition between spironolactone (or other MRAs) and digitalin analogs through the Na-K-ATPase. Indeed there is a strong structural similarity between these drugs. Hans Selye et al. reported in 1969 that spironolactone “protects the rat against the production of myocardial necroses and other manifestations of digitoxin poisoning”; this pioneering work proposed spironolactone as an antidote to digitoxin cardiac toxicity (Selye et al., 1969). Canrenone interacts with the cell membrane of cultured macrophages and vascular smooth muscle, and it partially reversed the disturbance of cation handling induced by high concentrations of ouabain, a response observed as early as 30 minutes after addition of drugs (Hannaert et al., 1986). A later study (Finotti and Palatini, 1981) concluded that canrenone is a partial agonist of brain Na-K-ATPase at the ouabain binding site. The question of direct interaction of canrenone and Na-K-ATPase has been explored (Tal and Karlish, 1988): although canrenone could displace ouabain binding to partially purified sodium pumps, it was concluded that canrenone does not react within the ouabain binding site itself but rather interacts allosterically with this site. Because spironolactone acts on Na-K-ATPase within minutes, one can wonder whether interaction with the sodium pump could explain, at least in part, the rapid effects of spironolactone (usually referred as nongenomic mineralocorticoid effects). More
recently, it has been reported that spironolactone can antagonize ouabain or analogs such as marinobufagenin; these observations suggest that some of the effects of spironolactone (reduction of experimental cardiac fibrosis) could rely on antagonism of marinobufagenin through the Na-K-ATPase (Balzan et al., 2003; Tian et al., 2009).

B. Nonsteroidal Compounds

In the last decade, the broader therapeutic indications of MRA in heart failure and possibly in kidney diseases, as well as the underuse of classic steroidal MRAs due to the risk of hyperkalemia has stimulated the search for novel MR antagonists with higher selectivity, higher potency, and, if possible, a reduced risk of hyperkalemia. Several nonsteroidal compounds acting as MRAs were identified. Dihydropyridines (L-type calcium channel antagonists) act as MRAs in vitro and in vivo (Kolkhof and Borden, 2012). Successful optimization of antagonists devoid of L-type calcium channel antagonist properties led to several new compounds and derivatives. For example, the Bayer BR-4628 compound was identified as a potent and selective MRA. Its mode of action differs from classic steroidal MRA, acting as a bulky antagonist, leading to the protrusion of MR helix 12 (which plays a major role in MR activation upon ligand binding (Hellal-Levy et al., 2000)). This causes the formation of an unstable receptor complex unable to recruit coregulators and is rapidly degraded. Optimization of BR-4628 led to BAY 94-8862 (Finerenone); this third generation MRA was used in phase 2a clinical trials (Pitt et al., 2013) in patients with heart failure and mild renal dysfunction. Finerenone was shown to be safe and led to lower rate of hyperkalemia than spironolactone, for a similar effect on the N terminal-proBNP surrogate marker (ARTS) (Pitt et al., 2013). Two clinical trials have been launched recently in heart failure (ARTS-HF) (Pitt et al., 2015) and in diabetic nephropathy (ARTS-DN) (Ruilope et al., 2014; Bakris et al., 2015) to further test safety and efficacy in phase 2b trials. The relative benefit of Finerenone over the classic steroidal antagonist spironolactone, regarding increased plasma potassium levels, may rely on a differential tissue distribution of the two compounds; while spironolactone (and eplerenone) accumulated three- to sixfold more in the kidney than in the heart, the distribution of finerenone appears equivalent in rat heart and kidney (Kolkhof et al., 2014). Therefore, low doses may allow sufficient MR antagonism outside of the kidney with less renal MR blockade and reduced K+ sparing effect. Other nonsteroidal MRAs such as PF-03882845 (Pfizer) and SM-368229 (Dainippon Sumitomo Pharma, Osaka, Japan), for example, have been generated and proven to be efficient in preclinical models and are currently going through clinical trials (Collin et al., 2014).

III. Mineralocorticoid Receptor and Cardiac Diseases

The combined inhibition of receptors of vasoactive hormones (angiotensin II, aldosterone, catecholamines) that are chronically activated in heart failure (HF) are now considered as main therapeutic tools. Several clinical studies have demonstrated that antagonism of MR is highly beneficial in mild to severe HF, suggesting that an excessive activation of the MR occurs over the course of the disease and plays an important role in the pathophysiology of HF. The mechanisms that explain the efficacy of MRAs in HF are likely multiple. Pharmacological blockade of MR limits the transition to heart failure in models of systolic left ventricular dysfunction (Kuster et al., 2005) and myocardial infarction (Wang et al., 2004a; Galuppo and Bauersachs, 2012) as well as in models of diastolic dysfunction in rats (Ohtani et al., 2007) and mice (Di Zhang et al., 2008). Clinical trials showed unambiguously that this benefit translates in patients with HF; the RALES, EPHESUS, and EMPHASIS trials demonstrated clear benefit of MR antagonists in HF, reducing morbidity and mortality, paving the way for broader clinical use of MRAs in cardiovascular (CV) diseases (Pitt et al., 1999, 2003; Zannad et al., 2011). The benefit of MRAs now extend to early phases of myocardial infarction (MI) (REMINDER study) (Montalescot et al., 2014) and possibly to HF with preserved ejection fraction (post hoc study of the TOPCAT trial) (Pfeffer et al., 2015).

In these trials, the beneficial effects of MR blockade are often observed in the absence of elevated plasma aldosterone levels, raising the question of the nature of the ligand that activates the MR excessively or inappropriately. It is generally considered that cortisol is the main MR ligand in the heart, because of the low level of the MR protecting enzyme 11 HSD2 (Funder, 2009). However, this view is challenged by reports showing that serum aldosterone and cortisol are independent predictors of increased mortality risk in heart failure (Guder et al., 2007, 2015). To assess the in vivo differential effects of aldosterone and glucocorticoids in heart, we compared the molecular cardiac signature of mouse heart after infusion of mice with aldosterone or with corticosterone in moderate amounts (fivefold increase in plasma aldosterone and twofold increase in corticosterone). We identified several genes that are specifically modified by aldosterone and unaffected by glucocorticoid treatment. For example, the connective tissue growth factor, involved in fibrosis, was upregulated specifically by aldosterone and not by glucocorticoid (Messaoudi et al., 2013; Gravez et al., 2015). Of note, aldosterone and not corticosterone, enhanced cell cycle-related gene networks and endothelial cell proliferation in myocardial capillaries, revealing a potential benefit for aldosterone stimulated-MR pathway to fight capillary rarefaction.
A. Mineralocorticoid Receptor and Extracellular Matrix Remodeling

Since the pioneering work of Weber and collaborators, it is now accepted that chronic MR activation is associated with fibrosis, extracellular matrix remodeling and cell growth and survival (Brilla and Weber, 1992; Brilla et al., 1993; Robert et al., 1994; Dooley et al., 2011). The onset of fibrosis may involve a number of cell types including fibroblasts but also other cells prone to secrete profibrotic factors (cardiomyocytes, vascular smooth muscle cells (VSMC), and inflammatory cells including macrophages and dendritic cells) that secondarily stimulate extracellular matrix production.

Chronic coadministration of aldosterone and NaCl in uninephrectomized rats (aldosterone-salt or deoxycorticosterone-salt (DOCA-salt) models) stimulates perivascular and interstitial cardiac fibrosis in left and right ventricles. This occurs independently of an increase in blood pressure, because MR blockade at subhypotensive doses could prevent fibrosis (Brilla et al., 1993; Robert et al., 1994). The development of cardiac fibrosis seems to start around vessels (associated with coronary and myocardial inflammation) and extends later to the interstitium (Robert et al., 1994). In myocardial infarction, reparative scar and interstitial fibrosis are improved by MR antagonism (Galuppo and Bauersachs, 2012). Deletion of cardiomyocyte MR ameliorates reverse remodeling after MI (Galuppo and Bauersachs, 2012). Clinical trials indicate that the benefit of MRA in the EPHESUS trial is associated with reduced plasma procollagen type I amino-terminal pro-peptide, a surrogate biomarker of fibrosis (Iraqi et al., 2009). One-month treatment with Spironolactone also reduced procollagen type I carboxyl-terminal propeptide in patients after stroke (Wong et al., 2013).

The mechanisms underlying the beneficial effects of MRA in cardiac diseases are complex and diverse. Aldosterone alone (ex vivo) or in association with salt (in vivo) stimulates the expression of proinflammatory molecules that may contribute to the pathogenesis of cardiac remodeling. For example, aldosterone increases endothelin 1 (ET1), transforming growth factor β, and (plasminogen activator inhibitor) PAI through MR-dependent mechanisms as well as collagen and metalloproteases (Marney and Brown, 2007). Oxidative stress is also induced, mainly through activation of the NADPH oxidases (Johar et al., 2006). Concomitant administration of NaCl is a prerequisite to induce cardiac fibrosis: aldosterone administration alone (or chronic increased aldosteronemia in models of hyperaldosteronism) is not sufficient to induce cardiac fibrosis in rats or mice (Brilla, 2000; Wang et al., 2004b). Cardiomyocyte-specific MR or aldosterone synthase overexpression did not induce fibrosis in the mouse (Garnier et al., 2004; Ouvrard-Pascaud et al., 2005), suggesting that one or more cofactors (salt, oxidative stress,…) are required to induce the profibrotic effects of aldosterone.

The interaction between MR and angiotensin II (AngII) receptor AT1R signaling cascades plays a key role in aldosterone-induced fibrosis. Spironolactone prevents AngII-induced cardiac and vascular remodeling (Virdis et al., 2002; Johar et al., 2006); AngII can activate the MR pathway indirectly via the EGFR pathway (Rautureau et al., 2011); expression of AT1R is obligatory for aldosterone activation of VSMC (Lemarie et al., 2009). These observations support the benefit of adjunct therapies employing MR and AT1R antagonists in cardiovascular pathologies.

Novel profibrotic targets modulated by MR were recently identified, including enzymes such as myocardin, lysyl oxidase involved in the maturation of procollagen (Lopez et al., 2009) or profibrotic molecules as cardiomyocytes, vascular smooth muscle cells (VSMC), and inflammatory cells including macrophages and dendritic cells] that secondarily stimulate extracellular matrix production.

