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# Central regulation of blood pressure by the Mineralocorticoid Receptor

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## Abstract

Addition of mineralocorticoid receptor (MR) antagonists to standard therapy for heart failure, kidney disease, metabolic syndrome, and diabetes is increasing steadily in response to clinical trials demonstrating clear benefits. In addition to blocking deleterious activity of MR within the heart, vessels and kidneys, MR antagonists target MR in hemodynamic regulatory centers in the brain, thereby decreasing excessive sympathetic nervous system drive, vasopressin release, abnormal baroreceptor function, and circulating and tissue pro-inflammatory cytokines. However, brain MR are also involved with cognition, memory, affect and functions yet to be determined. Understanding specific central mechanisms involved in blood pressure regulation by MR is necessary for the development of agents to target downstream events specific to central hemodynamic regulation, not only to avoid the hypokalemia caused by inhibition of renal tubular MR, but also to avoid untoward long term effects of inhibiting brain MR that are not involved in blood pressure control.

### Keywords

mineralocorticoid receptor; brain; hypertension; aldosterone; cortisol; corticosterone; inflammation; cardiovascular disease

## 1.0 The mineralocorticoid receptor

Mineralocorticoid receptor (MR) structure and biology is described in detail elsewhere in this issue of *Molecular and Cellular Endocrinology*, however a brief description of cellular activities is necessary for a review of its crucial role in the central control of hemodynamic and electrolyte homeostasis. The MR, like other members of the steroid nuclear receptor family, is a transcription factor which modulates gene transcription for the proteins that mediate its activities. It also produces more rapid non-genomic effects through second messenger systems [1,2]. Aldosterone also activates the G-protein receptor GPR30, leading to phosphatidylinositol 3-kinase-dependent ERK activation independently of the MR [3].

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While non-genomic effects are initiated more rapidly than genomic events which require protein synthesis, they may lead secondarily to alterations in gene transcription and have prolonged effects. Non-genomic and genomic actions mediated by MR probably occur concurrently in most cells [2,4–6] and are not differentiated in this review. Steroid-independent MR transactivation by other signal transduction pathways, in particular that of angiotensin II has been reported [7]. The potential activation of MR by an angiotensin II signaling pathway is germane to central hemodynamic control, as angiotensin II is also an important mediator of blood pressure, fluid and electrolyte homeostasis in the brain.

### 1.1 MR and Glucocorticoid receptor and ligand interactions

The structural similarities shared by the MR and glucocorticoid receptor (GR) complicate the elucidation of MR function. The MR has a similar affinity for aldosterone and the primary glucocorticoids, cortisol and corticosterone. The conformation of the ligand-bound receptor, thus efficiency with which cell-specific co-regulator proteins are recruited, appears to be influenced by the identity of the ligand. The MR:aldosterone complex appears to be more stable and have higher transcription efficiency than MR:cortisol [8–10]. GR are expressed in most, if not all cells, including those of the brain cells expressing MR. In addition to sharing a ligand, the MR and GR can form heterodimers, as well as homodimers [11–13] before binding to the same hormone response elements (HRE) [11,13–16]. Cell-specific expression of different co-activator proteins with different affinities for these complexes plays a role in determining transcriptional activity [15–18]. For example, the distinct anatomical distribution in the brain of different splice variants of Steroid Receptor Co-activator 1 (SRC-1) may be responsible for the opposite effect of stress levels of corticosterone on the release of corticotrophin releasing hormone from neurons of the paraventricular neuron (PVN) and central amygdala [17]. Analysis of co-regulator specificity is being studied to guide the development of MR:ligand-selective peptide antagonists to target MR in specific cells under specific contexts, for example, in the injured or poorly perfused cardiomyocyte [10].

