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Third Generation Mineralocorticoid Receptor Antagonists; Why We Need a Fourth

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Abstract

The first mineralocorticoid receptor (MR) antagonist, spironolactone, was developed almost 60 years ago to treat primary aldosteronism and pathological edema. Its use waned in part due to its lack of selectivity. Subsequently knowledge of the scope of MR function was expanded along with clinical evidence of the therapeutic importance of MR antagonists to prevent the ravages of inappropriate MR activation. Forty-two years elapsed between the first and MR-selective second generation of MR antagonists. Fifteen years later, despite serious shortcomings of the existing antagonists, a third generation antagonist has yet to be marketed. Progress has been slowed by the lack of appreciation of the large variety of cell types that express the MR and its diverse cell-type-specific actions, as well as its uniquely complex interactions actions at the molecular level. New MR antagonists should preferentially target the inflammatory and fibrotic effects of MR and perhaps its excitatory effects on sympathetic nervous system, but not the renal tubular epithelium or neurons of the cortex and hippocampus. This review briefly describes efforts to develop a third generation MR antagonist and why fourth generation antagonists and selective agonists based on structural determinants of tissue and ligand-specific MR activation should be contemplated.

Keywords

mineralocorticoid receptor; aldosterone; cortisol; hyperkalemia; sympathetic; cardiovascular

Introduction

Spironolactone, the first pharmacological antagonist of a steroid receptor approved for clinical use, was developed to treat primary aldosteronism, hypertension and the edema of chronic heart failure and cirrhosis. It was directed against the Mineralocorticoid Receptor (MR) 30 years before the MR was cloned¹ by basing its structure on that of progesterone, an endogenous antagonist of the MR. Spironolactone is an agonist of the progesterone receptor (PR), an antagonist of the androgen receptor (AR), and at diuretic doses reduces potassium excretion, thus can cause life-threatening hyperkalemia. Despite these serious side effects, it was 40 years before a second generation more selective MR antagonist was marketed. This was due in part to a lack of understanding of the basic biology of the MR, its ligands and

their regulation, as well as the mistaken belief that inhibition of the renin-angiotensin-aldosterone system sufficed to silence the many effects of inappropriate MR activation. Before truly selective antagonists, those that are not only selective for the MR and no other receptor, but also selectively inhibit some, but not all, MR functions can be developed, we need to understand how the MR becomes activated, particularly under circumstances in which its ligands are not particularly elevated.

The basic biology of the MR

MR are expressed in a wide variety of epithelial, endothelial and mesenchymal tissues, including cardiomyocytes, vascular endothelial and smooth muscle cells, renal tubular epithelia, neurons, macrophages, and adipocytes. It mediates a commensurately large variety of cell-type specific effects, many of which are unrelated to the vectorial transport of water and ions for which it was named^{2, 3}. The MR, like the other steroid receptors, is a ligand-activated nuclear transcription factor that regulates the expression of genes coding for proteins that mediate its functions. In addition, as do other steroid receptors, MR associated with the membrane initiate rapid events through common cell signaling pathways. While these signaling pathways are understood, the mechanisms by which they are activation by steroid receptors are not clear, nor is the mechanism by which the MR is associated with the membrane. Inactive steroid receptors destined for transcriptional function are bound to chaperone proteins that hold the receptor in a conformation suitable for ligand binding and are shed upon activation by a ligand. Receptors like the MR and glucocorticoid receptor (GR) that in the inactive state reside primarily in the cytoplasm are then transported to the nucleus where they form dimers and recruit transcription cofactors to form a transcription complex with DNA hormone response elements (HRE) to initiate transcription of cell type-specific effector proteins. Chaperone proteins, transcription co-factors and available HRE (those not masked by epigenetic changes) are cell and context specific, thus may provide a target for future manipulation of specific MR mediated functions⁴⁻⁷.

Structural similarities among the ligand binding domains(LBD) of the MR and glucocorticoid, progesterone, and androgen receptors (GR, PR and AR) and among their endogenous ligands have physiological implications and are important constraints to the development of selective synthetic agonists and antagonists^{3, 8}. The MR has similar affinity for its primary physiological agonists, aldosterone and the main endogenous glucocorticoids cortisol and corticosterone, as well as for 11-deoxycorticosterone (21-hydroxyprogesterone, deoxycorticosterone, DOC) and progesterone^{3, 9, 10}.

DOC was the first adrenal steroid identified and synthesized and its acetate, DOCA, has been used experimentally as a mineralocorticoid, in part due to its ease of synthesis and lower cost compared to aldosterone^{3, 11, 12}. However DOC is not equivalent to aldosterone; it also a glucocorticoid^{12, 13}. Despite ample evidence of its glucocorticoid properties, the belief that DOC is a mineralocorticoid equivalent to aldosterone has prevailed, apparently due to statements published over 50 years ago^{12, 13}. Nor is DOCA equivalent to DOC. DOCA is inactive and requires hydrolysis to DOC by esterases that are not ubiquitous, as once assumed, and are particularly limited in muscle¹². DOC is formed from progesterone by 21-hydroxylase in tissues expressing 21-hydroxylase including the adrenal gland,

placenta and ovary^{12, 13}. It is converted in the adrenal zona fasciculata to corticosterone by 11 β -hydroxylase (CYP11B1) and in the adrenal zona glomerulosa to aldosterone by aldosterone synthase (CYP11B2). In those species that express 17 α -hydroxylase in the adrenal, deoxycortisol is the primary substrate for CYP11B1 and cortisol is the primary glucocorticoid. In mammals DOC normally circulates at low concentrations in comparison to those of aldosterone, with a significant portion, >96% bound by corticosteroid binding globulin (CBG) and albumin, unavailable for receptor binding¹⁴. Accumulation of DOC in patients with 17 α -hydroxylase or 11 β -hydroxylase deficiency produces a mineralocorticoid excess hypertension^{12, 15}. While plasma concentration may increase by 10-fold in pregnancy due to the increase in its precursor progesterone, inactivation of DOC by the aldoketoreductase AKR1C3 is thought to prevent excessive activation of MR in colon and renal tubular epithelial cells by DOC¹⁵. Progesterone is an MR antagonist that binds the MR with similar or slightly higher affinity than aldosterone and attains physiological relevant inhibitory concentrations during pregnancy and at its zenith during the estrus cycle¹⁶.

Glucocorticoid synthesis in mammals is 2–3 orders of magnitude that of aldosterone. Most circulating cortisol and corticosterone, 96% or more, is bound to CBG or plasma albumin¹⁴, notwithstanding the amount of free glucocorticoid still greatly exceeds that of aldosterone even at the nadir of the circadian rhythm of the HPA axis when cortisol concentrations descend to the 3–10 μ g/dl range^{3, 17, 18}. Even in severe Primary aldosteronism (PA) aldosterone concentrations are in the 10s of ng/dl^{19–21}. CBG is a crucial regulator of glucocorticoid availability. Elastases produced by inflammatory cells and certain bacteria cleave CBG, releasing cortisol/corticosterone at the site of inflammation and infection^{22, 23} and globally in septic shock²⁴, thus maximizing MR occupation and increasing GR activation which tends to counter excessive inflammatory and repair response of MR activation. CBG is not merely a plasma transport protein; it is produced in various tissues and is an active participant in local glucocorticoid activity^{25–27}.

In aldosterone target cells, including renal tubular epithelial and vascular endothelial and smooth muscle cells, cortisol & corticosterone are converted to inactive 11 β -dehydro metabolites by the unidirectional 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) which thus provides pre-receptor selectivity for the lower concentrations of aldosterone. Glucocorticoids are thought to be the physiological MR ligand in most cells that do not express 11-HSD2, including the cardiomyocyte, mature adipocyte, macrophage, and most neurons^{10, 27–29}. A select few aldosterone target neurons of the nucleus tractus solitarius that mediate salt appetite co-express MR and 11 β -HSD2³⁰. 11 β -HSD1 is a bidirectional enzyme that usually acts as a reductase that activates the inactive metabolites 11-dehydrocorticosterone and cortisone and increases intra-cellular concentrations of active glucocorticoid. Selective HSD1 antagonists have been developed to decrease the effects of excessive glucocorticoid action, a major prejudicial factor in stress, obesity, metabolic syndrome, and dementia^{31, 32}. However 11-HSD1 acts as a dehydrogenase in the absence of hexose-6-phosphate dehydrogenase (H6PD) which is required to regenerate NADPH, the obligatory co-factor for reductase activity^{29, 33}. This occurs in a few types of cells; those of particular relevance to cardiovascular disease are preadipocytes and pre-sympathetic neurons of the paraventricular nucleus^{33, 34}. In cells expressing both MR and GR that do not have prereceptor selectivity conferred by an 11 β -HSD, for example many hippocampal

neurons and immune cells, it is thought that many or most MR are occupied by cortisol or corticosterone during most of the circadian cycle for glucocorticoids, most or all MR and some GR are occupied during the zenith of the cycle, and that most or all GR are activated by stress levels of glucocorticoids³⁵. The coordinated actions mediated by the MR and GR are critical to normal affect, response to stress, learning, and memory. The ultradian rhythm of glucocorticoid secretion mediates further change in relative MR and GR activation in hippocampal neurons³⁶. Such short term pulses of cort may be most relevant to the rapid non-transcriptional effects mediated by the MR and GR^{37, 38}. The balance between MR and GR mediated effects are crucially important in tissue repair, as well as neuronal function.

MR activation initiates inflammation and repair pathways, while GR activation tends to dampen the responses. Local treatment of a wound with an 11 β -HSD1 antagonist decreases local glucocorticoid activity at the site and accelerates wound healing, particularly in the stressed and aged patient^{39, 40}, while topical application of glucocorticoids suppresses excessive scar formation. Treatment with an MR antagonist also suppresses excessive inflammation and fibrosis after injury^{41, 42}.

Evidence for other mechanisms conferring ligand specificity to the MR has accumulated, but only recently have been identified⁴. For example tesmin is a co-activator for the MR when it is bound to aldosterone and deoxycorticosterone, but not to cortisol or spironolactone⁵. Work in the area of the structural requirements of ligand-discriminant co-activators may solve a mystery that is at the crux of the development of truly selective MR antagonists to treat cardiovascular, renal and metabolic diseases. Even in primary aldosteronism due to an aldosterone producing adenoma the amount of circulating aldosterone does not approximate that of endogenous glucocorticoid agonists, yet clearly patients with primary aldosteronism have significantly more severe cardiovascular remodeling for the same level and duration of hypertension and benefit from removal of the offending tumor or treatment with an MR antagonist^{3, 20, 43}. The MR is reported to be susceptible to activation in the absence of ligand under conditions of high reactive oxygen stress^{44, 45}, reviewed in reference 3, however there is evidence that the MR cardiomyocytes is quiescent when bound to glucocorticoids under normal conditions, but is activated by oxidative stress⁴⁶. This would explain the beneficial effects of MR antagonists in conditions of excessive MR activation in which aldosterone concentrations are either normal or reduced.

Increased binding of GR by glucocorticoid levels elevated by stress, inflammation or obesity may also play a significant role in increased or altered MR transcriptional activity. The affinity of the GR for cortisol and corticosterone is 10-fold less than that of the MR. Most cells that express MR also express GR. Therefore under non-stressed conditions glucocorticoid-targeted MRs such as those in cardiomyocytes or hippocampal neurons are generally fully occupied by glucocorticoids; both MR and GR are occupied at stress levels of glucocorticoids. In addition to forming homodimers, the activated MR forms heterodimers with ligand activated GR. MR:MR and MR:GR dimers have different transcriptional efficacy *in vivo* as well as *in vitro*⁴⁷⁻⁴⁹, reviewed in reference 10 and these differences may cause further dysfunction in injured tissue.