B. Mineralocorticoid Receptor and Electrophysiological Disorders

Recently, the deleterious role of aldosterone (and the beneficial effect of MR antagonists) was highlighted in the occurrence of atrial and ventricular arrhythmias.

Spironolactone prevents atrial remodeling (fibrosis and dilation) as well as apoptosis in an experimental model of atrial fibrillation (AF) and prevents the increased inducibility and duration of tachypacing-induced AF (Zhao et al., 2010a) as well as atrial remodeling and AF inducibility in a rat hypertensive model (Kimura et al., 2011). Fibrotic remodeling observed in a genetic mouse model of AF (constitutively active Rac1 under the control of the α myosin heavy chain) is prevented by MR antagonism (spironolactone or nonsteroidal BR-4628) (Lavall et al., 2014). An increased rate of AF was reported in patients with hyperaldosteronism (Milliez et al., 2005). Aldosteronemia is increased in patients with chronic AF and decreases rapidly after electrical cardioconversion (Goette et al., 2001). Atrial...
MR expression was increased in patients with AF (Tsai et al., 2010), and MRA was associated with reduced AF-related hospitalization (Williams et al., 2011). Eplerenone improved maintenance of sinus rhythm in patients with long standing AF (Ito et al., 2013), and post hoc analysis of the EMPHASIS-HF trial indicated that eplerenone reduced the incidence of new onset of AF (Swedberg et al., 2012).

MR activation can also lead to ventricular arrhythmias. Ex vivo, aldosterone increases T-type calcium channel expression and beating frequency in neonatal rat ventricular cardiomyocytes (Lalevee et al., 2005; Rossier et al., 2008). In vivo, aldosterone infusion or overexpression of MR in cardiomyocytes induces cardiac ion channel remodeling: the transient outward K⁺ current (Iₒ) is decreased, whereas the L type calcium current activity is increased; the activity of the ryanodine receptor is also impaired (Gomez et al., 2009; Ouivrard-Pascaud et al., 2005). This has important consequences in the control of calcium homeostasis, modulation of calcium transients, sarcoplasmic reticulum diastolic leaks, and initiating cardiac rhythm disorders. Indeed, conditional overexpression of the MR in cardiomyocytes is associated with ventricular extrasystoles and increased sensitivity to triggering ventricular arrhythmias (Ouvrard-Pascaud et al., 2005). In cardiomyopathic hamsters, eplerenone reduces cardiac remodeling and decreases the rate of spontaneous ventricular tachycardia. The spatial dispersion of QT interval is restored, whereas the ST segment depression and action potential propagation and conduction velocity are considerably improved (De Mello, 2006). Similarly, spironolactone treatment prevents gap junction remodeling and restores the decreased transverse conduction velocity in a thoracic aortic constriction model (Qu et al., 2009). Likewise, eplerenone reduces fibrosis-related arrhythmias in aged mice (Stein et al., 2010). Both spironolactone and eplerenone reduce the prolongation of QT intervals and diminishes the occurrence of ventricular premature beats or nonsustained ventricular tachycardia in aldosterone-infused rats (Dartsch et al., 2013). Spironolactone has similar effects on inducible ventricular tachyarrhythmia in rats with aldosterone-salt challenge (Deshmukh et al., 2011).

The beneficial effects of MRA on electrical remodeling may underlie the impressive beneficial effect of MR antagonism on lowering the rate of sudden death in the RALES and EPHESEUS trials (about 50% of the total benefit of MRA in these trials) (Pitt et al., 1999, 2003). Indeed, a meta-analysis of seven clinical trials including 8,635 patients showed that MRA reduced the risk of sudden death by 21% in patients with HF and lessened the risk of ventricular tachycardia by 72% (Wei et al., 2010). A recent dedicated clinical trial showed that administration of MRA at the early stage of MI (carriage bolus on admission for primary percutaneous coronary intervention followed by spironolactone 25 mg/ day) was associated with decreased life-threatening arrhythmia and cardiac arrest (Beygui et al., 2013).

C. Cardiac Effects of Noncardiomyocyte Mineralocorticoid Receptor

The MR is expressed in cardiomyocytes and also in other cardiac cell types: coronary endothelial and smooth muscle cells (VSMC) and inflammatory cells as macrophages, which participate in the remodeling process of cardiac pathologies (Nguyen Dinh Cat and Jaisser, 2012). The benefits of MRA in cardiac diseases may thus also be accounted for by MR blockade in these cellular targets.

The specific knockout of MR in macrophages prevents the development of cardiac interstitial fibrosis in the DOCA-salt model, although the cardiac recruitment of these cells was not prevented (Rickard et al., 2009). Myeloid MR can induce pro-inflammatory M1 macrophage polarization, and deletion of macrophage MR prevents interstitial fibrosis upon AngII infusion and chronic nitric oxide (NO) inhibition (Usher et al., 2010). These observations indicate that MR signaling in macrophages is required to elicit a full fibrotic response in the heart.

The importance of the coronary circulation in cardiac pathophysiology is well known, although its specific contribution to the harmful effects of mineralocorticoids has been scantily addressed. In a model of cardiomyocyte-specific overexpression of the aldosterone synthase, aldosterone concentration in cardiac tissue is moderately increased and there is no alteration of cardiac structure and function (Ambroisine et al., 2007). In contrast, coronary arteries develop a major NO-independent vascular dysfunction due to an altered expression and activity of the potassium channel BKCa expressed in VSMC (Ambroisine et al., 2007). Cardiomyocyte-specific overexpression of the MR is associated with local oxidative stress leading to coronary endothelial dysfunction (Favre et al., 2007). MR-dependent and aldosterone-induced coronary vessel alterations could increase the sensitivity of the heart to ischemia and predispose cardiac tissue to heart dysfunction. Unpublished data from our group showed that deletion of MR in VSMC is crucial in MI-induced heart dysfunction: deletion of the VSMC MR drastically improved coronary function after MI, as well as cardiac perfusion measured by magnetic resonance imaging, and MI-induced cardiac remodeling. MR antagonism with the nonsteroidal MRA finerenone had a similar effect, improving coronary reserve and coronary function in MI.

D. Mineralocorticoid Receptor and Cardiopathy of Metabolic Origin

The beneficial effects of MR blockade could extend to cardiopathies related to metabolic diseases. In an experimental model of type 2 diabetes (db/db mice),
Eplerenone normalized the cardiac expression of several adipokines (Guo et al., 2008). Eplerenone or spironolactone attenuated cardiac steatosis, apoptosis, and diastolic dysfunction in Zucker Diabetic Fatty rats (Ramirez et al., 2013; Bender et al., 2015a). Conversely, increased cardiac production of aldosterone is beneficial and protects against reduced capillarization in type I and type II diabetes (Messaoudi et al., 2009; Fazal et al., 2014). This response appears to be mediated by an increase in Akt phosphorylation (Fazal et al., 2014). Eplerenone improves coronary circulation (Joffe et al., 2007) and spironolactone likewise improves coronary reserve in type 2 diabetic patients (Garg et al., 2015).

Adipocytes are found in close proximity to blood vessels, where adipocyte-derived cytokines (adipokines) can directly influence vascular function (Nguyen Dinh Cat and Jaisser, 2012). Bender et al. (2015b) recently uncovered a specific role for MR as a mediator of coronary (not peripheral) vascular dysfunction in patients with obesity and diabetes. It is evident from these observations that the role of MR in epicardial or perivascular fat on coronary function deserves further analysis.

IV. Mineralocorticoid Receptor and Vascular Diseases

The pharmacological blockade of MR using spironolactone or eplerenone provides benefits for vascular tone and remodeling of the vascular wall (Virdis et al., 2002). Several studies in preclinical models have shown beneficial effects of MRA on endothelial dysfunction induced by diabetes (Schafer et al., 2010, 2013; Adel et al., 2014), by a high-fat diet (Schafer et al., 2013), or myocardial infarction (Sartorio et al., 2007). Eplerenone prevented the potentiation of agonist-induced vasoconstriction induced by aldosterone (Michea et al., 2005). In rats treated with aldosterone (Lacolley et al., 2002), eplerenone prevented blood pressure increase and reduced pulse pressure and elastic modulus of large vessels, two markers of vascular stiffness (Mitchell, 2014). In human hypertensive patients, MRA improved flow-mediated dilation, a mechanism contributing to vascular tone in hypertension (Fujimura et al., 2012). In patients with end-stage renal disease (ESRD), MRA also had favorable effects on intima-media remodeling (Vukusich et al., 2010). Of note, two studies reported a nondiuretic effect on blood pressure in patients with ESRD treated with spironolactone or eplerenone (Gross et al., 2005; Shavit et al., 2012).

More recently, the benefit of MRA has been reported in experimental models of pulmonary arterial hypertension, improving pulmonary vascular remodeling, right ventricular systolic pressure (Preston et al., 2013), and pulmonary artery systolic pressure (Maron et al., 2012). Importantly, this may translate to clinics, because in patients with pulmonary arterial hypertension, spironolactone as an adjunct to classic therapeutics has beneficial effects with improved exercise tolerance and decreased BNP plasmatic concentration (a marker of cardiac dysfunction) (Maron et al., 2013).

Although MR-mediated effects on arteries have been largely documented, only in abnormal situations such as venous arterialization do veins express functional MR and HSD2. MR expression may potentiate AngII activity in grafted veins (Bafford et al., 2011), whereas remodeling of venous wall is reduced by MRA (Ehsan et al., 2013). Veins exhibit a higher sensitivity to vasoconstrictor agents compared with arteries, which did not differ in their response between normal mice and DOCA-salt hypertension model (Perez-Rivera et al., 2004). Venous smooth muscle tone is increased in DOCA-salt hypertensive rats (Fink et al., 2000). Whether changes in venous contractility are secondary to the rise in blood pressure or depend directly on MR function is currently unknown.