### 1.2 MR polymorphisms

Polymorphisms of the MR gene and splice variants may confer tissue-specific intrinsic differences in ligand preference or specificity of gene transcription [19,20]. The very rare S810L substitution mutation of the MR, causes it to be constitutively active, producing pseudohyperaldosteronism. These patients suffer severe hypertension during pregnancy because progesterone, rather than being an antagonist, is a full agonist of MR S810L, [21]. Another recently described polymorphism of the human MR is the substitution of a cytosine for guanine in the MR Kozac consensus that causes less efficient translation of the MR gene [22]. Individuals with this c.-2G>C mutation had higher renin and aldosterone levels and a significant risk for mild hypertension [22].

### 1.3 11β-hydroxysteroid dehydrogenase type 2 and MR ligand specificity

Cortisol (humans and many other species) and corticosterone (rats, mice, several others), circulate at 100 (free) to 1000 (total)-fold the concentration of aldosterone. The affinity of the GR for cortisol and corticosterone is approximately one tenth that of the MR for these glucocorticoids; GR binding affinity for aldosterone is about one tenth that of cortisol and corticosterone. Accordingly, MR in the hippocampus are at least partially occupied by glucocorticoids under all physiological steroid concentrations, while GR in the brain are thought to be occupied by cortisol or corticosterone only at the zenith of the circadian cycle and during stress [23,24].

MR specificity for aldosterone in mineralocorticoid target cells of ion transport epithelia is generally attributed to the conversion of cortisol and corticosterone to the inactive

metabolites cortisone and 11-dehydrocorticosterone by the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) in the immediate vicinity of the MR, allowing aldosterone to bind the MR (reviewed in Gomez-Sanchez, [25]). Inactivating mutations of the 11β-HSD2 gene and pharmacological inhibition of the enzyme allow cortisol or corticosterone to activate aldosterone target MR, producing the syndrome of apparent mineralocorticoid excess while suppressing circulating aldosterone.

### 1.4 11β-HSD2 in the brain

11β-HSD2 is expressed throughout the brain in the rat fetus and is thought to protect the developing neurons from high glucocorticoid concentrations [26]. 11β-HSD2 is decreased dramatically in the neonate [26] and only limited 11β-HSD2 mRNA has been demonstrated by *in situ* hybridization and quantitative RT-PCR in several areas of the adult brain, including those in the hypothalamus and brain stem involved in hemodynamic regulation discussed below, [27–32]. Message for 11β-HSD2 was greatest in the hypothalamus, but clearly expressed in the brain stem, cerebellum, hippocampus and cortex as well [33]. Table 1 summarizes the expression of co-expression of 11β-HSD2 and MR. Note that most of the information for 11β-HSD2 expression in the adult rat brain is derived from RT-PCR, *in situ* hybridization and enzymatic activity, so the type of cell is not defined.

Co-expression of 11β-HSD2 and MR has been demonstrated definitively by immunohistochemistry in aldosterone target neurons of the nucleus tractus solitarius (NTS) that are important mediators of salt appetite [34–36] and also receive information from neurons of the PVN [37]. While there is debate about the extent of 11β-HSD2 and MR co-localization in the brain [35], considerable steroid dehydrogenase activity in the brain was demonstrated by incubating minced rat brain tissue with tritiated corticosterone [25]. While conversion to 11-dehydrocorticosterone was greatest in the brain stem, there was very significant dehydrogenase activity in all other areas tested, including the hypothalamus and hippocampus [38].