The First Generation Antagonists

Deoxycorticosterone was isolated 75 years ago based upon its mineral retaining properties, however studies with the purified compound soon demonstrated that in addition to stimulating sodium and water retention in exchange for the excretion of potassium and protons, it caused severe hypertension and heart failure^{11, 50, 51} preceded by increased vascular tone both due to direct action upon vessels and through an increase sympathetic drive^{51–55}. In the early 1960's, less than decade after the isolation of aldosterone⁵⁶, spironolactone (Aldactone) was developed and approved for the treatment of primary aldosteronism and its associated hypertension, hypokalemia and alkalosis⁵⁷, essential hypertension, and the edema of congestive heart failure and cirrhosis⁵⁸. It and canrenone, 7 α -thiomethyl spironolactone, one of several active spironolactone metabolites approved for clinical use in Europe, constitute the first generation of MR antagonists for clinical use⁵⁹. Notwithstanding growing evidence that mineralocorticoids acted directly in many tissues, including vessels, heart and brain^{60–65} and that spironolactone antagonized these effects, the prevailing dogma became that the antihypertensive effect of spironolactone was due solely or primarily to its diuretic and saluretic action⁶⁶, a misconception that lasted several decades. The structure of spironolactone resembles that of progesterone, an endogenous antagonist of the MR. Spironolactone is a PR agonist and AR antagonist within therapeutic ranges for MR blockade. The use of spironolactone and canrenone at doses for potassium sparing diuretic effects, was limited by significant hyperkalemia, as well as progestational and anti-androgen effects causing significant menstrual cycle disruption, gynecomastia and impotence. While lack of receptor selectivity is a significant problem for most uses of spironolactone, the anti-androgenic effect is useful in women with hirsutism, particularly when associated with hypertension, for example in polycystic ovarian syndrome^{67–69}. Drospirenone, one of a class of 17 α -pregnane-21,17-carbolactones with 15,16- β methylene modifications developed by Schering AG, now Bayer Healthcare^{59, 70} is a potent synthetic PR agonist and MR and AR antagonist currently used in birth control and menopausal hormone replacement regimens in combination with an estrogen. It is significantly more potent as an MR antagonist than spironolactone and has been suggested as a treatment for hypertension in women^{71, 72}. Thus lack of receptor selectivity is an advantage under select circumstances, however as with other oral contraceptives, the risk for thrombosis of estrogen +drospirenone preparations must be assessed for each patient^{73, 74}.

An effort was made by several laboratories during the 1980's to develop more selective MR antagonists^{59, 75}. Roussel-UCLAF developed highly soluble potent 7-alkyl spironolactone MR antagonists which were used for research but were not marketed for clinical use^{76, 77}. RU28318 was used to definitively demonstrate the critical importance of the MR in normal hippocampal neuronal function mediated by cortisol & corticosterone⁷⁸ and of MR in the central modulation of blood pressure by mineralocorticoid excess and in salt sensitive rats^{77, 79}. Ciba-Geigy produced a class of more selective MR antagonists by incorporating epoxy groups into spironolactone derivatives^{80, 81}, however testing and marketing of one of these, eplerenone, was delayed for 2 decades (and several pharmaceutical company restructurings), reviewed in reference 82.

Meanwhile, due to their side effects clinical use of spironolactone and canrenone as antihypertensive agents waned in favor of angiotensin converting enzyme inhibitors and later, angiotensin type 1 receptor (AT1R) antagonists that when paired with diuretics were thought to suppress the pernicious effects of excessive renin-angiotensin-aldosterone system (RAAS) activity, including that of aldosterone, notwithstanding the knowledge that aldosterone production often escaped control of the RAAS after chronic RAAS suppression^{82–84}. The accumulation of evidence from animal studies demonstrating that inappropriate activation of MR in the heart, vessels and kidneys led to inflammation, hypertrophy and fibrosis that were not prevented by angiotensin converting inhibition and were independent of hypertension^{85–90}, led to the Randomized Aldactone Evaluation Study (RALES). The RALES trial was stopped early when it became clear that addition of a low dose of Spironolactone to standard therapy of patients with severe congestive heart failure significantly reduced death from all causes, including sudden cardiac death, as well as improved quality of life⁹¹.

The second generation MR antagonist

Early results of the RALES trial spurred the completion of clinical testing and marketing in 2002 of 9–11 α -epoxymexrenone or eplerenone (Inspra). Eplerenone is selective for the MR, however its low potency and difficulty, thus expense, of synthesis in comparison to spironolactone had retarded its testing for market^{59, 92}. The Eplerenone Post-acute Myocardial Infarction Efficacy and Survival Study (EPHESUS)⁹³ and subsequent randomized clinical trials confirmed that the addition of either spironolactone or eplerenone to standard treatment regimens reduced arrhythmias and delayed pathological remodeling of the vessels, heart and kidneys in various clinical conditions, many of which are not associated with increased circulating aldosterone^{94–96}. The doses used were below those required to lower blood pressure or have a diuretic effect. As predicted from animal studies, some done more than 50 years earlier^{60, 97}, spironolactone and eplerenone were shown to decrease cardiac arrhythmias in patients through direct effects on cardiomyocyte excitation, as well as by decreasing sympathetic nervous system activation and normalizing hypokalemia and hypomagnesaemia, including in those with coronary artery disease and chronic kidney disease without heart failure^{96, 98–103}.

Evidence that mineralocorticoids enhanced central sympathetic excitation leading to hypertension accrued since the late 1970s^{63, 65, 104}. The advent of specific antibodies and tracers allowed the demonstration of MR in hypothalamic presympathetic neurons³⁴. Spironolactone and eplerenone are lipophilic and cross the blood brain barrier^{105, 106}. Lowering blood pressure with a thiazide diuretic as standard first line treatment of hypertension often activates the SNS, which among other effects, increases blood glucose. Addition of a low dose of Spironolactone to the diuretic or substituting the diuretic with the MR antagonist resulted in a similar decrease in blood pressure without increasing sympathoexcitation and producing insulin resistance^{107, 108}. Judicious use of an MR antagonist while monitoring serum potassium is now advocated in diabetic nephropathy to slow the progress of renal failure without further perturbing glycemic control⁹⁴. The potential for serious hyperkalemia must always be considered even in patients with no known renal impairment, particularly if the MR antagonist is added to an antihypertension

regimen that includes a moderate to high dose of an angiotensin II receptor blocker or converting enzyme inhibitor.

Comparison of the First and Second Generation MR antagonists

The clear advantage of eplerenone over spironolactone is its MR selectivity and lack of effect on the AR and PR. Both eplerenone over spironolactone increase the incidence of life-threatening hyperkalemia due to renal tubular effects that is mitigated by using lower doses. Some spironolactone metabolites are also potent MR antagonists that extend its effective half-life to about 16h and allow its once a day dosing with lower peak effects⁵⁹. Eplerenone is metabolized by the CYP3A4 and CYP3A5 enzymes to form inactive metabolites. While its half-life is only 3–4h, the natriuretic effects of eplerenone persist for 12h, perhaps because the eplerenone-bound MRs remain in an inert conformation that is less dynamic than of MR bound to an endogenous ligand that is released relatively rapidly after transcription, allowing the receptor to be translocated to the cytosol^{59, 109–111}, thus eplerenone is dosed twice a day despite its very short half-life. A comprehensive survey of clinically relevant interactions between eplerenone and other drugs that are metabolized by CYP3A enzymes has yet to be published⁹², however dosing of eplerenone is complicated by certain genetic polymorphisms of CYP3A and co-administration of eplerenone with other agents that alter CYP3A activity^{59, 92, 112, 113}. A major advantage of spironolactone over eplerenone is cost and once a day dosing. Spironolactone was a significantly better antihypertensive than eplerenone in two clinical studies, perhaps because of its greater potency and longer half-life^{59, 114, 115}. Despite side effects, results of clinical trials continue to show therapeutic benefits of MR antagonists in a growing list of conditions^{2, 96, 116, 117}, clearly indicating the need for better MR antagonists^{59, 75, 118–120}. The Dialysis Outcomes Heart Failure Aldactone Study and others have been demonstrated their utility in stemming the progression of chronic kidney disease^{121–123}. Another is the dramatic discovery that central serous chorioretinopathy, a rapidly progressing and heretofore untreatable cause of permanent destruction of the retina, is reversed by spironolactone and eplerenone^{124, 125}.

The need for better MR antagonists prompted John Funder to challenge the research community to develop third and fourth generations of MR antagonists, emphasizing that the new drugs should also have long patent lives to allow ample time for the recuperation of the cost of drug research and development at a reasonable price for the patient¹²⁶. Ideal third generation MR inhibitors were defined as being potent, selective and affordable, to be used in patients with primary aldosteronism, now recognized as comprising ~10% of treatment resistant hypertensive patients^{20, 127}. Fourth Generation MR Antagonists were defined as agents that target MR-mediated processes contributing to vascular dysfunction, but preserve the ability for MR to regulate renal excretion of potassium¹²⁶. I would propose that MR in neurons of the hippocampus and cerebral cortex should also be spared.

The third Generation MR Antagonists, some of which are now in clinical trials, are non-steroidal, potent, MR selective, and should be easier, thus less expensive, to synthesize than Eplerenone. None have been approved by the FDA for clinical use at this time, but not for lack of effort. At the time of this writing, 273 studies were listed for mineralocorticoid receptor antagonist on ClinicalTrials.gov. Some, in addition to good MR selectivity and

favorable metabolic profiles, are reported to have proportionately greater antagonism of MR-mediated cardiovascular inflammation, dysfunction and remodeling than upon MR-mediated exchange of sodium for potassium and hydrogen ions in renal tubular epithelia, however the mechanisms for this functional selectivity are not discussed.

The Dihydropyridine-like MR antagonists

It was shown 30 years ago that dihydropyridine voltage-dependent calcium channel blockers decreased MR activation by blunting the release of intracellular calcium that activates aldosterone synthesis by adrenal zona glomerulosa cells^{128–130}. It was later demonstrated that dihydropyridines also bind and antagonize the MR directly^{131–134}. Spironolactone, eplerenone and progesterone facilitate the translocation of MR from the cytosol to the nucleus, but prevent recruitment of transcriptional co-activators. In contrast, due to their size and conformation, dihydropyridine-bound MR, including MR with the S810L mutation, are not translocated to the nucleus^{59, 131, 133, 134}. This property is particularly useful in the treatment of patients with the MR S810L mutation responsible for a very severe form of familial pseudohyperaldosteronism which is greatly exacerbated by pregnancy. The constitutive activity of the MR S810L is significantly enhanced by progesterone, spironolactone and eplerenone which promote its translocation to the nucleus, but do not prevent its transcriptional activity¹³⁵. Dihydropyridine-type MR antagonists would be the first selective treatment for patients with the S810L mutation of the MR.

A class of **cyanoester dihydropyridines** MR antagonists that are potent, selective and orally available, as well as devoid of calcium channel-blocker activity, were developed at Pfizer Pharmaceutical research labs by introducing a cyano group at C3 and various R6 substitutions^{132, 136}. One of these compounds performed as well as eplerenone in decreasing hypertension in the Dahl salt sensitive rat¹³⁶. Bayer Pharm AG has also developed a class of cyano-dihydropyridines with similar properties and promising preclinical profiles¹³⁴. Among these are the BR-4628 (ethyl (4 R)-5-acetyl-2,6-dimethyl-4-(2-methyl-4-oxo-4 H -chromen-8-yl)-1,4-dihydropyridine-3-carboxylate) that performed very well in preventing adverse tissue remodeling in animal models of mineralocorticoid excess or salt-sensitivity despite mild progestational effects^{134, 137}. This led to the development of the dihydronaphthyridine BAY 94-8862, or finerenone^{138–140}, the only third generation non-steroidal MR antagonist registered for a phase 3 clinical trial as of 9/2015, though recruitment has not yet begun (ClinicalTrials.org).