A. Role of the Endothelial Mineralocorticoid Receptor

MR expression was demonstrated in the endothelial cells of rabbit aorta in the early 1990s (Lombes et al., 1992) and then later in bovine aortic endothelial cells (Leopold et al., 2007) and in endothelial cells from human coronary arteries and aorta (Caprio et al., 2008). Endothelial MR expression is increased in the microvasculature of spontaneously hypertensive Kyoto-Okamoto strain (SHR) rats (DeLano and Schmid-Schonbein, 2004). A mouse model with conditional and inducible endothelium-specific MR overexpression highlighted the involvement of the endothelial MR in blood pressure regulation (Nguyen Dinh Cat et al., 2010). Basal blood pressure was increased, as well as the blood pressure response to the acute infusion of the vasoconstrictors AngII and endothelin 1 (ET1), accompanied by an increased contractile response of mesenteric arteries to AngII and ET1 (Nguyen Dinh Cat et al., 2010). However, endothelial MR overexpression had no effect on endothelium-dependent or independent relaxation (Nguyen Dinh Cat et al., 2010). Endothelium-specific deletion of MR indicated that endothelial MR is not involved in deoxycorticosterone-salt (DOCA-salt)-mediated increase in systolic blood pressure (Rickard et al., 2014). Endothelial MR deletion could blunt obesity-induced endothelial dysfunction (Schafer et al., 2013); however, blood pressure was not assessed in this study. Both studies 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 challenges (Schafer et al., 2013; Rickard et al., 2014). MR deletion in endothelium has no consequences for systolic blood pressure under basal conditions, in contrast to the hypertensive responses observed with endothelial MR overexpression. This observation was confirmed recently in another model targeting MR in endothelial
cells (Mueller et al., 2015). 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 (Rickard et al., 2014). Indeed Mueller et al. (2015) recently reported that endothelial-MR participates in regulation of vasomotor function in a vascular bed-specific manner. Inducing angiotensin II-dependent hypertension caused an impaired endothelial-dependent relaxation that was prevented in endothelial MR-deficient mice in mesenteric vessels but not in coronary vessels. This vascular bed-specific contribution of endothelial MR is also suggested by the MR-induced vasoconstrictor phenotype reported in large arteries, contrasting with its vasodilatory effect on the choroid capillary network of the posterior eye (see below).

Aldosterone via MR activation modulates several endothelial functions and pathways, such as enhanced exocytosis of Weibel-Palade bodies containing proinflammatory cytokines (Jeong et al., 2009), cell adhesion of inflammatory cells (Caprio et al., 2008), and the production of reactive oxygen species (ROS). MR activation increases the activity of NADPH oxidase and reduces the degradation of ROS through reduced expression and activity of the antioxidant enzyme glucose-6-phosphate dehydrogenase (Leopold et al., 2007). This 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 (Leopold et al., 2007). In pulmonary artery endothelial cells, the high production of hydrogen peroxide induced by aldosterone induces a sulfenic posttranslational modification of the endothelin receptor type B, leading to reduced NO bioavailability in the pulmonary vasculature (Maron et al., 2012). 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 (Thum et al., 2007).

MR-modulated pathways in the endothelium may impact blood pressure or vascular properties in pathologies such as atherosclerosis or injury-induced remodeling (McGraw et al., 2013). Another recent issue is the involvement of MR signaling in vascular thrombosis. In mice with arterial injury or in normotensive rats, aldosterone was reported to favor thrombosis, whereas MR overexpression in endothelium impaired thrombus formation (Lagrangé et al., 2014). Endothelial MR activation may have a beneficial antithrombotic action in healthy vessels, whereas deleterious prothrombotic effects may occur only when the endothelium is injured (Lagrangé et al., 2014).

### B. Role of the Vascular Smooth Muscle Mineralocorticoid Receptor

MR is expressed in the vascular smooth muscle cells (VSMC) that play a crucial role in the regulation of vascular tone (Jaffe and Mendelsohn, 2005; Nguyen Dinh Cat et al., 2010). VSMC MR expression is increased in aging rats (Krug et al., 2010). VSMC MR is involved in blood pressure regulation, as shown in two different models of VSMC-specific MR gene inactivation (McCurley et al., 2012; Galmiche et al., 2014). The genetic inactivation of MR in 4-month-old adult mice prevented the increase in blood pressure occurring with aging in 9-month-old mice (McCurley et al., 2012). In a constitutive model of VSMC-specific MR inactivation, basal blood pressure was reduced in 4-month-old MR knockout (KO) mice (Galmiche et al., 2014). Interestingly, VSMC MR inactivation prevented the in vivo rise in blood pressure induced by AngII infusion but not by aldosterone-salt treatment (McCurley et al., 2012; Galmiche et al., 2014). VSMC MR gene inactivation blunted the contractile response to pressure and agonists (phenylephrine, AngII) in aged mice only (McCurley et al., 2012). This difference in the effect of VSMC MR deletion on blood pressure in mouse models with either constitutive or inducible MR deletion indicates that long-lasting MR inactivation is required to affect blood pressure control and decrease basal blood pressure: in both models, MR inactivation occurring during 4–5 months is required to result in lower basal blood pressure. Whether this reflects adaptation/compensatory mechanisms rather than a primary role of VSM MR in the control of blood pressure remains to be evaluated. 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 (Galmiche et al., 2014). As detailed below, activation of MR impacts on VSMC through ion channel regulation (McCurley et al., 2012; Tarjus et al., 2015a). MR activation in VSMC induces the expression of profibrotic markers (Jaffe and Mendelsohn, 2005; Kiyosue et al., 2011; Zhu et al., 2012; Calvier et al., 2013).

In pulmonary arteries, MR activation induced the proliferation of smooth muscle cells, an effect prevented by spironolactone (Preston et al., 2013). These effects could likely contribute to the development of pulmonary arterial hypertension and to the benefit of MRA in patients.

VSMC MR participates in vascular calcification (Jaffe et al., 2007) by regulating the expression of the phosphate transporter Pit1, which has an osteogenic function in the smooth muscle (Voelkl et al., 2013). In vivo administration of spironolactone blunts the vascular calcification observed in the Klotho deficient mouse model (Voelkl et al., 2013) and in uremic rats (Tatsunoto et al., 2015).

In conclusion, MR-mediated effects in VSMC may contribute to a variety of disease processes including vascular remodeling and stiffness, hypertension, and long-term consequences of aging.
C. Role of the Mineralocorticoid Receptor in the Crosstalk between Endothelium and Vascular Smooth Muscle Cells

The crosstalk between endothelium and vascular smooth muscle is crucial for vascular function. Indeed, VSMC responses are controlled by endothelial cells via complex intercellular signaling processes. Endothelial-dependent vascular relaxation is mediated by the release of nitric oxide (NO) or vasoactive prostanoids. Another pathway is associated with the hyperpolarization of both the endothelial cells and VSMC mediated by endothelium-derived hyperpolarizing factor(s) (EDHF) (Edwards et al., 2010). EDHF-mediated responses involve epoxidecysatrienoic acids, potassium ions and channels, reactive oxygen species (ROS), and myoendothelial junctions (Edwards et al., 2010). Moreover, endothelial-derived contractile factors are also important (Virdis et al., 2010).

MR activation clearly affects endothelium-VSMC crosstalk. Endothelial MR activation decreases NO synthesis by reducing the activity of endothelial NO synthase (eNOS) either directly or via eNOS uncoupling (Bauersachs and Fraccarollo, 2006). It also increases local ROS production and therefore decreases NO bioavailability. Aldosterone/MR also affects EDHF: endothelial MR increases calcium-activated potassium channel expression and activity (Zhao et al., 2012), resulting in membrane hyperpolarization leading to vasorelaxation. Aldosterone/MR also increased endothelial-derived contractile factors via increased synthesis of endothelin (Park and Schiffrin, 2001; Nguyen Dinh Cat et al., 2010). A novel mechanism of action of aldosterone/MR/MRA on the endothelin pathway has been recently highlighted: oxidative stress induced by mineralocorticoid activation resulted in an inactivating posttranslational modification of the endothelin 1 receptor type B (ET1RB) related to a cystein sulfenication (Maron et al., 2012; Barrera-Chimal et al., 2015b) MRAs prevented such inactivating modification of the ET1RB, allowing vasodilation to counterbalance the vasoconstriction induced by endothelin 1-mediated endothelin 1 receptor type A stimulation, leading to better local hemodynamics. These beneficial effects of MRA have been reported in pulmonary hypertension (Maron et al., 2012) as well as in renal ischemia reperfusion injury (Barrera-Chimal et al., 2015b).

D. Mineralocorticoid Receptor Affecting Microcirculation

Although most studies demonstrated MR-mediated effects on large vessels, for technical reasons (size of vessels), there is limited information on the microcirculation. However, the microcirculation plays a major role in tissue pathology (Granger et al., 2010). There is a longitudinal gradient of expression of MR, with higher expression in large arteries (Lombes et al., 1992), but MR is also expressed in arterioles and capillaries (DeLano and Schmid-Schönbein, 2004; Zhao et al., 2012), although to a lower extent, and perhaps essentially only in pathologic situations. Moreover, MR expression may depend on the precise location of the vessels within an organ (for example, in the renal glomerulus afferent versus efferent arteries or peritubular capillaries).