### 1.5 Reactive oxygen species and 11β-HSD2 action

Because of the magnitude of difference between circulating concentrations of aldosterone and those of cortisol or corticosterone [23], an alternative mechanism for 11β-HSD2 action in aldosterone target cells has been suggested based on the generation of NADH from $NAD^+$, the requisite cofactor for 11β-HSD2 dehydrogenase activity [39]. The alternative proposed is that MR:glucocorticoid complexes in non-aldosterone target tissues have limited activity. Even in aldosterone target tissues most MR are bound to glucocorticoids and NADH produced by 11β-HSD2 activity stabilizes the MR: glucocorticoid complex in a conformation with limited transcriptional activity. When 11β-HSD2 is inhibited, $NAD^+$ accumulates, producing a change in conformation of the glucocorticoid-bound MR that allows it to have the same transcription activity as the MR:aldosterone [39]. Reactive oxygen species that accumulate in injured or ischemic tissues, for example in the infarcted myocardium, are hypothesized to have a similar effect on the MR:glucocorticoid complex as does $NAD^+$, triggering the activation MR by glucocorticoids [39]. This is a compelling, if yet unproven, explanation for how MRs in the cardiomyocyte that does not express 11β-HSD2 are activated during pathological conditions, despite low to normal circulating aldosterone levels. However part of this hypothesis is based on whole kidney binding studies without consideration that not all MR in the kidney, those in the cortical collecting, distal convoluted and outer medullary tubules, are co-expressed with 11β-HSD2 [40–42]. Nor does this proposed mechanism explain the apparent mineralocorticoid excess produced by high levels of cortisol in ectopic ACTH syndrome which are thought to overwhelm 11β-HSD2 [43]. There is merit in this proposed mechanism, however, as will be described in the discussion of the role of reactive oxygen species in neuronal excitation below.

## 2.0 Mineralocorticoids, Hypertension and the Brain

### 2.1 Historical perspective

Soon after its isolation, deoxycorticosterone (desoxycorticosterone; DOC), the first mineralocorticoid to be isolated [44], was found to cause hypertension at excessive doses [45–48] (reviewed in Gomez-Sanchez [49]). Early studies in humans and animals demonstrated that, in addition to sodium and water retention by the kidney [45,50,51], mineralocorticoids increased vascular tone directly and indirectly through neural effects [52–54], and that bilateral nephrectomy did not prevent DOCA hypertension [55]. Shortly after the isolation of aldosterone [56–59], Jerome Conn described primary aldosteronism [60]. For many years considered a relatively rare disease, primary aldosteronism, is now recognized as the most frequent cause of secondary hypertension [61].

### 2.2 Distribution of adrenal corticosteroid binding in the brain

Autoradiographic studies demonstrated distinct binding of tritiated aldosterone and corticosterone, the primary glucocorticoid in rats and mice, in the brains of adrenalectomized rats in which glucocorticoid receptors (GR) were blocked by an unlabeled GR-specific ligand [62–64]. The greatest retention of both steroids was in the hippocampus, followed by the choroid plexus, anterior hypothalamus and select nuclei of the brain stem. Aldosterone binding in the cortex and thalamus was greater than that of corticosterone. Real time RT-PCR measurements of message for MR in the rat brain was congruent with the autoradiography information [33]. MR mRNA expression in the hippocampus was 3–4 times that in the cortex, cerebellum, hypothalamus and brain stem [33].

A similar relative distribution of aldosterone and corticosterone binding in the brains of intact rats was found by measuring endogenous steroids in the cell nucleus fractions obtained by after subcellular fractionation of different brain parts [65]. The heaviest site of endogenous corticosterone occupation was the hippocampus. Despite competition from corticosterone, aldosterone was also measured in cell nuclear fractions of all brain areas tested, with the highest concentration in the hypothalamus [65].

### 2.3 Brain MR and mineralocorticoid/salt excess hypertension

The hypertension of mineralocorticoid excess is associated with increased sympathetic drive to the periphery, vasopressin release and threshold for the baroreceptor reflex [66]. Sodium appetite is also increased and sodium depletion prevents mineralocorticoid hypertension [67].