Several multicenter, randomized, double-blind, placebo-controlled ARTS (mineralocorticoid Receptor antagonist Tolerability Studies) are in progress to determine the safety and efficacy of finerenone in heart failure, diabetes and nephropathy^{116, 139, 141, 142}. Only some studies have an active-comparator (spironolactone or eplerenone) arm. The phase 2a ARTS- HFrEF trial compared the effects of finerenone, spironolactone or placebo added to standard therapy of patients with heart failure with reduced ejection fraction and mild renal compromise^{143, 144}. The phase 2b ARTS-HF trial is to compare finerenone to eplerenone¹⁴¹. Finerenone and spironolactone decreased albuminuria and brain natriuretic peptide, markers of renal and heart function, respectively, by similar amounts, but the increase in serum potassium, incidence of hyperkalemia and

worsening of renal function were less in the finerenone group compared to spironolactone. The mechanisms for the decreased inhibition of MR mediated sodium/potassium homeostasis was not addressed. Spironolactone lowered the blood pressure to a significantly greater degree than finerenone in this study¹⁴³, so the lower effect on hyperkalemia might have been due to a lower effective dose of finerenone. However, spironolactone crosses the blood brain barrier and decreases peripheral sympathetic drive^{3, 34, 104, 105, 107}. This is particularly relevant to the potential for increased gluconeogenesis and insulin resistance due to sympathetic nervous system activation in patients that already have sympathoactivation^{3, 107, 108}. Whether finerenone crosses the blood brain barrier to access presympathetic neurons is not published. The authors of the phase 2a ARTS-HFrEF report point out that the hypertension in patients in this study was adequately controlled before the addition of the MR antagonists, however most were on a combination of anti-hypertensive drugs including β -blockers. A reassessment of the step-wise increase of drugs in multi-drug anti-hypertensive regimens is in order with what is now recognized as a clinically significant role of MR antagonists in the control of inappropriate activation of the sympathetic nervous system^{10, 107, 108, 145}. This may be particularly relevant for patients with type 2 diabetes¹⁰⁸. The ARTS-Diabetic Nephropathy (ARTS-DN; NCT01874431) compared the effect of the addition of finerenone to standard treatment in diabetics with mild-to-moderate chronic kidney disease receiving either an angiotensin type 2 receptor or converting enzyme inhibitor on albuminuria and serum potassium^{142, 144}. A steroidal MR antagonist was not included in the study, however finerenone decreased albuminuria to a similar degree as that reported for spironolactone in the previous ARTS-HF trial, and decreased the decline of renal function compared to placebo^{142, 146}. It would be very interesting to compare the effect of finerenone and spironolactone sympathetic activity and glycemic control.

The Pyrazoline-like MR antagonists

Pyrazoline derivative MR antagonists have been developed and tested at the Pfizer labs¹⁴⁷. One of these, PF-3882845 (3S, 3aR)-2-(3-chloro-4-cyanophenyl)-3-cyclopentyl-3,3a,4,5-tetrahydro-2H-benzo[g]indazole-7-carboxylic acid), is orally available, potent, selective for the MR, and significantly decreased the renal pathology and loss of function in an aldosterone + salt excess model with less risk for hyperkalemia as compared to eplerenone^{147, 148}. The first clinical phase I trial was terminated due to safety concerns. Results of another (NCT01314898) that evaluated urinary sodium/potassium ratio was presumably finished early in 2015 (<https://clinicaltrials.gov>), but results have not been published. Preclinical data for another promising pyrazoline derivative from Pfizer, (R)-14c, [(R)-6-(1-(4-cyano-3-methylphenyl)-5-cyclopentyl-4,5-dihydro-1H-pyrazol-3-yl)-2-methoxynicotinic acid], was recently described¹⁴⁹.

The Sulfonamide-based non-steroidal MR antagonists

The development of aryl sulfonamide-based non-steroidal MR antagonists by Pfizer that are smaller, more polar, with better pharmacodynamics and safety features than the pyrazoline PF-3882845 has been described, but is still in the pre-clinical phase of evaluation¹⁵⁰. Another sulfonamide MR antagonists has been studied further. SM-368229 (N-4,4-dimethyl-2-thioxo-1,4-dihydro-2H-3,1-benzoxazin-6-yl-thiophene-2-sulfonamide) is one of

a series of sulfonamide derived MR antagonists synthesized in by the Dainippon Sumitomo Pharma Research Laboratories¹⁵¹. SM-368229 is a selective MR partial agonist-antagonist about 10-fold more potent than spironolactone which was as effective as spironolactone in preventing hypertension, proteinuria and pathological cardiac and renal remodeling, as well as the increase in markers of oxidative stress, inflammation and fibrosis markers in the aldosterone + salt excess model. The authors credit the partial agonist activity of SM-368229 for the decreased potassium retention in animals receiving SM-368229 in comparison to spironolactone at the therapeutic doses^{152, 153}. SM-368229 has no PR activity, but is a weak AR antagonist¹⁵¹. This company has also developed an interesting compound, DSR-71167, (2-([(2,2-difluoroethyl)amino]methyl)-29-fluoro-N-(3-methoxy-4-sulfamoylphenyl)biphenyl-4-carboxamide hydrochloride) that is a selective MR antagonist and weak carbonic anhydrase inhibitor which lowered the blood pressure without increasing plasma potassium in the DOCA-salt and Dahl salt sensitive models¹⁵⁴. These two compounds are still in early development.

Other potential MR antagonists

Clinical trials for several other MR antagonists have been conducted for which details of the compound are not readily available. Two in early stages of clinical testing in patients with hypertension and diabetic nephropathy is MT-3995 (Mitsubishi) and SC-3150 (Daiichi-Sankyo)^{123, 140, 155}. Phase II clinical trials have been completed for LY2623091 (Eli Lilly) was compared to a placebo and spironolactone in patients with primary hypertension (NCT02194465) and to eplerenone, but not a placebo, in men and women with chronic renal disease (NCT01427972). Interaction of LY2623091 with drugs that commonly interfere with CYP450 metabolism was also tested (NCT02300259). While these studies have been closed (www.clinicaltrials.gov) data has yet to be published. **Oxazolidinedione derivative** MR antagonists are being developed and studied at the Merck Research Laboratories, but as yet published information is limited to interesting preclinical studies^{156–158}.

Despite ever growing evidence of the value of MR antagonists to treat diseases that are increasingly prevalent in the Developed World, major short comings of spironolactone and eplerenone, and concerted effort in drug development in this area for at least 20 years, third generation potent, non-steroidal, MR-selective antagonists have been created, but have yet to be marketed¹²⁰. The reasons surely vary, but probably combine a small improvement over the existing clinically approved MR antagonists and the cost of bringing a drug to market. The new generation MR antagonists appear to be selective for the MR, have a favorable pharmacokinetic and pharmacodynamic profiles with minimal interference with other potential therapeutic agents, and target excessive MR-mediated oxidative stress, inflammation and pathological structural remodeling. However evidence that any have less effect on potassium excretion than steroidal MR antagonists is not particularly strong, nor have mechanisms for sparing the effect of MR in renal tubular transport of ions been described even for finerenone, the closest candidate to becoming clinically available.

Partial MR agonists and those combining a third generation MR antagonist and carbonic anhydrase inhibitor are being developed to separate sodium excretion from potassium retention MR inhibition^{151–154}.

Perspective: Fourth Generation MR Antagonists?

In addition to sparing the MR-mediated regulation of ions in renal epithelia, inhibition of MR function in neurons is a complex issue that should be addressed in the development of antagonists of specific functions of the multi-tasking MR. The ability of non-steroidal third generation candidates to cross the blood brain barrier has not been reported, but lack of suppression of MR in pre-sympathetic neurons of the hypothalamus that are protected by the blood brain barrier may explain why finerenone was less effective than spironolactone in lowering the blood pressure in clinical studies. However, with the increasing incidence of obesity and metabolic syndrome, avoiding the effects of accelerated sympathetic drive on glycemic control should be a goal^{107, 108}. Conversely, the greatest number and concentration of MR in the body are in the hippocampus where they are required for a normal response to stress and for learning and memory^{3, 10, 32, 38, 159–161}. MR-mediated events are also required for neurogenesis, differentiation and migration, now known to be important for the adult, as well as developing human brain^{162, 163}. Neurons of the hippocampus express both MR and GR. As described above, both are thought to be occupied and activated by cortisol or corticosterone differentially depending on the circadian, metabolic and stress related changes in cort levels. A balance between activated MR and GR mediated function is crucial to normal affect and behavior. Abnormal HPA axis function and a decrease in the MR:GR ratio is seen in the brains of persons with major depressive disorder^{164–166}. Spironolactone impaired neuronal function and cognition in healthy humans and rats^{167–169}, as did MR gene deletion in mice^{162, 170, 171}. Aldosterone treatment restored impaired neuronal electrical activity underlying learning deficits in diabetic rats¹⁷² and addition of an MR agonist to standard SSRI and SNRI therapy was found to significantly ameliorate the response in patients with major depression¹⁷³. In both of these instances the balance between MR relative to GR actions was ostensibly restored. In stark contrast, treatment with spironolactone or eplerenone has been shown in multiple clinical studies to significantly improve mood, cognition and quality of life of patients with primary aldosteronism or severe cardiovascular disease^{141, 174, 175}. The difficulty in parsing the effects of an MR antagonist on cognition is in the separation of the effect of trophic actions of MR within neurons from the devastating effects of cardiovascular disease on the function and integrity of the cerebral vessels, the microvasculature in particular, that leads to neuronal dysfunction and death^{176–178}. Once the neuronal destruction of vascular dementia occurs, inhibitory effects on the MR within hippocampal and cortical neurons are probably moot. An agent that prevents MR mediated inflammation, loss of cerebrovascular autoregulation and pathological vascular remodeling would protect the neurons.

It is a daunting proposition to inhibit excessive MR-mediated activation of pre-sympathetic neurons of the hypothalamus while preserving MR function of the “learning & memory” neurons in the hippocampus and cortex, however there are differences in MR-mediated transcriptional function that may allow selective repression or stimulation of the transcription of specific genes. The MR antagonists developed so far act as passive antagonists that prevent binding of the activated receptor to transcription co-activators or do not allow the ligand-bound receptor to enter the nucleus¹³⁴. This approach does not take into consideration the many functions of the MR. Analysis of the structural basis for the

differential recruitment of co-transcription factors based upon whether the MR is bound to aldosterone or cortisol has progressed to the point that it may soon be feasible to develop active antagonists or partial agonists that produce a ligand-MR conformation that allows the recruitment of only certain co-factors, thus select the genes would be transcribed^{4–7, 179, 180}. For example, MR is a transcription factor for NADPH oxidase, crucial for many cell processes, which in excess is responsible in part for oxidative damage caused by excessive MR activation^{181–183}. MR also promotes the transcription of Nfr2, another transcription factor that promotes genes in major antioxidant pathways. However indirect stimulation of glutathione production by MR is not enough to neutralize the MR-mediated reactive oxygen species generation under pathological conditions¹⁸⁴. Might it be possible to make ligands that would selectively promote recruitment of co-activators for Nfr2 transcription? Such studies should also elucidate how injury to the heart resulting in the accumulation of reactive oxygen species propels glucocorticoid-bound MR into pro-inflammatory and pro-fibrotic transcriptional activities⁴⁶. Selective MR agonists are being considered in the treatment of depression and cognitive decline^{172, 173} (and Ronald de Kloet, personal communications). An approach that targets the conformation of the receptor which promotes or prevents the binding of specific co-transcription factors heralds a new age of MR agonists, as well as antagonists.

Another target that may be fruitful for the development of fourth generation MR antagonists (or agonists) is the mechanism for the formation of MR and GR homodimers and heterodimers, as these appear to have different transcriptional potential^{47–49, 185, 186}.

Two more strategies designed to alleviate excessive MR activation may be in the offing that deserve mention, though they are peripheral to the topic of MR antagonists. Specific antagonists of CYP11B2, or aldosterone synthase, the enzyme responsible for the last step in aldosterone synthesis are being developed that would be useful in the treatment of primary aldosteronism^{187–189}. However, as discussed above, aldosterone levels are not elevated or are even suppressed in many of the syndromes in which MR antagonists are effective, including in the Dahl Salt-Sensitive rat that was frequently used to test the Third Generation MR antagonists described above^{3, 10}. The second strategy involves the interaction between the MR and G protein-coupled protein receptor, GPER, formerly GPR30. GPER is activated by physiological levels of aldosterone, but only supra-physiological concentrations of estrogen^{190–192}. In addition to being a candidate for mediating those rapid non-genomic effects of aldosterone that are not inhibited by MR antagonists^{193, 194}, effects mediated by GPER activation are cell and context dependent and appear to act in opposition to some deleterious effects of MR activation^{191, 195–197}.