V. Mineralocorticoid Receptor and Metabolic Diseases

Experimental and clinical studies have highlighted aldosterone as a potential risk factor for diabetes and metabolic syndrome (MetS), through mechanisms at least partially independent of hypertension (Whaley-Connell et al., 2010). A new role for aldosterone/MR activation in adipose tissue has been highlighted (Zennaro et al., 2009; Marzolla et al., 2014; Gomez-Sanchez, 2015). MR is involved in the plasticity of white adipocyte and MR antagonism promotes “browning” of the white adipose tissue (i.e., increased presence of brown adipocytes within the white adipose tissue) through direct control of autophagy promoting increased metabolic activity of adipose depots (Armani et al., 2014). Ex vivo experiments indicate that activation of MR by aldosterone (Caprio et al., 2007) or glucocorticoids (Hirata et al., 2012) influence adipocyte differentiation and the secretion of adipokines as adiponectin and leptin as well as proinflammatory markers. Experimental studies in rodent models [db/db, ob/ob, or high-fat diet (HFD)-induced obese mice] indicate a specific role of aldosterone and/or MR activation. Indeed, MRA improves glucose tolerance and decreases insulin resistance, plasma levels of triglycerides, and proinflammatory cytokines (Guo et al., 2008; Hirata et al., 2009; Wada et al., 2010). In db/db mice, Guo et al. (2008) reported that pharmacological treatment with the selective MR antagonist eplerenone for 16 weeks reversed obesity-related changes in adipose tissue gene expression (as the increased expression of PAI-1, leptin, and proinflammatory cytokines tumor necrosis factor (TNF)-α and MCP-1 with a concomitant reduction of PPARα and adiponectin observed in db/db mice. Short-term eplerenone administration (3 weeks) in db/db and ob/ob mice also showed improved insulin sensitivity and MR antagonism restored the dysregulation of adipose gene expression in both models (Hirata et al., 2009). Eplerenone improved endothelial dysfunction induced by HFD (Schauer et al., 2013) and in streptozotocin-induced diabetic rats (Schauer et al., 2010) Spironolactone prevented HFD-induced arterial stiffening (DeMarco et al., 2015). Pharmacological approaches however do not discriminate between direct consequences of MR blockade in the adipose tissue and those related to global MR antagonism in other organs affected in MetS such as the heart, the vasculature, the
pancreas, the liver, or muscle, where MR is expressed and/or involved in insulin secretion/sensitivity and/or organ damage. The specific role of adipocyte MR was recently addressed using a novel transgenic mouse model, allowing inducible expression of MR in adipocytes only. Increased MR expression mimicking the increased expression observed in adipose tissues of experimental models of obesity (db/db, ob/ob, HFD) and human obese patients was associated with increased body weight, insulin resistance, and features of MetS (Urbanet et al., 2015). A novel MR target was identified: MR activation modulated adipocyte expression of the prostaglandin D2 synthase that was an absolute requirement for the ex vivo adipogenic effects of aldosterone (Urbanet et al., 2015). Interestingly, adipose tissue expression of MR correlated with prostaglandin D2 synthase expression in human fat depots (Urbanet et al., 2015).

Aldosterone-induced MR activation is associated with impaired insulin sensitivity in adipocytes, skeletal muscle, and the vasculature (Garg and Adler, 2012; Bender et al., 2013). Plasma aldosterone is correlated with body mass index and insulin resistance and Conn and Fajans (1956) already reported in the 1950s that patients with primary aldosteronism had increased risk of diabetes. A high prevalence (10–50%) of glucose intolerance and/or diabetes has been reported in primary aldosteronism, and these metabolic disturbances could be corrected by surgical removal of the aldosterone-producing adenoma (Fallo et al., 2012). The underlying mechanisms remain partially known and may converge on the insulin receptor/insulin growth factor receptor/insulin receptor substrate/AKT pathways (Garg and Adler, 2012; Bender et al., 2013; Luther, 2014). Locally synthesized aldosterone may also intervene; aldosterone production by adipocytes has been demonstrated (Briones et al., 2012) and may affect vascular function and insulin response by a paracrine mechanism. Cultured human adipocytes can secrete factors that stimulate adrenal production of aldosterone (Ehrhart-Bornstein et al., 2003).

Aldosterone may also affect insulin secretion (Luther, 2014). MR is expressed in rodent pancreatic islets and aldosterone impairs glucose-stimulated insulin secretion in vivo in mice and in murine islets (Luther et al., 2011). The potential role of inflammatory cells (essentially macrophages) in the adipose tissue and the implication of MR activation in macrophages has been raised recently (Marzolla et al., 2014). Indeed MR activation, affecting macrophages polarization toward a proinflammatory phenotype, may participate in the development of dysfunctional adipose tissue upon corticosteroid stimulation. Conversely, MR blockade may affect adipose metabolic function by reducing adipose tissue inflammation, now recognized as an important factor in metabolic diseases contributing to insulin resistance and dysfunctional adipocytes (Toubal et al., 2013). Despite strong evidence for a deleterious role of MR activation and a beneficial role of MR antagonism in metabolic diseases in preclinical models, results from human studies remain equivocal and the benefit of pharmacological MR antagonism to improve metabolic diseases has not been established. For example, although spironolactone improves coronary microvascular function in type 2 diabetic patients without clinical ischemic disease, MRA has no effect on lipids, body mass index, or fasting glucose (Garg et al., 2015). This is consistent with a pilot study from the same group using eplerenone (Joffe et al., 2007). However, in patients with moderate chronic kidney disease and increased HOMoetatic Model Assessement (HOMA) index, there is a significant reduction of HOMA index and fasting insulin after 6 months spironolactone treatment (Hosoya et al., 2015).

VI. Mineralocorticoid Receptor and Renal Diseases

In 1964, Conn et al. (1964) described the first 145 cases of proven primary hyperaldosteronism associated with hypertension and where proteinuria was present in 85% of the patients. The proteinuria was considered as a consequence of hypertension until the 1990s, when experiments on the remnant kidney model in rats revealed that mineralocorticoid hormones can induce proteinuria in the absence of hypertension. In the salt-sensitive hypertensive Dahl rat, renal failure, proteinuria, and histologic renal lesions could be fully prevented by low-dose eplerenone, whereas blood pressure remained very high (230 mmHg). The benefit of MRA in renal damage occurred independently of blood pressure modulation (Kobayashi et al., 2005). The group of T. Fujita highlighted recently a novel pathway involved in renal MR signaling whereby the small GTPase Rac1 potentiates the activity of MR, contributing to ligand-independent MR activation in preclinical models of kidney injury (Nagase and Fujita, 2013). Therefore, although aldosterone exerts primarily physiologic homeostatic responses, allowing maintenance of extracellular volume in response to acute volume loss or salt depletion, a sustained MR activation, in some pathologic situations, can lead to pressure-independent renal damage.

In addition to functional MR in the epithelial cells of the distal nephron, MR is also expressed in vascular endothelial cells and, to a lesser extent, in vascular smooth muscle cells of interlobar renal arteries in the mouse (Nguyen Dinh Cat et al., 2010). Under physiologic conditions, MR expression is not detectable in the glomerulus (Farman et al., 1982a,b, 1991) but MR expression has been demonstrated ex vivo in cultured podocytes, mesangial cells, and renal fibroblasts (Nishiyama et al., 2005; Shibata et al., 2008). In vivo, it is possible that MR, in nonclassic target tissues such as
podocytes or mesangial cells, reaches a significant level of expression only during pathologic situations as in type I diabetes observed in the rat (Lee et al., 2009) and in spontaneous hypertensive rats with metabolic syndrome (Nagase et al., 2006). Increased MR expression also occurs in patients with chronic renal disease of various origins (Quinkler et al., 2005), but the affected cell type is unknown.

A. Experimental Studies

Experimental evidence points to a benefit of MR antagonism in models of chronic kidney diseases (nephron reduction, diabetic nephropathy, glomerulopathies) and has been reviewed (Bertocchio et al., 2011; Ritz and Tomaschitz, 2014). Potential novel therapeutic indications of MRA have emerged from situations of renal ischemia (Juncos and Juncos, 2015). MRAs have proven to be highly efficient in preventing ischemic reperfusion injury; MRAs administered before or just after ischemic injury fully prevented acute renal injury in rat (Mejia-Vilet et al., 2007; Sanchez-Pozos et al., 2012; Barrera-Chimal et al., 2013). This has been confirmed recently in the mouse in which the nonsteroidal antagonist BR-4628 was also shown to be efficient (Barrera-Chimal et al., 2015b). Of major interest, short-term MRA treatment, flanking the ischemic period, also had long-term beneficial effects after the therapeutic intervention, because it prevented the delayed occurrence of chronic renal failure and interstitial fibrosis (Barrera-Chimal et al., 2013, 2015a). The role of renal inflammation and activation of MR in macrophages has been underlined in an elegant study using mouse models with genetic MR inactivation in the myeloid lineage. Glomerulopathy induced by antigelomular basement membrane antibody is blunted in mice with MR deletion in the myeloid lineage (Huang et al., 2014), underlying the role of macrophage MR activation in the inflammatory process associated with renal injury. Whether this notion could apply to renal injury of other origins remains to be tested. The role of vascular MR in the renal vascular bed has been highlighted recently by observations that CIN can limit cyclosporine-induced nephrotoxicity (CIN) (Bobadilla and Gamba, 2007). The nephrotoxicity of cyclosporine involves vasoconstriction and altered renal hemodynamics (Amador, 2015). CIN was prevented in mice with genetic VSMC MR inactivation but not when endothelial MR was deleted (Amador, 2015). The hemodynamic alterations related to MR activation may have important impact in renal transplantation outcomes and MRA may prevent or slow down the progression of CIN. Indeed, spironolactone improves transplant vasculopathy in rats with renal transplant (Waanders et al., 2009). MRA may therefore limit delayed graft dysfunction by moderating the ischemia-reperfusion injury occurring during organ preservation/transplantation.

B. Clinical Studies

An increase in plasma aldosterone has been reported to be a risk factor for kidney injury in clinical studies; chronic kidney disease (CKD) can be considered as a state of relative hyperaldosteronism (Schwenk et al., 2015), and possible benefit of MRA has been explored. In 2001, a brief description of the use of MRA in eight proteinuric patients reported 54% decrease in proteinuria after 4 weeks administration of spironolactone (Chrysostomou and Becker, 2001). Since then, several studies have questioned the role of aldosterone and MR in proteinuria and in the progression of CKD, as reviewed recently (Bertocchio et al., 2011; Ritz and Tomaschitz, 2014; Schwenk et al., 2015). A meta-analysis showed that MRA, in addition to angiotensin converting enzyme inhibitors and angiotensin receptor blockers (or both), reduced proteinuria (Bolignano et al., 2014); however, their consequences on progression toward end-stage renal disease (ESRD) or major cardiovascular events need to be further investigated. A small decrease in estimated glomerular filtration rate (eGFR) was frequently reported, especially in diabetic patients, probably reflecting reversal of hyperfiltration (Bolignano et al., 2014). The decrease in eGFR has also been reported in large CV trials (RALES, EPHESUS, or EMPHASIS-HF) but did not lead to harmful alteration of kidney function, even in patients with pre-existing altered renal function. Importantly, the long-term benefit of MRAs in these patients occurred despite eGFR reduction (Vardeny et al., 2012; Rossignol et al., 2014). Several clinical trials are now ongoing or were recently completed in diabetic nephropathy: EVALUATE (Ando et al., 2014) and ARTS-DN trials (Bakris et al., 2015). The variable effects of MRA on renal function in diabetic patients to slow down CKD progression were recently reviewed (Mavrakanas et al., 2014).