### 2.4 Location of centers for hemodynamic control

Ablation studies in rats identified areas of the anterior hypothalamus and brain stem involved in mineralocorticoid modulation of blood pressure, including the organum vasculosum lamina terminalis, subfornical organ, area postrema, subcommissural organ, the paraventricular and supraoptic nuclei (sites of vasopressin synthesis in magnocellular neurons), nucleus tractus solitarius (NTS), and rostral ventrolateral medulla [68–71]. Changes in osmotic pressure and concentrations of sodium, hormones and cytokines in the blood and cerebrospinal fluid are detected in the organum vasculosum lamina terminalis, subfornical organ and area postrema, circumventricular organs outside the blood brain barrier [72]. The tissue anteroventral to the third ventricle, the AV3V area, comprises the organum vasculosum of the lamina terminalis, ventral portion of the median preoptic nucleus, anteroventral periventricular nucleus, and periventricular nucleus, as well as nerve fibers that relay information between these structures and areas receiving and integrating hemodynamic information. Among the latter are the paraventricular, supraoptic and median preoptic nuclei of the hypothalamus, and NTS and lateral parabrachial nucleus in the

medulla [73]. AV3V area lesions prevent mineralocorticoid-salt and renovascular forms of hypertension by disrupting multiple regulatory systems. Among these are osmoreception, water and sodium homeostasis (thirst and sodium appetite), integration of baroreceptor and autonomic nervous system information, and secretion of vasopressin and natriuretic factor [69,74].

### 2.5 Effectors of MR mediated hypertension

As in chronic systemic mineralocorticoid excess, the chronic lateral intracerebroventricular (icv) infusion of aldosterone or DOC acetate at doses too small to have an effect when infused systemically produces hypertension associated with an increase in central sympathetic activation and release of vasopressin and a decrease in baroreceptor sensitivity [75,76] (reviewed in [77]. Salt loading and uninephrectomy accelerate the development of hypertension, however it also occurs in intact rats on a standard rodent chow (0.3% Na) [78]. The icv infusion of a MR, but not GR, antagonist, at a dose too small to have an effect when infused systemically prevents mineralocorticoid excess-salt hypertension [79]. Preventing the synthesis of MR protein with the icv infusion of a viral vector carrying siRNA for MR confirmed the specificity of the effect of the pharmacological antagonists [80]. The sympathoexcitation and hypertension produced by systemic aldosterone-salt excess was also mitigated by the icv infusion of the NADPH oxidase inhibitor apocynin or the reactive oxygen species scavenger tempol, however reducing reactive oxygen species did not decrease sodium appetite in this experimental model [80].

### 2.6 Synergy between brain MR and AT1R

In excess, the primary effectors of the renin-angiotensin-aldosterone-system, angiotensin II and aldosterone, have similar deleterious effects on the blood pressure, vessels, heart and kidneys. Separating their effects has been difficult because angiotensin II is a main secretagogue for aldosterone and aldosterone increases the expression of angiotensin converting enzyme [81,82]. The icv infusion of pharmacological inhibitors and siRNA for both MR and the AT1R has demonstrated that both receptors work synergistically in the brain to increase sympathetic drive and blood pressure in mineralocorticoid excess hypertension [80].

### 2.7 Ligand specificity

MR activation in transport epithelia resulting in the increase in the vectorial transport of ions and water is similar whether the ligand is aldosterone, cortisol or corticosterone. In other tissues, MR mediated effects are ligand specific; excessive aldosterone interferes with normal MR:cort function in the hippocampus [83]. Pharmacological inhibitors of 11β-HSD2 increase the blood pressure when administered orally or subcutaneously, or icv at lower, systemically inactive, concentrations [84,85]. The icv infusion of a MR antagonist prevents the hypertension produced by the systemic administration of an 11β-HSD2 inhibitor, suggesting that 11β-HSD2 prevents the activation of the MR by glucocorticoids in neurons controlling blood pressure, as it is thought to do in the kidney [84,85]. However, while the icv infusion of corticosterone at an equimolar concentration as the hypertensinogenic dose of aldosterone did not alter the blood pressure, it mitigated the increase in blood pressure produced by the concomitant icv infusion of aldosterone [86]. The mechanism of this inhibition has not been ascertained. The elevated local corticosterone concentrations might lead to the increased formation of transcriptionally inactive MR:cort complexes or differences in conformation of MR:aldo and MR:cort may lead to the recruitment of different co-regulatory proteins, resulting in the transcription of different genes, some with competing functions [10]. The increased corticosterone may increase GR:cort transcriptional activity producing gene products that interfere with MR-mediated functions. Alternatively, it has been suggested that MR in these neurons may be primarily occupied by glucocorticoids