Conclusion

More than half a century after developing the first MR antagonist we have yet to develop an ideal antagonist that will mitigate the cardiovascular, renal and metabolic pathology associated with inappropriate MR activation without interfering with important homeostatic functions. This is due in great measure to the many roles of the MR and the complexity of its interaction with different physiological ligands under differing redox conditions, as well as its molecular and functional interaction with the GR with which it shares ligands. Thus it

does not suffice to have a potent selective MR antagonist with excellent pharmacokinetic and pharmacodynamic properties. A better understanding of the molecular constraints of co-factor binding and how pathological concentrations of reactive oxygen species alter the structure of the ligand-activated MR and dictate its transcriptional activity should provide the basis for developing targeted MR antagonists and agonists.

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References

1. Arriza JW, Weinberger C, Cerelli G, Glaser TM, Handelin BL, Housman DE, Evans RM. Cloning of human mineralocorticoid receptor complementary DNA: Structural and functional kinship with the glucocorticoid receptor. *Science*. 1987; 237:268–275. [PubMed: 3037703]
2. Bauersachs J, Jaissier F, Toto R. Mineralocorticoid receptor activation and mineralocorticoid receptor antagonist treatment in cardiac and renal diseases. *Hypertension*. 2015; 65:257–263. [PubMed: 25368026]
3. Gomez-Sanchez E, Gomez-Sanchez CE. The multifaceted mineralocorticoid receptor. *Comprehensive Physiology*. 2014; 4:965–994. [PubMed: 24944027]
4. Fuller PJ. Novel interactions of the mineralocorticoid receptor. *Mol Cell Endocrinol*. 2015; 408:33–37. [PubMed: 25662276]
5. Rogerson FM, Yao YZ, Young MJ, Fuller PJ. Identification and characterization of a ligand-selective mineralocorticoid receptor coactivator. *FASEB J*. 2014; 28:4200–4210. [PubMed: 24907116]
6. Fuller PJ, Yao Y, Yang J, Young MJ. Mechanisms of ligand specificity of the mineralocorticoid receptor. *J Endocrinol*. 2012; 213:15–24. [PubMed: 22159507]
7. Yang J, Chang CY, Safi R, Morgan J, McDonnell DP, Fuller PJ, Clyne CD, Young MJ. Identification of ligand-selective peptide antagonists of the mineralocorticoid receptor using phage display. *Mol Endocrinol*. 2011; 25:32–43. [PubMed: 21106883]
8. Petit-Topin I, Fay M, Resche-Rigon M, Ulmann A, Gainer E, Rafestin-Oblin ME, Fagart J. Molecular determinants of the recognition of ulipristal acetate by oxo-steroid receptors. *J Steroid Biochem Mol Biol*. 2014; 144(Pt B):427–435. [PubMed: 25204619]
9. Arriza JL, Simerly RB, Swanson LW, Evans RM. The neuronal mineralocorticoid receptor as a mediator of glucocorticoid response. *Neuron*. 1988; 1:887–900. [PubMed: 2856104]
10. Gomez-Sanchez EP. Brain mineralocorticoid receptors in cognition and cardiovascular homeostasis. *Steroids*. 2014; 91C:20–31. [PubMed: 25173821]
11. Kuhlman D, Ragan C, Ferrebee JW, Atchley DW, Loeb RF. Toxic effects of deoxycorticosterone esters in dogs. *Science*. 1939; 90:496–497. [PubMed: 17769062]
12. Vinson GP. The mislabelling of deoxycorticosterone: Making sense of corticosteroid structure and function. *J Endocrinol*. 2011; 211:3–16. [PubMed: 21715433]
13. Failla D, Tomkins GM, Santi DV. Partial purification of a glucocorticoid receptor. *Proc Natl Acad Sci U S A*. 1975; 72:3849–3852. [PubMed: 172887]
14. Dunn JF, Nisula BC, Rodbard D. Transport of steroid hormones: Binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. *J Clin Endocrinol Metab*. 1981; 53:58–68. [PubMed: 7195404]
15. Sharma KK, Lindqvist A, Zhou XJ, Auchus RJ, Penning TM, Andersson S. Deoxycorticosterone inactivation by *akr1c3* in human mineralocorticoid target tissues. *Mol Cell Endocrinol*. 2006; 248:79–86. [PubMed: 16337083]
16. Ahmed AH, Gordon RD, Taylor PJ, Ward G, Pimenta E, Stowasser M. Are women more at risk of false-positive primary aldosteronism screening and unnecessary suppression testing than men? *J Clin Endocrinol Metab*. 2011; 96:E340–346. [PubMed: 20962019]

17. Samuels MH, Brandon DD, Isabelle LM, Cook DM, Graham KE, Purnell JQ, Loriaux DL. Cortisol production rates in subjects with suspected cushing's syndrome: Assessment by stable isotope dilution methodology and comparison to other diagnostic methods. *J Clin Endocrinol Metab.* 2000; 85:22–28. [PubMed: 10634358]
18. Reul JMH, De Kloet ER. Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. *Endocrinology.* 1985; 117:2505–2511. [PubMed: 2998738]
19. Satoh F, Morimoto R, Ono Y, Iwakura Y, Omata K, Kudo M, Takase K, Seiji K, Sasamoto H, Honma S, Okuyama M, Yamashita K, Gomez-Sanchez CE, Rainey WE, Arai Y, Sasano H, Nakamura Y, Ito S. Measurement of peripheral plasma 18-oxocortisol can discriminate unilateral adenoma from bilateral diseases in patients with primary aldosteronism. *Hypertension.* 2015; 65:1096–1102. [PubMed: 25776074]
20. Gomez-Sanchez CE, Rossi GP, Fallo F, Mannelli M. Progress in primary aldosteronism: Present challenges and perspectives. *Horm Metab Res.* 2010; 42:374–381. [PubMed: 20091458]
21. Nguyen G, Blanchard A, Curis E, Bergerot D, Chambon Y, Hirose T, Caumont-Prim A, Tabard SB, Baron S, Frank M, Totsune K, Azizi M. Plasma soluble (pro)renin receptor is independent of plasma renin, prorenin, and aldosterone concentrations but is affected by ethnicity. *Hypertension.* 2014; 63:297–302. [PubMed: 24218434]
22. Hammond GL, Smith CL, Paterson NA, Sibbald WJ. A role for corticosteroid-binding globulin in delivery of cortisol to activated neutrophils. *J Clin Endocrinol Metab.* 1990; 71:34–39. [PubMed: 2370299]
23. Simard M, Hill LA, Underhill CM, Keller BO, Villanueva I, Hancock RE, Hammond GL. Pseudomonas aeruginosa elastase disrupts the cortisol-binding activity of corticosteroid-binding globulin. *Endocrinology.* 2014; 155:2900–2908. [PubMed: 24848868]
24. Beishuizen A, Thijs LG, Vermes I. Patterns of corticosteroid-binding globulin and the free cortisol index during septic shock and multitrauma. *Intensive care medicine.* 2001; 27:1584–1591. [PubMed: 11685298]
25. Moisan MP, Minni AM, Dominguez G, Helbling JC, Foury A, Henkous N, Dorey R, Beracochea D. Role of corticosteroid binding globulin in the fast actions of glucocorticoids on the brain. *Steroids.* 2013
26. Mattos GE, Heinzmann JM, Norkowski S, Helbling JC, Minni AM, Moisan MP, Touma C. Corticosteroid-binding globulin contributes to the neuroendocrine phenotype of mice selected for extremes in stress reactivity. *J Endocrinol.* 2013; 219:217–229. [PubMed: 24048966]
27. Deak T, Nguyen KT, Cotter CS, Fleshner M, Watkins LR, Maier SF, Spencer RL. Long-term changes in mineralocorticoid and glucocorticoid receptor occupancy following exposure to an acute stressor. *Brain Research.* 1999; 847:211–220. [PubMed: 10575090]
28. Gomez-Sanchez EP, Gomez-Sanchez MT, de Rodriguez AF, Romero DG, Warden MP, Plonczynski MW, Gomez-Sanchez CE. Immunohistochemical demonstration of the mineralocorticoid receptor, 11 β -hydroxysteroid dehydrogenase-1 and -2, and hexose-6-phosphate dehydrogenase in rat ovary. *J Histochem Cytochem.* 2009; 57:633–641. [PubMed: 19255253]
29. Odermatt A, Atanasov AG. Mineralocorticoid receptors: Emerging complexity and functional diversity. *Steroids.* 2009; 74:163–171. [PubMed: 19022273]
30. Shekhtman E, Geerling JC, Loewy AD. Aldosterone-sensitive neurons of the nucleus of the solitary tract: Multisynaptic pathway to the nucleus accumbens. *J Comp Neurol.* 2007; 501:274–289. [PubMed: 17226797]
31. Scott JS, Bowker SS, Deschoolmeester J, Gerhardt S, Hargreaves D, Kilgour E, Lloyd A, Mayers RM, McCoull W, Newcombe NJ, Ogg D, Packer MJ, Rees A, Revill J, Schofield P, Selmi N, Swales JG, Whittamore PR. Discovery of a potent, selective, and orally bioavailable acidic 11 β -hydroxysteroid dehydrogenase type 1 (11 β -hsd1) inhibitor: Discovery of 2-[(3s)-1-[5-(cyclohexylcarbamoyl)-6-propylsulfanylpiperidin-2-yl]-3-piperidyl]acetic acid (azd4017). *J Med Chem.* 2012; 55:5951–5964. [PubMed: 22691057]
32. Harris AP, Holmes MC, de Kloet ER, Chapman KE, Seckl JR. Mineralocorticoid and glucocorticoid receptor balance in control of hpa axis and behaviour. *Psychoneuroendocrinology.* 2013; 38:648–658. [PubMed: 22980941]