Another target of MRA in CKD are the CV events associated with CKD progression or ESRD. Patients with CKD present an increased risk of CV events, LV hypertrophy, increased vascular stiffness, stroke, and sudden death. Of note, the 4D study indicated that aldosterone levels were powerful predictors of sudden death in hemodialyzed patients with type 2 diabetes (Drechsler et al., 2013). Because MRA treatment has now been clearly established as beneficial in CV diseases, the added value of MRAs on CV events in CKD and ESRD patients has been proposed. A positive impact of MRA on CV events has been reported in hemodialyzed patients (Matsumoto et al., 2014), but the number of patients with CV events in the placebo group was low; therefore a larger clinical trial is needed (Pitt and Rossignol, 2014). Such a clinical trial (Alchemist, NCT 01848639) is ongoing and will assess CV events and morbidity-mortality in hemodialyzed patients treated or not with spironolactone.
Only scarce data are available in transplanted patients. Addition of spironolactone to the blockade of the renin-angiotensin system with angiotensinogen converting enzyme inhibitors and angiotensin receptor blockers provided added value, decreasing severe proteinuria in 11 renal transplant patients, without significant effect on renal function (Gonzalez Monte et al., 2010). Of interest, spironolactone given 1 day before and 3 days after transplantation can reduce oxidative stress in renal transplant patients from living donors without affecting renal function (Ojeda-Cervantes et al., 2013).

When using MRA in patients prone to hyperkalemia (CKD, ESRD, or renal graft), a major issue is the safety concern regarding the increase in plasma potassium concentration. Hyperkalemia is often observed in patients treated with MRAs, reflecting efficacy of MRA in reducing urinary K+ secretion. Life-threatening hyperkalemia have been reported, particularly when using high dosage of MRAs (Schwenk et al., 2015). Therefore close monitoring of kaliemia in patients with impaired renal function is required to avoid clinically meaningful hyperkalemia above 5.5 mmol/l. The benefit of MRAs may nevertheless be greater than the risk associated with increased plasma potassium levels.

VII. Mineralocorticoid Receptor and Ocular Diseases

Recent reports have demonstrated that the retina is a target tissue for mineralocorticoids, with specific involvement in eye pathology (Wilkinson-Berka et al., 2012; Zhao et al., 2012). Whether MR antagonists may exert beneficial effects in retinal diseases is a novel concept in ophthalmology. In clinical ophthalmology, high doses of glucocorticoids (injected into the vitreous cavity of patients with macular edema) are currently used for anti-inflammatory and antiedematous effects on the retina; this treatment is accompanied by frequent and sometimes severe side effects such as intraocular hypertension (glaucoma), cataract, or toxicity. In addition to GR-mediated events, excess glucocorticoids may activate the ocular MR, leading to pathology, and inappropriate MR signaling may be deleterious for the retina.

Retinal Muller glial cells are essential for retinal water and K+ homeostasis. These cells express the MR, together with the GR and the MR-protector enzyme 11 HSD2 (Zhao et al., 2010b, 2011). In retinal Muller glial cells, the MR is functional, as 24-hour aldosterone treatment promotes upregulation of ion and water channels (potassium channel Kir 4.1, epithelial sodium channel ENaC, and water channel AQP4); aldosterone also increases retinal thickness, reminiscent of a proedematous effect (Zhao et al., 2010b). The retinal pigmented epithelium (RPE) controls the movements of fluid from the inner retina toward the choroid vessels and blood stream, and retinal detachment may occur in pathology between the apical side and the neighboring photoreceptors. We showed that retinal pigmented epithelium cells express both MR and GR (Zhao et al., 2010b), but the role of MR/GR pathways in this epithelium is unknown.

Few reports have addressed the functional role of MR of retinal vessels in pathology. Activation (either systemic or intraocular) of the renin-angiotensin-aldosterone system is a key feature of diabetes, and blockers of the renin-angiotensin-aldosterone system appear to be effective in reducing the risk of ophthalmological complications of diabetes in some patients (Fletcher et al., 2010; Wilkinson-Berka et al., 2012). MR antagonism attenuates pathologic angiogenesis of rat retinal vessels in neonatal oxygen-induced retinopathy, a model of retinopathy of prematurity (Wilkinson-Berka et al., 2009), indicating a direct pathologic role of aldosterone (Wilkinson-Berka et al., 2009). It should be noted that, in the absence of induced pathology, aldosterone per se did not promote retinal angiogenesis. Low-salt diet appeared protective for the retina in ischemic retinopathy, despite elevated plasma aldosterone (Deliyaniti et al., 2014).

Choroid vessels form a rich vascular network plexus lying behind the retina and choroid blood flow is one of the highest of the body (together with the renal blood flow, and 10-fold higher than in the brain) (Nickla and Wallman, 2010). The choroidal endothelium expresses the MR and this tissue is mineralocorticoid sensitive (Zhao et al., 2012; Bousquet et al., 2013). We have shown that a single injection of aldosterone into the rat vitreous cavity leads to choroidal vasodilation (Zhao et al., 2012). A similar phenotype was observed in mice with overexpression of the MR in endothelial cells (our unpublished results). In choroid vessels, MR activation enhanced the expression of the endothelial calcium-activated KCa2.3 channel and intravitreous injection of the KCa2.3 channel inhibitor apamine prevented the aldosterone-induced vasodilation. In addition to retinal detachment, dilation of choroid vessels is a feature of central serous chorioretinitis (CSCR), an eye disease affecting stressed young men, and triggered by or aggravated by glucocorticoids. CSCR can become chronic and possibly lead to blindness, with no current gold standard treatment. We showed in pilot clinical studies that short-term (1–3 months) treatment of the patients with MR antagonist leads to spectacular resolution of the disease and recovery of vision (Zhao et al., 2012; Bousquet et al., 2013). These pilot studies were confirmed in a randomized clinical trial using spironolactone (Bousquet et al., 2015). CSCR disease is the first indication of an effective MRA therapy in ophthalmology (Daruich et al., 2015).

The retinal ganglion cells (RGC) are neurons that relay visual information to the central nervous system. The MR is expressed by retinal ganglion cells (Wilkinson-Berka et al., 2009; Zhao et al., 2010b) but its function remains largely unknown. Interestingly it has been reported that intravitreal injection of aldosterone reduces the number
of RGC (Liu et al., 2012). Chronic aldosterone infusion leads to RGC cell death and degeneration of the optic nerve (without affecting intraocular pressure), raising the question of a role for MR in normotensive glaucoma (Nitta et al., 2013). A subset of retinal ganglion cells (the melanopsin light-sensitive RGC) participates in the synchronization of circadian rhythms in response to light. Whether nonvisual functions such as endogenous clock and chronobiology (Lahouaoui et al., 2014) may be influenced by MR activation is currently unknown.

VIII. Mineralocorticoid Receptor and Skin Diseases

Evidence for MR expression in the human epidermis and hair follicle (as well as in sebaceous and sweat glands) is available but with limited knowledge of its cutaneous role (Kenouch et al., 1994; Zennaro et al., 1997; Farman et al., 2010). Glucocorticoids are efficient drugs to treat chronic inflammatory or autoimmune skin diseases. The skin may be damaged by glucocorticoid excess (Schoepf et al., 2006), as observed with locally applied dermocorticoids or per os administration, endogenous secretion (Cushing disease), or enhanced local biosynthesis (Slominski et al., 2013). Glucocorticoid excess can lead to a thin and fragile skin and delayed wound healing, which is of great concern for patients (Schoepf et al., 2006). Such side-effects have similarities with skin defects occurring during aging (Tiganescu et al., 2013). Glucocorticoids may bind to the cutaneous MR, in addition to the GR, and contribute to glucocorticoid-induced side effects in the skin (Sainte Marie et al., 2007). Aldosterone and MR antagonists modulate elastin and collagen content of human skin (Mitts et al., 2010). Restoration of dermal homeostasis by MR antagonism may rely on anti-inflammatory and anti-inflammatory actions, leading to improved collagen structural organization and remodeling and cellularity. In a mouse model of aging skin, associating ultraviolet irradiation and metabolic syndrome, MR blockade could improve some aging-like features (Nagase et al., 2013). Genetic inactivation of the glucocorticoid-activating enzyme 11HSD1 (or its local blockade) leads to reduced endogenous glucocorticoid production, thus limiting the possibility of MR occupancy. In this situation, the aging-dependent dermal atrophy and delayed wound healing in mice were reversed (Tiganescu et al., 2013).

The epidermis is a mineralocorticoid-sensitive epithelium. MR-mediated sodium absorption through the epidermis is a key feature of amphibian skin to adapt from aqueous to terrestrial environment. Pioneer studies demonstrated aldosterone-stimulated sodium absorption across the skin of Rana temporaria and Bufo marinus (Crabbe and Deweer, 1964). This function was lost during evolution of higher vertebrates. Thus one can wonder whether the mammalian epidermal MR may exert another function in normal states or in pathology. Of note, the activity of the MR-protecting enzyme 11HSD2 is minimal in human epidermis (Kenouch et al., 1994), permitting MR occupancy by glucocorticoids. Excess glucocorticoid activation of the MR is most likely to occur in the face of local application of dermocorticoids. Whether and how this mechanism could contribute to some of the deleterious side effects of dermocorticoids, such as skin atrophy and delayed wound healing, is an important therapeutical issue, because it could be limited by local coadministration of MR antagonists.