and quiescent until the inhibition of the 11β-HSD2 leads to the accumulation of $NAD^+$, leading to a change in the MR:cort transcriptional activity, producing hypertension [39].

### 3.1 Aldosterone target neurons of the NTS and sodium appetite

Until recently, little was known about specific functions or connections of aldosterone target neurons with other neurons. Geerling and Loewy identified a small population of neurons of the NTS that co-express MR and 11β-HSD2 and are distinct from the other neurons of the NTS that are crucial for the response to sodium deprivation [35,87,88]. These aldosterone-target neurons receive information about sodium intake and plasma sodium concentration from the vagus nerve [89] and about osmolality from area postrema neurons [35,88]. They project to neurons in the parabrachial nuclei, which in turn project to the central nucleus of the amygdala [87], another area of the brain crucial for salt appetite. The amygdala also expresses MR, inhibition of which, either by infusion of an antagonist or antisense oligonucleotides, inhibits the salt appetite produced by mineralocorticoid excess [90].

### 3.2 Does aldosterone enter the brain?

Despite being lipophilic, steroid accumulation in the brain is controlled by multiple drug resistance, or p-glycoproteins, that actively pump specific compounds out of cells and may alter the intra-cellular aldosterone and glucocorticoid concentration ratio. P-glycoproteins are expressed in select neurons, as well as in brain microvascular endothelial cells and podocytes of astrocytes where they contact microvessels [91]. Transport (thus exclusion) by the human p-glycoprotein is significantly less for aldosterone than for cortisol, while corticosterone extrusion is minimal, suggesting that corticosterone, which circulates at 5–10% the level of cortisol in the human, is a significant ligand for the MR and GR in the human brain [92]. Levels of corticosterone in the brain would still be expected to greatly exceed those of aldo. Because p-glycoprotein expression in neurons is specific to the type of neuron, for example it is relatively abundant in granule cells of the dentate gyrus [91], it may provide a degree of ligand specificity for certain neurons. While p-glycoproteins have been cited as a major impediment for aldosterone binding of MR in the brain [35], aldosterone concentrations in the normal rat brain after extensive perfusion with heparinized saline are only slightly lower than those in plasma, though still 2 orders of magnitude less than levels of corticosterone [93]. The consistent finding of aldosterone in brain cell nuclei from all parts of the brain studied [65] suggests that aldosterone is able to access MR throughout the brain, not only in circumventricular areas unprotected by the blood brain barrier.

### 3.3 Synthesis of aldosterone in the brain

One way in which physiologically relevant intracellular aldosterone levels might be attained is as a neurosteroid produced and acting locally. All of the components required for *de novo* aldosterone synthesis from cholesterol are present in rat and human brains [33,94–96]. The last and unique enzyme in the synthetic pathway for aldosterone, aldosterone synthase, has been demonstrated in neurons [97] and brain tissue from adrenalectomized and intact rats can synthesize aldosterone from endogenous, as well as tritiated, substrates *in vitro* [98,99]. The amount of aldosterone synthesized in the brain is so small in comparison to that produced by the adrenal gland that it must act as a paracrine or autocrine hormone if it is physiologically relevant. Because most of the aldosterone in the brain is derived from the circulation and local synthesis is so limited, synthesis of aldosterone in the brain has only been reliably measured after adrenalectomy. Thus the function and regulation of extra-adrenal aldosterone synthesis has been difficult to ascertain [96,100,101].