33. Bujalska IJ, Walker EA, Hewison M, Stewart PM. A switch in dehydrogenase to reductase activity of 11 beta-hydroxysteroid dehydrogenase type 1 upon differentiation of human omental adipose stromal cells. *J Clin Endocrinol Metab.* 2002; 87:1205–1210. [PubMed: 11889189]
34. Chen J, Gomez-Sanchez CE, Penman A, May PJ, Gomez-Sanchez E. Expression of mineralocorticoid and glucocorticoid receptors in preautonomic neurons of the rat paraventricular nucleus. *Am J Physiol Regul Integr Comp Physiol.* 2014; 306:R328–340. [PubMed: 24381176]
35. de Kloet ER, Karst H, Joels M. Corticosteroid hormones in the central stress response: Quick-and-slow. *Front Neuroendocrinol.* 2008; 29:268–272. [PubMed: 18067954]
36. Conway-Campbell BL, Sarabdjitsingh RA, McKenna MA, Pooley JR, Kershaw YM, Meijer OC, De Kloet ER, Lightman SL. Glucocorticoid ultradian rhythmicity directs cyclical gene pulsing of the clock gene period 1 in rat hippocampus. *J Neuroendocrinol.* 2010; 22:1093–1100. [PubMed: 20649850]
37. Sarabdjitsingh RA, Joels M, de Kloet ER. Glucocorticoid pulsatility and rapid corticosteroid actions in the central stress response. *Physiology & behavior.* 2012; 106:73–80. [PubMed: 21971364]
38. Joels M, Sarabdjitsingh RA, Karst H. Unraveling the time domains of corticosteroid hormone influences on brain activity: Rapid, slow, and chronic modes. *Pharmacol Rev.* 2012; 64:901–938. [PubMed: 23023031]
39. Youm JK, Park K, Uchida Y, Chan A, Mauro TM, Holleran WM, Elias PM. Local blockade of glucocorticoid activation reverses stress- and glucocorticoid-induced delays in cutaneous wound healing. Wound repair and regeneration: official publication of the Wound Healing Society [and] the European Tissue Repair Society. 2013; 21:715–722.
40. Tiganescu A, Tahrani AA, Morgan SA, Otranto M, Desmouliere A, Abrahams L, Hassan-Smith Z, Walker EA, Rabbitt EH, Cooper MS, Amrein K, Lavery GG, Stewart PM. 11beta-hydroxysteroid dehydrogenase blockade prevents age-induced skin structure and function defects. *J Clin Invest.* 2013; 123:3051–3060. [PubMed: 23722901]
41. Fraccarollo D, Galuppo P, Schraut S, Kneitz S, van Rooijen N, Ertl G, Bauersachs J. Immediate mineralocorticoid receptor blockade improves myocardial infarct healing by modulation of the inflammatory response. *Hypertension.* 2008; 51:905–914. [PubMed: 18299485]
42. Ward MR, Kanellakis P, Ramsey D, Funder J, Bobik A. Eplerenone suppresses constrictive remodeling and collagen accumulation after angioplasty in porcine coronary arteries. *Circulation.* 2001; 104:467–472. [PubMed: 11468211]
43. Gomez-Sanchez, EP.; Gomez-Sanchez, CE. Primary aldosteronism. In: Re, RN.; DiPette, DJ.; Schiffrin, EL.; Sowers, JR., editors. *Molecular mechanisms in hypertension*. London and New York: Taylor & Francis; 2006. p. 113-121.
44. Ueda K, Nagase M. Mineralocorticoid receptor activation as an etiological factor in kidney diseases. *Clinical and experimental nephrology.* 2014; 18:16–23. [PubMed: 23832326]
45. Nagase M, Fujita T. Role of rac1-mineralocorticoid-receptor signalling in renal and cardiac disease. *Nature reviews. Nephrology.* 2013; 9:86–98. [PubMed: 23296296]
46. Mihailidou AS, Loan Le TY, Mardini M, Funder JW. Glucocorticoids activate cardiac mineralocorticoid receptors during experimental myocardial infarction. *Hypertension.* 2009; 54:1306–1312. [PubMed: 19841288]
47. Trapp T, Rupprecht R, Castren M, Reul JM, Holsboer F. Heterodimerization between mineralocorticoid and glucocorticoid receptor: A new principle of glucocorticoid action in the CNS. *Neuron.* 1994; 13:1457–1462. [PubMed: 7993637]
48. Farman N, Rafestin-Oblin ME. Multiple aspects of mineralocorticoid selectivity. *Am J Physiol Renal Physiol.* 2001; 280:F181–192. [PubMed: 11208593]
49. Ackermann D, Gresko N, Carrel M, Loffing-Cueni D, Habermehl D, Gomez-Sanchez C, Rossier BC, Loffing J. In vivo nuclear translocation of mineralocorticoid and glucocorticoid receptors in rat kidney: Differential effect of corticosteroids along the distal tubule. *Am J Physiol Renal Physiol.* 2010; 299:F1473–1485. [PubMed: 20861076]
50. Steiger M, Reichstein T. Desoxy-corticosterone (21-oxy-progesterone). *Aus d-3-oxo-etiocholensäure. Helv Chim Acta.* 1937; 20:1164.

51. Rodbard S, Freed SC. The effect of desoxycorticosterone acetate on the blood pressure of the dog. *Endocrinology*. 1942; 30:365–368.
52. Relman AJ, Schwartz WB. The effect of doca on electrolyte balance in normal man and its relation to sodium intake. *Yale J Biol Med*. 1952; 24:540–556. [PubMed: 14951401]
53. Raab W, Humphreys RJ, Lepeschkin E. Potentiation of pressor effects of nor-epinephrine and epinephrine in man by desoxycorticosterone acetate. *J Clin Invest*. 1950; 29:1397–1404. [PubMed: 14778903]
54. Vanatta JC, Cottle KE. Effect of desoxycorticosterone acetate on the peripheral vascular reactivity of dogs. *Am J Physiol*. 1955; 151:119–122. [PubMed: 14376581]
55. Hall CE, Hall O. Interaction between deoxycorticosterone treatment, fluid intake, sodium consumption, blood pressure, and organ changes in rats during drinking water, saline, or sucrose solution. *Journal of physiology and pharmacology: an official journal of the Polish Physiological Society*. 1969; 47:81–86.
56. Simpson SA, Tait JF, Wettstein A, Neher R, von Euw J, Schindler O, Reichstein T. Konstitution des aldosterons, des neuen mineralocorticoids. *Experientia*. 1953; 10:132–133. [PubMed: 13161890]
57. Conn JW. Primary aldosteronism, a new clinical syndrome. *The Journal of laboratory and clinical medicine*. 1955; 45:3–7. [PubMed: 13233623]
58. Hood WG Jr, Hill R Jr, Pittman JA Jr, Farmer TA Jr. Studies on the metabolic effects of spironolactone in man. *Ann N Y Acad Sci*. 1960; 88:864–880. [PubMed: 13715691]
59. Kolkhof P, Borden SA. Molecular pharmacology of the mineralocorticoid receptor: Prospects for novel therapeutics. *Mol Cell Endocrinol*. 2012; 350:310–317. [PubMed: 21771637]
60. Tanz RD. Studies on the inotropic action of aldosterone on isolated cardiac tissue preparations: Including the effect of ph, ouabain and sc-8109. *J Pharmacol Exp Ther*. 1962; 135:71–78. [PubMed: 13919589]
61. Jones AW, Hart RG. Altered ion transport in aortic smooth muscle during deoxycorticosterone acetate hypertension in rats. *Circ Res*. 1975; 37:333–341. [PubMed: 1157222]
62. Reid JL, Zivin JA, Kovin IF. Central and peripheral adrenergic mechanisms in the development of deoxycorticosterone-saline hypertension in rats. *Circ Res*. 1975; 37:569–579. [PubMed: 1192556]
63. Kubo T, Hashimoto M. Effects of intraventricular and intraspinal 6-hydroxydopamine and blood pressure of doca-saline hypertensive rats. *Arch Int Pharmacodyn*. 1979; 238:50–59. [PubMed: 485704]
64. Brody MJ, Haywood JR, Touw KB. Neural mechanisms in hypertension. *Ann Rev Physiol*. 1978; 42:441–453. [PubMed: 6996591]
65. Berecek KH, Murray RD, Gross F. Significance of sodium, sympathetic innervation and central adrenergic structures on renal vascular responsiveness in doca-treated rats. *Circ Res*. 1980; 47:675–683. [PubMed: 7418127]
66. Guyton AC. Dominant role of the kidneys and accessory role of whole-body autoregulation in the pathogenesis of hypertension. *Am J Hypertens*. 1989; 2:575–585. [PubMed: 2667575]
67. Cascella T, Palomba S, Tauchmanova L, Manguso F, Di Biase S, Labella D, Giallauria F, Vigorito C, Colao A, Lombardi G, Orio F. Serum aldosterone concentration and cardiovascular risk in women with polycystic ovarian syndrome. *J Clin Endocrinol Metab*. 2006; 91:4395–4400. [PubMed: 16940454]
68. Shibata H, Itoh H. Mineralocorticoid receptor-associated hypertension and its organ damage: Clinical relevance for resistant hypertension. *Am J Hypertens*. 2012
69. Kelestimur F. Diane 35 and spironolactone combination in the treatment of hirsutism. *Clin Endocrinol (Oxf)*. 2001; 54:699–700. [PubMed: 11380503]
70. Oelkers WK. Effects of estrogens and progestogens on the renin-aldosterone system and blood pressure. *Steroids*. 1996; 61:166–171. [PubMed: 8732994]
71. Boschitsch E, Mayerhofer S, Magometschnigg D. Hypertension in women: The role of progesterone and aldosterone. *Climacteric*. 2010; 13:307–313. [PubMed: 20443718]
72. Caprio M, Antelmi A, Chetrite G, Muscat A, Mammi C, Marzolla V, Fabbri A, Zennaro MC, Feve B. Antiadipogenic effects of the mineralocorticoid receptor antagonist drospirenone: Potential

implications for the treatment of metabolic syndrome. *Endocrinology*. 2011; 152:113–125. [PubMed: 21084448]

73. Nisenbaum MG, de Melo NR, Giribela CR, de Morais TL, Guerra GM, de Angelis K, Mostarda C, Baracat EC, Consolim-Colombo FM. Effects of a contraceptive containing drospirenone and ethinyl estradiol on blood pressure and autonomic tone: A prospective controlled clinical trial. *European journal of obstetrics, gynecology, and reproductive biology*. 2014; 175:62–66.
74. Stegeman BH, de Bastos M, Rosendaal FR, van Hylckama Vlieg A, Helmerhorst FM, Stijnen T, Dekkers OM. Different combined oral contraceptives and the risk of venous thrombosis: Systematic review and network meta-analysis. *Bmj*. 2013; 347:f5298. [PubMed: 24030561]
75. Luther JM. Is there a new dawn for selective mineralocorticoid receptor antagonism? *Curr Opin Nephrol Hypertens*. 2014; 23:456–461. [PubMed: 24992570]
76. Torrelli V, Hardy M, Nedelec L, Tournemine C, Deraedt R, Philibert D. 7-alkyl spironolactones as potent aldosterone antagonists. *J Steroid Biochem*. 1982; 17(Suppl III):17. [PubMed: 7109588]
77. Gomez-Sanchez EP, Fort CM, Gomez-Sanchez CE. Intracerebroventricular infusions of ru28318 blocks aldosterone-salt hypertension. *Am J Physiol*. 1990; 258:E482–E484. [PubMed: 2316642]
78. van den Berg DTWM, De Kloet ER, van Dijken HH, de Jong W. Differential central effects of mineralocorticoid and glucocorticoid agonists and antagonists on blood pressure. *Endocrinology*. 1990; 126:118–124. [PubMed: 2293978]
79. Gomez-Sanchez EP, Fort C, Thwaites D. Central mineralocorticoid receptor antagonism blocks hypertension in dahl s/jr rats. *Am J Physiol*. 1992; 262:E96–E99. [PubMed: 1310219]
80. de Gasparo M, Joss U, Ramjoue HP, Whitebread SE, Haenni H, Schenkel L, Kraehenbuehl C, Biollaz M, Grob J, Schmidlin J, et al. Three new epoxy-spirolactone derivatives: Characterization in vivo and in vitro. *J Pharmacol Exp Ther*. 1987; 240:650–656. [PubMed: 2949071]
81. de Gasparo M, Whitebread SE, Preiswerk G, Jeunemaitre X, Corvol P, Menard J. Antialdosterones: Incidence and prevention of sexual side effects. *J Steroid Biochem*. 1989; 32:223–227. [PubMed: 2913412]
82. Borghi C, Boschi S, Ambrosioni E, Melandri G, Branzi A, Magnani B. Evidence of a partial escape of renin-angiotensin-aldosterone blockade in patients with acute myocardial infarction treated with ace inhibitors. *Journal of Clinical Pharmacology*. 1993; 33:40–45. [PubMed: 8429112]
83. Pitt B. “Escape” of aldosterone production in patients with left ventricular dysfunction treated with an angiotensin converting enzyme inhibitor: Implications for therapy. *Cardiovasc Drugs Ther*. 1995; 9:145–149. [PubMed: 7786835]
84. Sato A, Saruta T. Aldosterone escape during angiotensin-converting enzyme inhibitor therapy in essential hypertensive patients with left ventricular hypertrophy. *The Journal of international medical research*. 2001; 29:13–21. [PubMed: 11277342]
85. Brilla CG, Matsubara LS, Weber KT. Anti-aldosterone treatment and the prevention of myocardial fibrosis in primary and secondary hyperaldosteronism. *J Mol Cell Card*. 1993; 25:563–575.
86. Brilla CG, Matsubara LS, Weber KT. Antifibrotic effects of spironolactone in preventing myocardial fibrosis in systemic arterial hypertension. *Am J Cardio*. 1993; 71:12A–16A.
87. Brilla CG, Zhou G, Matsubara L, Weber KT. Collagen metabolism in cultured adult rat cardiac fibroblasts: Response to angiotensin ii and aldosterone. *J Mol Cell Card*. 1994; 26:809–820.
88. Young MJ, Funder JW. Mineralocorticoids, salt, hypertension: Effects on the heart. *Steroids*. 1996; 61:233–235. [PubMed: 8733007]
89. Gomez-Sanchez EP, Zhou MY, Gomez-Sanchez CE. Mineralocorticoids, salt and high blood pressure: Causes. *Steroids*. 1996; 61:184–188. [PubMed: 8732997]
90. Rocha R, Chander PN, Khanna K, Zuckerman A, Stier CT Jr. Mineralocorticoid blockade reduces vascular injury in stroke-prone hypertensive rats. *Hypertension*. 1998; 31:451–458. [PubMed: 9453344]
91. Pitt B, Zannad F, Cody R, Castaigne APA, Palensky J, Wittes J. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized aldactone evaluation study investigators. *New Engl J Med*. 1999; 341:709–717. [PubMed: 10471456]