Unexpected roles of MR in keratinocytes have been highlighted by a conditional mouse model expressing the human MR under the control of a keratinocyte-specific promotor (K5-MR mice) (Sainte Marie et al., 2007). Gestational expression of the MR led to premature skin barrier establishment and epidermal atrophy reminiscent of the glucocorticoid-induced atrophy (Sainte Marie et al., 2007). The pathways regulated by epidermal MR are currently unknown. Of note, the three subunits of the epithelial sodium channel ENaC are overexpressed in the skin of K5-MR mice at birth (Maubec et al., 2015). Enhanced ENaC expression in K5-MR pups may depend on glucocorticoid-activated MR, because of the perinatal rise in plasma glucocorticoid concentration. In addition, blockade of MR during gestation (carbonate given to the mother) prevented the neonatal epidermal atrophy and ENaC overexpression (Sainte Marie et al., 2007; Maubec et al., 2015), pointing at mineralocorticoid signaling as a novel epidermal modulator. The hypothesis that inappropriate/excessive activation of the cutaneous MR by glucocorticoids leading to atrophy may be extended to human skin. The potent glucocorticoid clobetasol induces epidermal atrophy in cultured human skin explants and in subjects with local application of the glucocorticoid as a gel. Importantly, the coadministration of MRA could limit the glucocorticoid-induced atrophy (Maubec et al., 2015). Although the recovery was partial, the improvement may be important for patients, because there is no alternative treatment other than glucocorticoid withdrawal. Therefore, the combination of MR antagonists with local corticoid treatment in humans presents a novel promising therapeutical approach to limit epidermal atrophy (Maubec et al., 2015). The mechanisms at the origin of this beneficial effect include correction of impaired keratinocyte proliferation and are far from being elucidated. The blockade of the activity of ENaC also improved epidermal atrophy and fully restored keratinocyte proliferation rate, suggesting that ENaC activation may participate in the glucocorticoid-stimulated epidermal MR signaling cascade (Maubec et al., 2015).

Postnatal induction of MR in K5-MR mice was associated with progressive loss of fur (alopecia) and dysmorphism of hair follicles (Sainte Marie et al., 2007). Elevated plasma aldosterone levels were noted in
subjects with androgenetic alopecia (Arias-Santiago et al., 2009), suggesting that excessive MR signaling may alter hair growth. On the other hand, some clinical reports mentioned that spironolactone can be used to treat hirsutism or, conversely, female pattern hair loss. Future studies should help to elucidate these effects.

**IX. Paradoxical Effects of Mineralocorticoid Receptor Activation**

In contrast with the above-mentioned deleterious effects of MR activation, some reports indicate beneficial effects of aldosterone in specific pathologic conditions.

The increased cardiac production of aldosterone in the mouse heart is accompanied by beneficial effects on peripheral capillarization in type I and type II diabetes (Messaoudi et al., 2009; Fazal et al., 2014). Aldosterone also improved neovascularization in a model of limb ischemia in mice (Michel et al., 2004). The expression of the MR in neutrophils may transduce an anti-inflammatory response to aldosterone mediated by inhibition of nuclear factor κB, leading to reduced neutrophil adhesion to endothelium (Bergmann et al., 2010). In a rat model of endotoxin-induced uveitis, intraocular injection of aldosterone restored the clinical intensity of the early phase of uveitis and restored the downregulated MR brought on by inflammation in the iris and ciliary body (Bousquet et al., 2012). Activation of MR appears to have anticoagulant properties in mice with intact endothelium (Lagrange et al., 2014). Thus, although MR activation is generally considered as detrimental, it is important to realize that this pathway may be beneficial in some specific pathologic situations.

**X. Common Mechanisms Involved in Pathologic Consequences Of Mineralocorticoid Receptor Activation**

**A. Mineralocorticoid Receptor is a Regulator of Ion Channels in Multiple Tissues**

Aldosterone and MR modulate various ion channels (Table 2) (Pearce et al., 2015; Penton et al., 2015). The prototype of aldosterone action in the kidney collecting duct principal cell is upregulation of the epithelial sodium channel ENaC, the rate-limiting step of Na⁺ reabsorption. Aldosterone and MR also control potassium secretion through the regulation of apical K⁺ channel ROMK and fluid absorption through aquaporin 2 water channels. Disturbance of these signaling pathways is responsible for altered Na⁺ and K⁺ homeostasis and hypertension. These regulatory pathways will not be reviewed here (Frindt et al., 2008; Soundararajan et al., 2010). The notion that aldosterone regulates ion channels now extends far beyond classic MR epithelial cells targets (kidney, distal colon, sweat and salivary gland ducts).

Evidence has been provided that MR signaling leads to changes in ion channel expression or activity in vascular endothelium and smooth muscle (DuPont et al., 2014). Ion channels may be direct MR targets in the vascular endothelium. The Ca²⁺-activated K⁺ channel (KCa2.3) is upregulated in human umbilical vein endothelial cells by aldosterone. In the retinal choroid vascular bed, the activity of the KCa2.3 K⁺ channel is requisite for the vasodilatory effect of aldosterone (Zhao et al., 2012). The epithelial sodium channel (ENaC) is also modulated by aldosterone via MR in the endothelium (Kusche-Vihrog et al., 2008; Warnock et al., 2014). Because endothelial ENaC contributes to the stiffening

<table>
<thead>
<tr>
<th>Ion Channel</th>
<th>Tissue</th>
<th>Reference</th>
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<tbody>
<tr>
<td><strong>Na channel</strong></td>
<td></td>
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</tr>
<tr>
<td>ENaC</td>
<td>Kidney</td>
<td>Pearce et al., 2015</td>
</tr>
<tr>
<td>Distal colon</td>
<td></td>
<td>Escoubet et al., 1997</td>
</tr>
<tr>
<td>Endothelium (HUVEC/EaHy)</td>
<td></td>
<td>Kusche-Vihrog et al., 2008</td>
</tr>
<tr>
<td>Epidermis</td>
<td></td>
<td>Maubec et al., 2015</td>
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<tr>
<td><strong>K channel</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROMK</td>
<td>Kidney</td>
<td>Penton et al., 2015</td>
</tr>
<tr>
<td>Kir 4.1</td>
<td>Eye (retina)</td>
<td>Zhao et al., 2010b</td>
</tr>
<tr>
<td>KCa 2.3</td>
<td>Endothelium (choroid, HUVEC)</td>
<td>Zhao et al., 2012; Jaisser, unpublished data</td>
</tr>
<tr>
<td>KCa 2.2</td>
<td>Endothelium (choroid)</td>
<td>Farman, unpublished data</td>
</tr>
<tr>
<td>Transient outward (Ito)</td>
<td>Ventricular cardiomyocyte</td>
<td>Benitah and Vassort, 1999; Benitah et al., 2001; Ouvrard-Pascaud et al., 2005</td>
</tr>
<tr>
<td>BKCa</td>
<td>Coronary artery (vascular smooth muscle cell)</td>
<td>Ambroisine et al., 2007</td>
</tr>
<tr>
<td><strong>Ca channel</strong></td>
<td></td>
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<tr>
<td>Cav 1.2</td>
<td>Ventricular cardiomyocyte</td>
<td>Benitah and Vassort, 1999; Benitah et al., 2001; Ouvrard-Pascaud et al., 2005; Perrier et al., 2005</td>
</tr>
<tr>
<td><strong>Hyperpolarization-activated current</strong></td>
<td></td>
<td>McCurley et al., 2012</td>
</tr>
<tr>
<td>HCN1</td>
<td>Embryonic stem cell-derived cardiomyocytes</td>
<td>Le Menuet et al., 2010</td>
</tr>
<tr>
<td>HCN 2, HCN4</td>
<td>Cardiomyocyte</td>
<td>Muto et al., 2007</td>
</tr>
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</table>
of endothelial cell membranes (Jeggle et al., 2013) and to the activity of eNOS (Perez et al., 2009), aldosterone/MR modulation of endothelial ENaC expression/activity is an important issue (Warnock et al., 2014). The ENaC channel is also expressed in the VSMC and contributes to myogenic tone (Drummond, 2012). Whether ENaC is regulated by MR in the VSMC (in addition to the endothelium) is unknown. The role of ENaC channel is also expressed in the VSMC and contributes to myogenic tone (Drummond, 2012). Indeed, the Cav1.2 agonist BayK8644 (1,4-Dihydro-2,6-dimethyl-5-nitro-4- [2-(trifluoromethyl)phenyl]-3-pyridinecarboxylic acid, methyl ester) had lower vasoconstrictive effects in mesenteric arteries from aged VSMC-MR KO mice (McCurley et al., 2012). The impaired Ca2+ signaling can affect the contractile machinery, as demonstrated by reduced phosphorylation of myosin phosphatase-targeting subunit 1, myosin light chain kinase, and myosin light chain 2 (Tarjus et al., 2015b).

Mineralocorticoid stress modifies cardiac ion channels (Laszlo et al., 2011; Gomez et al., 2013), providing a cellular substrate for arrhythmia. Aldosterone increases L-type Ca2+ current and decreases transient outward K+ current (Ito) in cultured ventricular myocytes (Benitah and Vassort, 1999; Benitah et al., 2001) and the hyperpolarization-activated cyclic nucleotide-gated (HCN2 and 4) channels (Muto et al., 2007). In coronary artery, aldosterone regulates the calcium-activated potassium (BKCa) channel (Ambroisine et al., 2007). MR (not GR) enhances the cardiac calcium current (Rougier et al., 2008). Cardiac MR overexpression has a major impact on Ca2+ currents together with decreased K+ transient outward current (Ito) (Ouvrard-Pascaud et al., 2005). Enhanced MR expression in cardiomyocytes promotes cardiac ryanodine receptor opening (Gomez et al., 2009). There is a strong correlation between aldosterone levels and L-type Ca2+ currents in ventricular myocytes freshly isolated from mouse heart overexpressing the MR and submitted to aldosterone infusion (Perrier et al., 2005).

The epidermis expresses ENaC (Roudy-Pujol et al., 1996; Brouard et al., 1999; Mauro et al., 2002) and appears as a novel target of the MR in the skin (Farman et al., 2010; Maubec et al., 2015). The MR-ENaC cascade participates to the regulation of keratinocyte homeostasis through control of keratinocyte proliferation (Maubec et al., 2015).

Ion channels (ENaC, Kir4.1) are also aldosterone/MR targets in the neuroretina (as mentioned above), thus playing a role in the hydration of the retina (Zhao et al., 2010b) and vasodilation of the choroid capillaries depends on the activity of KCa2.3 channel (Zhao et al., 2012).

Altogether, from these data (and evidence that MR activates neuronal channels in the brain) we would like to suggest that ion channel remodeling is a general feature of mineralocorticoid signaling, however, the nature of affected ion channels depends on the cell context and pathology.

B. Mineralocorticoids Induces Oxidative Stress

Oxidative stress appears as a central mechanism in the effects of aldosterone and MR activation. This has been reviewed recently in excellent publications (Queisser and Schupp, 2012; Even et al., 2014). As mentioned above, mineralocorticoid-induced oxidative stress may lead to reversible and irreversible post-translational modifications (carbonylation, sulfenification, ...) of important downstream pathways, as exemplified for the inactivating sulfenification of the ET1RB occurring in renal ischemia-reperfusion injury for example (Barrera-Chimal et al., 2015b). Aldosterone stimulates oxidative stress in classic and nonclassic target cells, such as collecting duct cells, cardiomyocytes, endothelial and vascular smooth muscle cells, adipocytes, and macrophages. Aldosterone/MR upregulates the expression of NADPH oxidase subunits, such as Nox 2, Nox4 (Bayorh et al., 2011; Brown, 2013), p47phox, p67phox (Queisser and Schupp, 2012), and rac1 (Iwashima et al., 2008), thereby affecting Nox activity. Cellular accumulation of Zn2+ and Ca2+ ions in the heart after aldosterone-salt challenge contributes to cardiac oxidative stress and mitochondrial dysfunction, an effect prevented by spironolactone (Kamalov et al., 2009). The consequences of oxidative stress are multiple and are involved in some of the pathologic effects of MR activity. Oxidative stress increases DNA damage (Schupp et al., 2010) and protein carbonylation (Di Zhang et al., 2008). Uncoupling of the NO synthase decreases the availability of NO for vasorelaxation, as well as increased production of hydrogen peroxide, and activation of the nuclear factor xB pathway leading to inflammation and fibrosis (Mayyas et al., 2013; Karbach et al., 2014).

C. Mineralocorticoid Receptor Activation Leads to Fibrosis

A growing body of evidence indicates that MR plays an important role in CV and renal diseases by promoting fibrosis (Thomas et al., 2010; Young and Rickard, 2012). In randomized clinical trials, the beneficial effects of MRA in heart failure were associated with a reduction of fibrosis surrogate markers (Iraqi et al., 2009). MRA also induced arterial destiffening (Lacolley et al., 2009). Several profibrotic factors (NGAL, CT1, Gal3, osteopontin) are direct MR targets in the CV system. Inappropriate MR activation has been shown to promote CV and renal tubulointerstitial fibrosis (Bauersachs et al., 2015). Moreover, fibrosis is increasingly appreciated as a major player in adipose tissue dysfunction (Divoux and Clement, 2011). Recent reports indicate that spironolactone limits peritoneal fibrosis in rats, opening up the possibility to improve the efficiency
of peritoneal dialysis (Vazquez-Rangel et al., 2014; Yelken et al., 2014; Zhang et al., 2014). In addition, spironolactone may be beneficial for the cardiovascular function of these patients (Ito et al., 2014). MR activation may be involved in liver fibrosis, as inferred from the beneficial effect of MRA in mice with nonalcoholic steatohepatitis (Pizarro et al., 2015). Spironolactone was also shown to limit skin (dermal) fibrosis (Mitts et al., 2010). In the lung, hypoxia-induced local secretion of aldosterone by pulmonary artery endothelial cells leads to pulmonary vascular fibrosis (Maron et al., 2014). Thus fibrotic actions of MR appear as a general feature that can be prevented by MRA.

D. Mineralocorticoid Receptor and Inflammation

Low-grade inflammation is a hallmark of cardiovascular, renal, and metabolic diseases. In pathology, inflammatory biomarkers (C-reactive protein, cytokines, and their receptors) are associated with poor clinical outcomes and prognosis (Schiffrin, 2013). Patients with hypertension and CV diseases present with a chronic vascular inflammatory state that may depend on adaptive immune response mechanisms (Schiffrin, 2013). Pharmacological MR blockade correlates with the prevention/improvement of the chronic inflammatory state associated with CV dysfunction (Herrada et al., 2010, 2011). Chronic MR activation may interfere with inflammatory mechanisms. Macrophages, dendritic cells, and T lymphocytes were recently identified as MR target cells (Bene et al., 2014). MR activation leads to MR-dependent potentiation of interleukin (IL)-6 and tumor necrosis factor-α (TNF-α) expression and nuclear factor κB activation in both immune and nonimmune cells (Bene et al., 2014).

Vascular inflammation and the infiltration of the arterial wall and perivascular space by immune cells is a relatively early event after MR activation (Kasal and Schiffrin, 2012). Monocyte/macrophage deficiency in osteopetrotic mice results in the absence of aldosterone-induced oxidative stress and endothelial dysfunction (De Ciuceis et al., 2005). T regulatory cells prevent aldosterone-induced vascular injury in mice (Kasal et al., 2012). Macrophages participate to the hypertensive and CV remodeling effects of aldosterone-salt challenge, as shown using macrophage-specific MR KO mice (Rickard et al., 2009). Macrophage MR deletion also protects against cardiac fibrosis induced by L-nitroarginine methyl ester-Ang II pharmacological treatment (Usher et al., 2010) or thoracic aortic constriction (Li et al., 2014). In adipose tissue, aldosterone regulates secretion of proinflammatory adipokines and MR activation stimulates production of TNFα, MCP1, PAI-1, IL-6, an effect prevented by pharmacological MR antagonism (Guo et al., 2008). MR activation also promotes a switch in macrophage polarization toward a proinflammatory phenotype (Marzolla et al., 2014). Aldosterone activation of dendritic cells increases IL-6 and transforming growth factor β expression as well as dendritic cell-mediated Th17 polarization of T lymphocytes (Herrada et al., 2010, 2011). Aldosterone promotes autoimmune renal damage by enhancing Th17-mediated immunity (Herrada et al., 2010) that was blocked by MR antagonism (Amador et al., 2014). These data suggest that aldosterone/MR modulates innate and adaptive immunity, which may have a critical role in initiating/maintaining vascular remodeling as well as hypertension and organ damage in response to exogenous aldosterone and/or MR activation. Recent studies focused on a potential role of DAMPs (danger-associated molecular pattern molecules), which can initiate and perpetuate immune responses in the noninfectious inflammatory response (Prantz et al., 2014). Several of these factors (tenascine-C, osteopontin, galectin3, collagen/fibronectin peptides) have been clearly identified as MR targets, although the link between MR activation and DAMPs awaits clarification.

In conclusion, although aldosterone has proinflammatory properties, there is a large body of evidence showing that MRA can prevent/limit the inflammation that precedes the development of fibrosis. Because MR is expressed in innate and adaptive immune systems and can modulate immune functions, it suggests that the beneficial effects of MRA in cardiovascular, renal, and metabolic diseases could in part rely on their anti-inflammatory properties.

E. Mineralocorticoid Receptor and Aging

Glucocorticoid-mediated MR activation occurring in aging persons should be considered in light of the risks for development of heart failure and aging-associated pathologies (Pitt, 2012). Although plasma aldosterone concentration declines with age, cortisol concentration, and the cortisol/cortisone ratio (an indicator of reduced 11HSD2 activity) increases (Henschkowski et al., 2008; Pitt, 2012). A cross sectional study of normotensive subjects indicated that 11HSD2 activity declines after the 4th decade of life, favoring cortisol activation of MR and onset of hypertension (Campino et al., 2013). Considering the bulk of aging-associated pathologies, particularly heart failure, it is worth considering MR antagonism in elderly patients.

MR activation is associated with cellular aging in the kidney, with the induction of senescence-associated β galactosidase, of the cyclin-dependent kinase inhibitor (p21) and decreased expression of SIRT1 (Fan et al., 2011). In VSMC, aldosterone stimulates senescence and p21, p53, P16, p27, and Ki-ras2 expression (Min et al., 2007). Of note, the crossstalk between AngII and aldosterone plays a central role in this process (Min et al.,
Aldosterone induces oxidative stress as well as DNA strand breaks and chromosomal damage that may promote senescence (Schupp et al., 2010). Interestingly, this is independent of blood pressure as subhypotensive doses of spironolactone could prevent renal DNA damage in vivo in rats challenged with aldosterone and salt without uninephrectomy (Queisser and Schupp, 2012).

MR expression is also elevated in the aorta of old rats (34 months), and increased MR signaling may promote and amplify age-associated inflammation (Krug et al., 2010). VSMC MR is important for the control blood pressure and vasoreactivity in aged mice (McCurley et al., 2012). The role of MR in aging probably extends to other tissues besides the vasculature. In the skin, aging-like changes have been linked to excessive MR signaling (Nagase et al., 2013).

XI. Dysregulations of Mineralocorticoid Receptor Activity

A. Regulation of Mineralocorticoid Receptor Expression Levels

It has been recognized only recently that the level of MR expression can be enhanced in pathology (Table 3). Assessment of MR is most frequently performed by detection of its mRNA (because of the convenience of quantitative-polymerase chain reaction assay), sometimes at the protein level, and scarcely by measurements of MR binding capacity with radioactive ligands. In vivo enhanced MR expression has been reported in several experimental models, explaining the efficacy of MRA:

- in the heart of rodents with myocardial infarction (Milik et al., 2007; Takeda et al., 2007), with diastolic heart failure (Ohtani et al., 2007), or with hypertension (Silvestre et al., 2000; Konishi et al., 2003);
- in vessels of hypertensive animals (SHR) (DeLano and Schmid-Schonbein, 2004) or vascular cells in a model of normal aging (30-month-old Fisher344 cross-bred Brown Norway rats) (Krug et al., 2010);
- in hypoxic pulmonary artery vascular endothelial cells (Maron et al., 2014);
- in the kidney of Brown Norway rats (Cavallari et al., 2008) and in the renal collecting duct of spontaneously hypertensive rats (Farman and Bonvalet, 1985);
- in adipose tissue from diabetic animal models (obese db/db and ob/ob mice, HFD) (Guo et al., 2008; Hirata et al., 2009, 2012); in kidney from db/db mice and streptozotocin-treated rats (Guo et al., 2006);
- in skin of mice with ultraviolet irradiation and metabolic syndrome (Nagase et al., 2013).