92. Danjuma MI, Mukherjee I, Makaronidis J, Osula S. Converging indications of aldosterone antagonists (spironolactone and eplerenone): A narrative review of safety profiles. *Curr Hypertens Rep.* 2014; 16:414. [PubMed: 24407447]
93. Pfeffer MA. New treasures from old? Ephesus. Eplerenone post-ahf heart failure efficacy and survival study. *Cardiovasc Drugs Ther.* 2001; 15:11–13. [PubMed: 11504157]
94. Mavrakanas TA, Gariani K, Martin PY. Mineralocorticoid receptor blockade in addition to angiotensin converting enzyme inhibitor or angiotensin ii receptor blocker treatment: An emerging paradigm in diabetic nephropathy: A systematic review. *European journal of internal medicine.* 2014; 25:173–176. [PubMed: 24315413]
95. Hargovan M, Ferro A. Aldosterone synthase inhibitors in hypertension: Current status and future possibilities. *JRSM cardiovascular disease.* 2014; 3:2048004014522440. [PubMed: 24570839]
96. Tamargo J, Solini A, Ruilope LM. Comparison of agents that affect aldosterone action. *Semin Nephrol.* 2014; 34:285–306. [PubMed: 25016400]
97. Mokler CM. Antiarrhythmic activity of various steroidal spiro lactones in dogs. *Proc Soc Exp Biol Med.* 1960; 105:257–259. [PubMed: 13771762]
98. Simopoulos V, Tagarakis G, Hatziefthimiou A, Skoularigis I, Triposkiadis F, Trantou V, Tsilimangas N, Aidonidis I. Effectiveness of aldosterone antagonists for preventing atrial fibrillation after cardiac surgery in patients with systolic heart failure: A retrospective study. *Clinical research in cardiology: official journal of the German Cardiac Society.* 2015; 104:31–37. [PubMed: 25134923]
99. Liu X, Yu H, Pei J, Chu J, Pu J, Zhang S. Clinical characteristics and long-term prognosis in patients with chronic heart failure and reduced ejection fraction in china. *Heart, lung & circulation.* 2014
100. Beygui F, Vicaute E, Ecollan P, Machecourt J, Van Belle E, Zannad F, Montalescot G. Rationale for an early aldosterone blockade in acute myocardial infarction and design of the albatross trial. *American heart journal.* 2010; 160:642–648. [PubMed: 20934557]
101. Le Menuet D, Munier M, Meduri G, Viengchareun S, Lombes M. Mineralocorticoid receptor overexpression in embryonic stem cell-derived cardiomyocytes increases their beating frequency. *Cardiovasc Res.* 2010; 87:467–475. [PubMed: 20299331]
102. Le Menuet D, Viengchareun S, Muffat-Joly M, Zennaro MC, Lombes M. Expression and function of the human mineralocorticoid receptor: Lessons from transgenic mouse models. *Mol Cell Endocrinol.* 2004; 217:127–136. [PubMed: 15134811]
103. Ramires FJ, Mansur A, Coelho O, Maranhao M, Gruppi CJ, Mady C, Ramires JA. Effect of spironolactone on ventricular arrhythmias in congestive heart failure secondary to idiopathic dilated or to ischemic cardiomyopathy. *American Journal of Cardiology.* 2000; 85:1207–1211. [PubMed: 10802002]
104. Xue B, Beltz TG, Yu Y, Guo F, Gomez-Sanchez CE, Hay M, Johnson AK. Central interactions of aldosterone and angiotensin ii in aldosterone- and angiotensin ii-induced hypertension. *Am J Physiol Heart Circ Physiol.* 2011; 300:H555–564. [PubMed: 21112947]
105. Schmiedek P, Sadee W, Baethmann A. Cerebral uptake of a 3 h-labelled spiro lactone compound in the dog. *Eur J Pharmacol.* 1973; 21:238–241. [PubMed: 4696105]
106. Kang YM, Zhang ZH, Johnson RF, Yu Y, Beltz T, Johnson AK, Weiss RM, Felder RB. Novel effect of mineralocorticoid receptor antagonism to reduce proinflammatory cytokines and hypothalamic activation in rats with ischemia-induced heart failure. *Circ Res.* 2006; 99:758–766. [PubMed: 16960100]
107. Menon DV, Arbique D, Wang Z, Adams-Huet B, Auchus RJ, Vongpatanasin W. Differential effects of chlorthalidone versus spironolactone on muscle sympathetic nerve activity in hypertensive patients. *J Clin Endocrinol Metab.* 2009; 94:1361–1366. [PubMed: 19158191]
108. Raheja P, Price A, Wang Z, Arbique D, Adams-Huet B, Auchus RJ, Vongpatanasin W. Spironolactone prevents chlorthalidone-induced sympathetic activation and insulin resistance in hypertensive patients. *Hypertension.* 2012
109. Pascual-Le Tallec L, Lombes M. The mineralocorticoid receptor: A journey exploring its diversity and specificity of action. *Mol Endocrinol.* 2005; 19:2211–2221. [PubMed: 15802372]

110. Tirard M, Almeida OF, Hutzler P, Melchior F, Michaelidis TM. Sumoylation and proteasomal activity determine the transactivation properties of the mineralocorticoid receptor. *Mol Cell Endocrinol.* 2007; 268:20–29. [PubMed: 17314004]
111. Yokota K, Shibata H, Kurihara I, Kobayashi S, Suda N, Murai-Takeda A, Saito I, Kitagawa H, Kato S, Saruta T, Itoh H. Coactivation of the n-terminal transactivation of mineralocorticoid receptor by ubc9. *J Biol Chem.* 2007; 282:1998–2010. [PubMed: 17105732]
112. Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. Influence of cytochrome p450 polymorphisms on drug therapies: Pharmacogenetic, pharmacoeypigenetic and clinical aspects. *Pharmacol Ther.* 2007; 116:496–526. [PubMed: 18001838]
113. Thomas M, Burk O, Klumpp B, Kandel BA, Damm G, Weiss TS, Klein K, Schwab M, Zanger UM. Direct transcriptional regulation of human hepatic cytochrome p450 3a4 (cyp3a4) by peroxisome proliferator-activated receptor alpha (pparalpha). *Mol Pharmacol.* 2013; 83:709–718. [PubMed: 23295386]
114. Weinberger MH, Roniker B, Krause SL, Weiss RJ. Eplerenone, a selective aldosterone blocker, in mild-to-moderate hypertension. *Am J Hypertens.* 2002; 15:709–716. [PubMed: 12160194]
115. Parthasarathy HK, Menard J, White WB, Young WF Jr, Williams GH, Williams B, Ruilope LM, McInnes GT, Connell JM, MacDonald TM. A double-blind, randomized study comparing the antihypertensive effect of eplerenone and spironolactone in patients with hypertension and evidence of primary aldosteronism. *J Hypertens.* 2011; 29:980–990. [PubMed: 21451421]
116. Miller RJ, Howlett JG. Evolving role for mineralocorticoid receptor antagonists in heart failure with preserved ejection fraction. *Curr Opin Cardiol.* 2015
117. Azizi M, Perdrix L, Bobrie G, Frank M, Chatellier G, Menard J, Plouin PF. Greater efficacy of aldosterone blockade and diuretic reinforcement vs. Dual renin-angiotensin blockade for left ventricular mass regression in patients with resistant hypertension. *J Hypertens.* 2014; 32:2038–2044. [PubMed: 24983187]
118. Funder JW. Mineralocorticoid-receptor blockade, hypertension and heart failure. *Nat Clin Pract Endocrinol Metab.* 2005; 1:4–5. [PubMed: 16929357]
119. Funder JW. Rales, ephesus and redox. *J Steroid Biochem Mol Biol.* 2005; 93:121–125. [PubMed: 15860254]
120. Collin M, Niemann F, Jaisser F. Mineralocorticoid receptor modulators: A patent review (2007 – 2012). *Expert Opin Ther Pat.* 2014; 24:177–183. [PubMed: 24215301]
121. Vukusich A, Kunstmann S, Varela C, Gainza D, Bravo S, Sepulveda D, Cavada G, Michea L, Marusic ET. A randomized, double-blind, placebo-controlled trial of spironolactone on carotid intima-media thickness in nondiabetic hemodialysis patients. *Clin J Am Soc Nephrol.* 2010; 5:1380–1387. [PubMed: 20522535]
122. Epstein M. Mineralocorticoid receptor antagonists: Part of an emerging treatment paradigm for chronic kidney disease. *The lancet. Diabetes & endocrinology.* 2014; 2:925–927. [PubMed: 25466241]
123. Perez-Gomez MV, Sanchez-Nino MD, Sanz AB, Martin-Cleary C, Ruiz-Ortega M, Egido J, Navarro-Gonzalez JF, Ortiz A, Fernandez-Fernandez B. Horizon 2020 in diabetic kidney disease: The clinical trial pipeline for add-on therapies on top of renin angiotensin system blockade. *Journal of clinical medicine.* 2015; 4:1325–1347. [PubMed: 26239562]
124. Bousquet E, Beydoun T, Rothschild PR, Bergin C, Zhao M, Batista R, Brandely ML, Couraud B, Farman N, Gaudric A, Chast F, Behar-Cohen F. Spironolactone for nonresolving central serous chorioretinopathy: A randomized controlled crossover study. *Retina.* 2015
125. Zhao M, Celerier I, Bousquet E, Jeanny JC, Jonet L, Savoldelli M, Offret O, Curan A, Farman N, Jaisser F, Behar-Cohen F. Mineralocorticoid receptor is involved in rat and human ocular chorioretinopathy. *J Clin Invest.* 2012
126. Funder JW. Reconsidering the roles of the mineralocorticoid receptor. *Hypertension.* 2009; 53:286–290. [PubMed: 19139379]
127. Gomez-Sanchez CE. Primary aldosteronism: A channelopathy? *Hypertension.* 2014; 63:668–669. [PubMed: 24420546]

128. Schiebinger RJ, Braley LM, Menachery A, Williams GH. Unique calcium dependencies of the activating mechanism of the early and late aldosterone biosynthetic pathways in the rat. *J Endocrinol.* 1986; 110:315–325. [PubMed: 2427632]
129. Barrett PQ, Ertel EA, Smith MM, Nee JJ, Cohen CJ. Voltage-gated calcium currents have two opposing effects on the secretion of aldosterone. *Am J Physiol.* 1995; 268:C985–992. [PubMed: 7733247]
130. Rossier MF, Burnay MM, Maturana A, Capponi AM. Duality of the voltage-dependent calcium influx in adrenal glomerulosa cells. *Endocr Res.* 1998; 24:443–447. [PubMed: 9888523]
131. Dietz JD, Du S, Bolten CW, Payne MA, Xia C, Blinn JR, Funder JW, Hu X. A number of marketed dihydropyridine calcium channel blockers have mineralocorticoid receptor antagonist activity. *Hypertension.* 2008; 51:742–748. [PubMed: 18250364]
132. Arhancet GB, Woodard SS, Dietz JD, Garland DJ, Wagner GM, Iyanar K, Collins JT, Blinn JR, Numann RE, Hu X, Huang HC. Stereochemical requirements for the mineralocorticoid receptor antagonist activity of dihydropyridines. *J Med Chem.* 2010; 53:4300–4304. [PubMed: 20408553]
133. Kosaka H, Hirayama K, Yoda N, Sasaki K, Kitayama T, Kusaka H, Matsubara M. The l-, n-, and t-type triple calcium channel blocker benidipine acts as an antagonist of mineralocorticoid receptor, a member of nuclear receptor family. *Eur J Pharmacol.* 2010; 635:49–55. [PubMed: 20307534]
134. Fagart J, Hillisch A, Huyet J, Barfacker L, Fay M, Pleiss U, Pook E, Schafer S, Rafestin-Oblin ME, Kolkhof P. A new mode of mineralocorticoid receptor antagonism by a potent and selective nonsteroidal molecule. *J Biol Chem.* 2010; 285:29932–29940. [PubMed: 20650892]
135. Geller DS, Farhi A, Pinkerton N, Fradley M, Moritz M, Spitzer A, Meinke G, Tsai FT, Sigler PB, Lifton RP. Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy. *Science.* 2000; 289:119–123. [PubMed: 10884226]
136. Arhancet GB, Woodard SS, Iyanar K, Case BL, Woerndle R, Dietz JD, Garland DJ, Collins JT, Payne MA, Blinn JR, Pomposiello SI, Hu X, Heron MI, Huang HC, Lee LF. Discovery of novel cyanodihydropyridines as potent mineralocorticoid receptor antagonists. *J Med Chem.* 2010; 53:5970–5978. [PubMed: 20672820]
137. Lavall D, Selzer C, Schuster P, Lenski M, Adam O, Schafers HJ, Bohm M, Laufs U. The mineralocorticoid receptor promotes fibrotic remodeling in atrial fibrillation. *J Biol Chem.* 2014; 289:6656–6668. [PubMed: 24469458]
138. Barfacker L, Kuhl A, Hillisch A, Grosser R, Figueroa-Perez S, Heckroth H, Nitsche A, Erguden JK, Gielen-Haertwig H, Schlemmer KH, Mittendorf J, Paulsen H, Platzeck J, Kolkhof P. Discovery of bay 94-8862: A nonsteroidal antagonist of the mineralocorticoid receptor for the treatment of cardiorenal diseases. *ChemMedChem.* 2012; 7:1385–1403. [PubMed: 22791416]
139. Kolkhof P, Delbeck M, Kretschmer A, Steinke W, Hartmann E, Barfacker L, Eitner F, Albrecht-Kupper B, Schafer S. Finerenone, a novel selective nonsteroidal mineralocorticoid receptor antagonist protects from rat cardiorenal injury. *J Cardiovasc Pharmacol.* 2014; 64:69–78. [PubMed: 24621652]
140. Kolkhof P, Nowack C, Eitner F. Nonsteroidal antagonists of the mineralocorticoid receptor. *Curr Opin Nephrol Hypertens.* 2015
141. Pitt B, Anker SD, Bohm M, Gheorghiadu M, Kober L, Krum H, Maggioni AP, Ponikowski P, Voors AA, Zannad F, Nowack C, Kim SY, Pieper A, Kimmeskamp-Kirschbaum N, Filippatos G. Rationale and design of mineralocorticoid receptor antagonist tolerability study-heart failure (arts-hf): A randomized study of finerenone vs. Eplerenone in patients who have worsening chronic heart failure with diabetes and/or chronic kidney disease. *Eur J Heart Fail.* 2015; 17:224–232. [PubMed: 25678098]
142. Ruilope LM, Agarwal R, Chan JC, Cooper ME, Gansevoort RT, Haller H, Remuzzi G, Rossing P, Schmieder RE, Nowack C, Ferreira AC, Pieper A, Kimmeskamp-Kirschbaum N, Bakris GL. Rationale, design, and baseline characteristics of arts-dn: A randomized study to assess the safety and efficacy of finerenone in patients with type 2 diabetes mellitus and a clinical diagnosis of diabetic nephropathy. *American journal of nephrology.* 2014; 40:572–581. [PubMed: 25591469]
143. Pitt B, Kober L, Ponikowski P, Gheorghiadu M, Filippatos G, Krum H, Nowack C, Kolkhof P, Kim SY, Zannad F. Safety and tolerability of the novel non-steroidal mineralocorticoid receptor antagonist bay 94-8862 in patients with chronic heart failure and mild or moderate chronic

kidney disease: A randomized, double-blind trial. *Eur Heart J*. 2013; 34:2453–2463. [PubMed: 23713082]

144. Pitt B, Filippatos G, Gheorghiade M, Kober L, Krum H, Ponikowski P, Nowack C, Kolkhof P, Kim SY, Zannad F. Rationale and design of arts: A randomized, double-blind study of bay 94-8862 in patients with chronic heart failure and mild or moderate chronic kidney disease. *Eur J Heart Fail*. 2012; 14:668–675. [PubMed: 22562554]
145. Kontak AC, Wang Z, Arbique D, Adams-Huet B, Auchus RJ, Nesbitt SD, Victor RG, Vongpatanasin W. Reversible sympathetic overactivity in hypertensive patients with primary aldosteronism. *J Clin Endocrinol Metab*. 2010; 95:4756–4761. [PubMed: 20660053]
146. Bakris GL, Agarwal R, Chan JC, Cooper ME, Gansevoort RT, Haller H, Remuzzi G, Rossing P, Schmieder RE, Nowack C, Kolkhof P, Joseph A, Pieper A, Kimmeskamp-Kirschbaum N, Ruilope LM. Mineralocorticoid Receptor Antagonist Tolerability Study-Diabetic Nephropathy Study G. Effect of finerenone on albuminuria in patients with diabetic nephropathy: A randomized clinical trial. *JAMA*. 2015; 314:884–894. [PubMed: 26325557]
147. Meyers MJ, Arhancet GB, Hockerman SL, Chen X, Long SA, Mahoney MW, Rico JR, Garland DJ, Blinn JR, Collins JT, Yang S, Huang HC, McGee KF, Wendling JM, Dietz JD, Payne MA, Homer BL, Heron MI, Reitz DB, Hu X. Discovery of (3s,3ar)-2-(3-chloro-4-cyanophenyl)-3-cyclopentyl-3,3a,4,5-tetrahydro-2h-benzo[g] indazole-7-carboxylic acid (pf-3882845), an orally efficacious mineralocorticoid receptor (mr) antagonist for hypertension and nephropathy. *J Med Chem*. 2010; 53:5979–6002. [PubMed: 20672822]
148. Orena S, Maurer TS, She L, Eudy R, Bernardo V, Dash D, Loria P, Banker ME, Tugnait M, Okerberg CV, Qian J, Boustany-Kari CM. Pf-03882845, a non-steroidal mineralocorticoid receptor antagonist, prevents renal injury with reduced risk of hyperkalemia in an animal model of nephropathy. *Frontiers in pharmacology*. 2013; 4:115. [PubMed: 24133446]
149. Casimiro-Garcia A, Piotrowski DW, Ambler C, Arhancet GB, Banker ME, Banks T, Boustany-Kari CM, Cai C, Chen X, Eudy R, Hepworth D, Hulford CA, Jennings SM, Loria PM, Meyers MJ, Petersen DN, Raheja NK, Sammons M, She L, Song K, Vrieze D, Wei L. Identification of (r)-6-(1-(4-cyano-3-methylphenyl)-5-cyclopentyl-4,5-dihydro-1h-pyrazol-3-yl)-2-methoxynicotinic acid, a highly potent and selective nonsteroidal mineralocorticoid receptor antagonist. *J Med Chem*. 2014; 57:4273–4288. [PubMed: 24738581]
150. Futatsugi K, Piotrowski DW, Casimiro-Garcia A, Robinson S, Sammons M, Loria PM, Banker ME, Petersen DN, Schmidt NJ. Design and synthesis of aryl sulfonamide-based nonsteroidal mineralocorticoid receptor antagonists. *Bioorganic & medicinal chemistry letters*. 2013; 23:6239–6242. [PubMed: 24157365]
151. Nariai T, Fujita K, Mori M, Katayama S, Hori S, Matsui K. Sm-368229, a novel selective and potent non-steroidal mineralocorticoid receptor antagonist with strong urinary na⁺ excretion activity. *Journal of pharmacological sciences*. 2011; 115:346–353. [PubMed: 21358118]
152. Nariai T, Fujita K, Mori M, Katayama S, Hori S, Matsui K. Antihypertensive and cardiorenal protective effects of sm-368229, a novel mineralocorticoid receptor antagonist, in aldosterone/salt-treated rats. *Pharmacology*. 2012; 89:44–52. [PubMed: 22302095]
153. Nariai T, Fujita K, Mori M, Katayama S, Hori S, Matsui K. Sm-368229, a novel promising mineralocorticoid receptor antagonist, shows antihypertensive efficacy with minimal effect on serum potassium level in rats. *J Cardiovasc Pharmacol*. 2012; 59:458–464. [PubMed: 22240917]
154. Nariai T, Fujita K, Kawane K, Mori M, Nakayama R, Matsuda K, Katayama S, Fukuda N, Hori S, Iwata M, Hasegawa F, Suzuki K, Kato H. Dsr-71167, a novel mineralocorticoid receptor antagonist with carbonic anhydrase inhibitory activity, separates urinary sodium excretion and serum potassium elevation in rats. *J Pharmacol Exp Ther*. 2015; 354:2–9. [PubMed: 25922341]
155. Fernandez-Fernandez B, Ortiz A, Gomez-Guerrero C, Egidio J. Therapeutic approaches to diabetic nephropathy--beyond the ras. *Nature reviews. Nephrology*. 2014; 10:325–346. [PubMed: 24802062]
156. Cox JM, Chu HD, Yang C, Shen HC, Wu Z, Balsells J, Crespo A, Brown P, Zamlynny B, Wiltsie J, Clemas J, Gibson J, Contino L, Lisnock J, Zhou G, Garcia-Calvo M, Bateman T, Xu L, Tong X, Crook M, Sinclair P. Mineralocorticoid receptor antagonists: Identification of heterocyclic amide replacements in the oxazolidinedione series. *Bioorganic & medicinal chemistry letters*. 2014; 24:1681–1684. [PubMed: 24630411]