Although MR expression in human tissues is difficult to assess because of limited availability of premortem organs, some studies reported enhanced MR levels in cardiac tissue from patients with heart failure (Yoshida et al., 2005)

### TABLE 3

<table>
<thead>
<tr>
<th>Species</th>
<th>Organ</th>
<th>Pathology</th>
<th>Increase in MR</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD rat</td>
<td>heart</td>
<td>infarct</td>
<td>MR mRNA LV, RV protein only in LV</td>
<td>Milik et al., 2007</td>
</tr>
<tr>
<td>SD rat</td>
<td>heart</td>
<td>infarct</td>
<td>MR and HSD2 mRNA</td>
<td>Takeda et al., 2007</td>
</tr>
<tr>
<td>SS-Dahl rat</td>
<td>heart</td>
<td>Hypertension diastolic heart failure</td>
<td>MR protein</td>
<td>Ohtani et al., 2007</td>
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<tr>
<td>Wistar rat</td>
<td>heart</td>
<td>DOCA salt hypertension</td>
<td>MR mRNA, protein, in LV (no change in RV or K)</td>
<td>Silvestre et al., 2000</td>
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<tr>
<td>Wistar rat</td>
<td>heart</td>
<td>Ang II infusion 7 days</td>
<td>MR mRNA, protein, in LV (no change in RV or K)</td>
<td>Silvestre et al., 2000</td>
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<td>hypertension</td>
<td>MR mRNA, protein, in LV (no change in RV or K)</td>
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<td>db mice and STZ rats</td>
<td>kidney</td>
<td>metabolic syndrome</td>
<td>MR mRNA</td>
<td>Nagase et al., 2006</td>
</tr>
</tbody>
</table>

LV, left ventricle; RV: right ventricle; K: kidney; SD, Sprague Dawley; SS-Dahl, salt-sensitive Dahl; SHR, spontaneously hypertensive Kyoto-Okamoto strain; BN, Brown-Norway; STZ, streptozotocine-induced diabetes.
B. Mechanisms Modulating Mineralocorticoid Receptor

The MR may be subjected to genetic and epigenetic changes as well as posttranscriptional/posttranslational alterations, underlying the importance of elucidating the regulation of the receptor itself. The mechanisms underlying alterations of cell/tissue-specific MR expression or activity include genetic polymorphisms, epigenetic changes, mRNA stabilization/destabilization, and posttranslational modifications of the MR.

Human mutations of the MR affecting its function have been reported. These include loss of function mutations (Zennaro et al., 2012) leading to neonatal salt-wasting syndrome, featured by renal type I pseudohypaldosteronism, and a gain of function mutation leading to severe hypertension (Geller et al., 2000). Mutations affecting downstream pathways also affect renal sodium handling and blood pressure levels (Lifton et al., 2001; Shibata et al., 2013). In addition, various genetic determinants can contribute to variations in plasma aldosterone (Zennaro et al., 2013). Because excellent reviews have detailed these features, they will not be addressed here.

Several functional MR polymorphisms (i.e., affecting its activity or expression levels) have been identified, as reviewed by Dalila et al. (2015). The MR1180V (rs5522) polymorphism results in an amino acid change in the N terminal domain of the MR, which is present in 7–13% of the population and affects MR activity and ligand-mediated MR activation in a reporter cell system (DeRijk et al., 2006). The MR-2GC (rs2070951) polymorphism is located inside the Kozak translation regulatory sequence and affects MR translation efficiency and MR expression level (van Leeuwen et al., 2011). It is present in 50% of the population. MR gene haplotypes constituted of MR-2GC and I1180V polymorphisms strongly modulate cortisol-induced MR transcription and protein expression (van Leeuwen et al., 2011). Some studies analyzed the association between the presence of MR polymorphisms and diseases: MR polymorphisms are correlated to obesity and low-density lipoprotein-cholesterol (I1180V) or blood pressure (I1180V and MR-2GC) (Fernandes-Rosa et al., 2010). The MRI1180V polymorphism was shown to affect blood pressure response to enalapril treatment and may serve as a useful pharmacogenomic marker of antihypertensive response to enalapril in essential hypertension patients (Luo et al., 2014). The MR-2GC polymorphism was associated with higher hyperkalemic response to spironolactone in heart failure patients (Cavallari et al., 2010). The rs3857080 polymorphism, localized in intron 3, was associated with urinary electrolyte excretion (Dalila et al., 2015). This polymorphism was previously associated with nocturnal systolic blood pressure (Tobin et al., 2008).

Epigenetic events may modulate nuclear receptors, leading to deregulation of their activity. In utero exposure to di-(2-ethylhexyl) phthalate leads to loss of methylation of MR gene promotor and reduced MR mRNA expression in testes (Martinez-Arguelles et al., 2009). Histone deacetylase 3 and 4 complexes have been reported to regulate MR transcriptional activity in cultured cells (Lee et al., 2015). Epigenetic mechanism may regulate MR expression via expression of micro-RNAs (miRNAs/miRs) and short regulatory RNAs. In silico prediction identified the MR as a target of miR-135a and miR-124 (Sober et al., 2010; Mannironi et al., 2013). These miRNAs were shown to decrease MR transactivation in a reporter assay (Sober et al., 2010; Mannironi et al., 2013). Upregulation of miRNA 124 was also associated with a decrease in MR expression in podocytes upon mechanical stretch (Li et al., 2013).

Interestingly a polymorphism (rs5534) located in the 3’ untranslated region of the MR was recently identified and predicted to modify hsa-miR-383 binding and possibly involved in the decrease of MR expression mediated by this miRNA. This polymorphism was associated with an increased risk of myocardial infarction (Nossent et al., 2011). Whether and how such phenomena could participate in pathologic MR down-regulation remain to be explored.

Posttranscriptional events modulating MR activity have been reviewed (Faresse, 2014). These include phosphorylation, ubiquitylation, sumoylation, and oxidation. Posttranscriptional modifications affecting the stability of the mRNAs encoding the MR have been reported in the kidney. Hypertonicity induces the mRNA-destabilizing protein Tis11b, which interacts with the adenylate/uridylate-rich elements present in the 3’ untranslated region of the MR mRNA, increasing MR mRNA turnover and accelerating mRNA destabilization, therefore reducing MR expression (Viengchareun et al., 2014). Ubiquitylation of the MR has been demonstrated to be modulated by phosphorylation, leading to enhanced MR degradation and therefore affecting MR signaling (Fairesse et al., 2010, 2012). Whether this phenomenon occurs in vivo remains to be demonstrated. A spontaneous mutation of a potential phosphorylation site of the MR in Brown Norway rats (Y73C) has been identified as a gain of function mutation modulating transactivation activity in the presence of aldosterone (and progesterone) (Marissal-Arvy et al., 2004). Phosphorylation of a critical residue in the ligand binding domain preventing ligand-mediated MR activation has been identified in the renal intercalated cell but its consequences on MR stability have not been reported (Shibata et al., 2013).
The notion of excessive MR activity leading to organ pathology is expanding rapidly, raising the possibility of several unexpected novel indications of MR antagonism. However, several aspects of MR action in disease remain to be elucidated.

It is important to recall that the transactivation activity of the MR is highly dependent on the nature of the bound ligand (Hellal-Levy et al., 1999; Fuller et al., 2012). Each ligand (agonist or antagonist) induces a unique conformational change that drives interactions with receptor coregulators and tissue-specific transcriptional factors. Thus the ligand-receptor complexes may have distinct (sometimes opposite) tissue-specific target genes and therefore distinct downstream effects depending on the steroid accommodated in the ligand binding pocket of the MR.

In pathologic situations, it is often considered that glucocorticoids most likely occupy the MR rather than aldosterone. In addition, the amount of ligand activating the MR in target cells may be different from their plasma concentrations. Indeed aldosterone or glucocorticoids may be secreted locally, and local production may be altered (reduced or increased) in pathologic situations (Taves et al., 2011).

Glucocorticoids may be inactivated within a tissue in the presence of 11HSD2; conversely, the enzyme 11HSD1 (reductase) allows regeneration of active glucocorticoids from dehydrogenated inactive forms. The cellular cortisol can originate from circulating cortisone, thus providing excessive MR activation. Regulation of 11HSD2-11HSD1 expression and activity, which could contribute significantly to altered MR signaling, are far from being completely elucidated, particularly in the context of diseases (Odermatt and Kratschmar, 2012; Chapman et al., 2013).

Another issue possibly modifying local MR ligands or antagonists, in a specific cell context, is related to efflux pumps that limit cellular accumulation and therefore biologic activity. Although corticosteroids exhibit lipophilic structures favoring their diffusion through the cell membrane, their entry or efflux into and out of cells is influenced by ATP-binding cassette transporters (ABC transporters) including P-gp/multidrug resistance (MDR), or MDR-associated proteins. Several excellent reviews detail the characteristics of the superfAMILY of efflux pumps (Deeley et al., 2006; Klaassen and Aleksunes, 2010). There is a wide variation in tissue distribution, species differences, and sex specificity of efflux pump expression (Cui et al., 2009), but there is limited literature on the functional interactions of P-gp/MDR and corticosteroid hormones. Interest in the role of efflux pumps to regulate corticosteroid hormone functions has been renewed by the evidence that these pumps are important to restrict access of corticosteroid hormones to the brain (Geerling and Loewy, 2009). P-gp can transport aldosterone, cortisol, dexamethasone, and corticosterone (Ueda et al., 1992). Spironolactone could interfere with efflux pumps, thus modifying accumulation/elimination of corticosteroids in cells. Whether and how efflux pumps modify corticosteroids or MRA concentrations in specific tissues in pathology are open questions. The involvement of ABC pumps to regulate blood pressure and salt sensitivity was recently addressed with specific focus on biologic interactions between P-gp and drugs influencing the renin-angiotensin-aldosterone system (Bochud et al., 2011). The search for genetic variants of P-gp/MDR genes in aldosterone-related diseases, other than hypertension, may be a worthwhile avenue of investigation.

In conclusion, we reported some aspects of inappropriate MR activation in cardiovascular, renal, metabolic, ocular, and skin diseases that illustrate situations where MR blockade is now recognized to bring clear clinical benefit or should do so in the near future. This list is not complete (for instance the impact of MRA in the central nervous system and brain diseases has not been addressed) and we apologize for the publications that have not been cited because of space limitations. Based upon the recent and impressive accumulation of knowledge, including novel MR target diseases, a new era for pharmacological MR antagonism is open in medicine.

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