157. Yang C, Balsells J, Chu HD, Cox JM, Crespo A, Ma X, Contino L, Brown P, Gao S, Zamylnny B, Wiltsie J, Clemas J, Lisnock J, Gibson J, Zhou G, Garcia-Calvo M, Bateman TJ, Tong V, Xu L, Crook M, Sinclair P, Shen HC. Discovery of benzimidazole oxazolidinediones as novel and selective nonsteroidal mineralocorticoid receptor antagonists. *ACS medicinal chemistry letters*. 2015; 6:461–465. [PubMed: 25941555]
158. Yang C, Shen HC, Wu Z, Chu HD, Cox JM, Balsells J, Crespo A, Brown P, Zamylnny B, Wiltsie J, Clemas J, Gibson J, Contino L, Lisnock J, Zhou G, Garcia-Calvo M, Bateman T, Xu L, Tong X, Crook M, Sinclair P. Discovery of novel oxazolidinedione derivatives as potent and selective mineralocorticoid receptor antagonists. *Bioorganic & medicinal chemistry letters*. 2013; 23:4388–4392. [PubMed: 23777778]
159. Karst H, Berger S, Turiault M, Tronche F, Schutz G, Joels M. Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone. *Proc Natl Acad Sci U S A*. 2005; 102:19204–19207. [PubMed: 16361444]
160. Hamstra DA, de Kloet ER, van Hemert AM, de Rijk RH, Van der Does AJ. Mineralocorticoid receptor haplotype, oral contraceptives and emotional information processing. *Neuroscience*. 2015; 286:412–422. [PubMed: 25497375]
161. Kruk MR, Haller J, Meelis W, de Kloet ER. Mineralocorticoid receptor blockade during a rat's first violent encounter inhibits its subsequent propensity for violence. *Behav Neurosci*. 2013; 127:505–514. [PubMed: 23895062]
162. Gass P, Kretz O, Wolfer DP, Berger S, Tronche F, Reichardt HM, Kellendonk C, Lipp HP, Schmid W, Schutz G. Genetic disruption of mineralocorticoid receptor leads to impaired neurogenesis and granule cell degeneration in the hippocampus of adult mice. *EMBO Rep*. 2000; 1:447–451. [PubMed: 11258486]
163. Le Menuet D, Lombes M. The neuronal mineralocorticoid receptor: From cell survival to neurogenesis. *Steroids*. 2014
164. Qi XR, Kamphuis W, Wang S, Wang Q, Lucassen PJ, Zhou JN, Swaab DF. Aberrant stress hormone receptor balance in the human prefrontal cortex and hypothalamic paraventricular nucleus of depressed patients. *Psychoneuroendocrinology*. 2012; 38:863–870. [PubMed: 23137715]
165. Xing GQ, Russell S, Webster MJ, Post RM. Decreased expression of mineralocorticoid receptor mRNA in the prefrontal cortex in schizophrenia and bipolar disorder. *Int J Neuropsychopharmacol*. 2004; 7:143–153. [PubMed: 14741058]
166. De Kloet ER. Hormones and the stressed brain. *Ann N Y Acad Sci*. 2004; 1018:1–15. [PubMed: 15240347]
167. Cornelisse S, Joels M, Smeets T. A randomized trial on mineralocorticoid receptor blockade in men: Effects on stress responses, selective attention, and memory. *Neuropsychopharmacology*. 2011; 36:2720–2728. [PubMed: 21881569]
168. Otte C, Moritz S, Yassouridis A, Koop M, Madrischewski AM, Wiedemann K, Kellner M. Blockade of the mineralocorticoid receptor in healthy men: Effects on experimentally induced panic symptoms, stress hormones, and cognition. *Neuropsychopharmacology*. 2007; 32:232–238. [PubMed: 17035932]
169. Yau JL, Noble J, Seckl JR. Continuous blockade of brain mineralocorticoid receptors impairs spatial learning in rats. *Neurosci Lett*. 1999; 277:45–48. [PubMed: 10643894]
170. Wintermantel TM, Berger S, Greiner EF, Schutz G. Evaluation of steroid receptor function by gene targeting in mice. *J Steroid Biochem Mol Biol*. 2005; 93:107–112. [PubMed: 15860252]
171. Berger S, Bleich M, Schmid W, Greger R, Schutz G. Mineralocorticoid receptor knockout mice: Lessons on Na⁺ metabolism. *Kidney Int*. 2000; 57:1295–1298. [PubMed: 10760057]
172. Stranahan AM, Arumugam TV, Lee K, Mattson MP. Mineralocorticoid receptor activation restores medial perforant path LTP in diabetic rats. *Synapse*. 2010; 64:528–532. [PubMed: 20196138]
173. Otte C, Hinkelmann K, Moritz S, Yassouridis A, Jahn H, Wiedemann K, Kellner M. Modulation of the mineralocorticoid receptor as add-on treatment in depression: A randomized, double-blind, placebo-controlled proof-of-concept study. *J Psychiatr Res*. 2010; 44:339–346. [PubMed: 19909979]

174. Kunzel HE, Apostolopoulou K, Pallauf A, Gerum S, Merkle K, Schulz S, Fischer E, Brand V, Bidlingmaier M, Endres S, Beuschlein F, Reincke M. Quality of life in patients with primary aldosteronism: Gender differences in untreated and long-term treated patients and associations with treatment and aldosterone. *J Psychiatr Res.* 2012; 46:1650–1654. [PubMed: 23017810]
175. Kunzel HE. Psychopathological symptoms in patients with primary hyperaldosteronism--possible pathways. *Horm Metab Res.* 2012; 44:202–207. [PubMed: 22351473]
176. Popa-Wagner A, Buga AM, Popescu B, Muresanu D. Vascular cognitive impairment, dementia, aging and energy demand. A vicious cycle. *Journal of neural transmission.* 2013
177. Pabbidi MR, Mazur O, Fan F, Farley JM, Gebremedhin D, Harder DR, Roman RJ. Enhanced large conductance K^+ channel activity contributes to the impaired myogenic response in the cerebral vasculature of fawn hooded hypertensive rats. *Am J Physiol Heart Circ Physiol.* 2014; 306:H989–H1000. [PubMed: 24464756]
178. Fan F, Geurts AM, Murphy SR, Pabbidi MR, Jacob HJ, Roman RJ. Impaired myogenic response and autoregulation of cerebral blood flow is rescued in cyp4a1 transgenic dahl salt-sensitive rat. *Am J Physiol Regul Integr Comp Physiol.* 2014 ajpregu 00256 02014.
179. Le Billan F, Khan JA, Lamribet K, Viengchareun S, Bouligand J, Fagart J, Lombes M. Cistrome of the aldosterone-activated mineralocorticoid receptor in human renal cells. *FASEB J.* 2015
180. Viengchareun S, Le Menuet D, Martinerie L, Munier M, Pascual-Le Tallec L, Lombes M. The mineralocorticoid receptor: Insights into its molecular and (patho)physiological biology. *Nucl Recept Signal.* 2007; 5:e012. [PubMed: 18174920]
181. Kitada K, Nakano D, Liu Y, Fujisawa Y, Hitomi H, Shibayama Y, Shibata H, Nagai Y, Mori H, Masaki T, Kobori H, Nishiyama A. Oxidative stress-induced glomerular mineralocorticoid receptor activation limits the benefit of salt reduction in dahl salt-sensitive rats. *PLoS ONE.* 2012; 7:e41896. [PubMed: 22911865]
182. Bienvenu LA, Morgan J, Rickard AJ, Tesch GH, Cranston GA, Fletcher EK, Delbridge LM, Young MJ. Macrophage mineralocorticoid receptor signaling plays a key role in aldosterone-independent cardiac fibrosis. *Endocrinology.* 2012; 153:3416–3425. [PubMed: 22653557]
183. Zhu X, Manning RD Jr, Lu D, Gomez-Sanchez CE, Fu Y, Juncos LA, Liu R. Aldosterone stimulates superoxide production in macula densa cells. *Am J Physiol Renal Physiol.* 2011; 301:F529–535. [PubMed: 21270097]
184. Queisser N, Oteiza PI, Link S, Hey V, Stopper H, Schupp N. Aldosterone activates transcription factor nrf2 in kidney cells both in vitro and in vivo. *Antioxidants & redox signaling.* 2014
185. Ou XM, Storrer JM, Kushwaha N, Albert PR. Heterodimerization of mineralocorticoid and glucocorticoid receptors at a novel negative response element of the 5-ht1a receptor gene. *J Biol Chem.* 2001; 276:14299–14307. [PubMed: 11278286]
186. Savory JG, Prefontaine GG, Lamprecht C, Liao M, Walther RF, Lefebvre YA, Hache RJ. Glucocorticoid receptor homodimers and glucocorticoid-mineralocorticoid receptor heterodimers form in the cytoplasm through alternative dimerization interfaces. *Mol Cell Biol.* 2001; 21:781–793. [PubMed: 11154266]
187. Yin L, Hu Q, Emmerich J, Lo MM, Metzger E, Ali A, Hartmann RW. Novel pyridyl- or isoquinolinyl-substituted indolines and indoles as potent and selective aldosterone synthase inhibitors. *J Med Chem.* 2014; 57:5179–5189. [PubMed: 24899257]
188. Namsolleck P, Unger T. Aldosterone synthase inhibitors in cardiovascular and renal diseases. *Nephrol Dial Transplant.* 2014; 29(Suppl 1):i62–i68. [PubMed: 24493871]
189. Hu Q, Yin L, Hartmann RW. Aldosterone synthase inhibitors as promising treatments for mineralocorticoid dependent cardiovascular and renal diseases. *J Med Chem.* 2014; 57:5011–5022. [PubMed: 24422519]
190. Gros R, Ding Q, Sklar LA, Prossnitz EE, Arterburn JB, Chorazyczewski J, Feldman RD. Gpr30 expression is required for the mineralocorticoid receptor-independent rapid vascular effects of aldosterone. *Hypertension.* 2011; 57:442–451. [PubMed: 21242460]
191. Gros R, Ding Q, Davis M, Shaikh R, Liu B, Chorazyczewski J, Pickering JG, Feldman RD. Delineating the receptor mechanisms underlying the rapid vascular contractile effects of aldosterone and estradiol. *Can J Physiol Pharmacol.* 2011; 89:655–663. [PubMed: 21854125]

192. Barton M. Position paper: The membrane estrogen receptor gper--clues and questions. *Steroids*. 2012; 77:935–942. [PubMed: 22521564]
193. Meinel S, Gekle M, Grossmann C. Mineralocorticoid receptor signaling: Crosstalk with membrane receptors and other modulators. *Steroids*. 2014
194. Wendler A, Albrecht C, Wehling M. Nongenomic actions of aldosterone and progesterone revisited. *Steroids*. 2012; 77:1002–1006. [PubMed: 22285849]
195. Feldman RD, Gros R. Unraveling the mechanisms underlying the rapid vascular effects of steroids: Sorting out the receptors and the pathways. *Br J Pharmacol*. 2011; 163:1163–1169. [PubMed: 21545416]
196. Zhao Z, Wang H, Jessup JA, Lindsey SH, Chappell MC, Groban L. Role of estrogen in diastolic dysfunction. *Am J Physiol Heart Circ Physiol*. 2014; 306:H628–640. [PubMed: 24414072]
197. Ren Y, D'Ambrosio MA, Garvin JL, Leung P, Kutskill K, Wang H, Peterson EL, Carretero OA. Aldosterone sensitizes connecting tubule glomerular feedback via the aldosterone receptor gpr30. *Am J Physiol Renal Physiol*. 2014; 307:F427–434. [PubMed: 24966088]

Clinical Studies for New Mineralocorticoid Receptor Antagonists: 2011–2015

	trials	subjects	compared	outcomes	Notes
Phase 1					
PF-03882845 (pyrazoline~) Pfizer	4 different safety, PK/PD trials	100 H +/-simvastatin	placebo	4 Short studies PK/PD potassium	5 completed
PF-03882845		12 DM	placebo	Serum K	safety concern terminated
PF-03882845	NCT01488877	6 DM+DN	placebo spironolactone	PK/PD Serum K albuminuria	>1 severe hyperK Terminated
LY2623091 Eli Lilly (structure not published)	NCT02300259	48 H		PK/PD (CYP3A –drug interactions)	nsrp
LY2623091	NCT02242981	6 H m	14C labeled-LY2623091	PK/PD	nsrp
LY2623091	NCT01237899	32 H, m&f	placebo	Serum K	nsrp
Phase 2					
BAY 94-8862 Finerenone (dihydropyridine)	NCT01345656	CHF+CKD 2a) 60 2b)360	a. placebo b. spironolactone	PK/PD Serum K; CV & renal biomarkers	nsrp
	NCT01955694	CHF+ LVSD +/-DM2 +/- CKD 72	placebo or * eplerenone 25mg q48h *	N-t BNP Serum K	nsrp
	NCT01807221	worsening HF+CKD 1058	a. placebo or spironolactone b. placebo or eplerenone	CV & renal biomarkers serum K	nsrp
	NCT01874431	DM2+DN 823	(+ RAS inhibitor) placebo	Albuminuria, serum K	nsrp
	NCT01968668	P 2a; DM2+DN (Japan) 96	placebo	Albuminuria, serum K	nsrp
LY2623091 Eli Lilly	NCT02194465	306 hypertension	Placebo, spironolactone +/- tadafafi	Change in BP	nsrp
LY2623091	NCT01427972	48; CKD; m&f	placebo or eplerenone	Proteinuria; PK/PD Serum K	nsrp
MT-3995	NCT01889277	DM2+DN	placebo	Albuminuria, serum K	nsrp
Phase 3					
BAY 94-8862 finerenone	NCT02540993	P3: DM2+DN 4800 planned	placebo	Albuminuria, serum K	Enrollment not started

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* dose of eplerenone low even for CKD

ClinicalTrials.gov + references; H-healthy volunteers; DM diabetes mellitus; CHF cardiac failure; LVSD-left ventricular systolic dysfunction; DN-diabetic nephropathy; CKD-chronic kidney failure; RAS inhibitor- angiotensin converting enzyme or receptor inhibitor; nsrp: no study results posted