Inappropriate aldosterone production within the brain appears to be involved in the pathogenesis of hypertension in the Dahl salt sensitive (SS) rat. Hypertension in these

animals is due to multiple genetic factors leading to problems with sodium and hemodynamic regulation. Despite suppressed circulating aldosterone, salt-induced hypertension in SS rats is prevented by the same measures that prevent mineralocorticoid excess-salt hypertension, including the central infusion of MR and epithelial sodium channel antagonists, as well as AV3V area ablation and central sympathectomy (reviewed in [33]). Expression of mRNA encoding the MR and 11β-HSD1 & 2 in different brain parts of the SS rat is similar to that of the normotensive Sprague-Dawley (SD) rat, however the *Cyp11b2* gene encoding for aldosterone synthase, is greater in the brains and less in the adrenal glands of the SS compared to Sprague-Dawley rats. SS have higher aldosterone concentrations in their brains than SD and Dahl salt resistant (SR) rats, yet similar or lower plasma aldosterone and total aldosterone production as reflected by 24 hour excretion compared to SD and SR rats [33]. Salt-sensitive hypertension in the SS rat is mitigated by the central infusion of several inhibitors of steroid synthesis, including one specific for aldosterone synthase [25,102]. This suggests that the dysregulation of aldosterone synthesis in or near aldosterone target neurons that are important for blood pressure regulation is a factor in the hypertension of the SS rats. Whether extra-adrenal aldosterone synthesis is relevant to normal physiology is not known.

### 3.4 A role for endogenous ouabain?

There is substantial evidence for an endogenous ouabain-like factor with an important role in the central control of the blood pressure [103–107]. Endogenous ouabain inhibits the Na/K ATPase pump as do therapeutic cardenolides, however it differs from these in that it increases the activity of other ion channels in vascular smooth muscle and other excitable cells [107]. Endogenous ouabain activity has been found to increase with sodium over-load and cause hypertension and vascular pathology that are prevented by MR antagonists. Despite more than 30 years of concerted effort, the mechanism for the synthesis of ouabain in a mammal has not been elucidated nor have intermediates been isolated [108,109]. It is postulated that ouabain is synthesized in the adrenal gland using the enzymes required for aldosterone, cortisol and corticosterone synthesis through the step requiring 3β-hydroxysteroid dehydrogenase activity, where it diverges. We infused trilostane, an inhibitor of 3β-hydroxysteroid dehydrogenase, icv to test the hypothesis that decreasing the synthesis of deoxycorticosterone, the precursor for aldosterone would inhibit salt-induced hypertension in the Dahl SS rat [110]. Trilostane effectively prevented the development of hypertension and decreased the pressure in those SS in which salt-induced hypertension had been established. However, ouabain is also implicated in the hypertension of the Dahl SS rat [104,111]. Interpretation of this model awaits precise measurement of ouabain, corticosterone and aldosterone, as the synthesis of all three are dependent upon 3β-hydroxysteroid dehydrogenase activity. The role of the inhibition of the action of endogenous ouabain as a mechanism of action of MR antagonists in human heart failure is yet to be definitively resolved [112].

## 4.0 Clinical relevance

Primary aldosteronism is produced by an aldosterone-producing tumor or idiopathic hyperplasia with unregulated aldosterone production by adrenal zona glomerulosa cells. It is the most frequent cause of secondary hypertension and associated with suppressed renin, hypokalemia, alkalosis, and cardiovascular remodeling with hypertrophy [61,77]. It should be noted that even in primary aldosteronism, concentrations of aldosterone are still at least 2 orders of magnitude less than those of cortisol. Even small amounts of aldosterone may increase the risk of hypertension. Aldosterone values in the highest quartile of the normal range predict the future development of hypertension in normotensive subjects, demonstrating the importance of seemingly insignificant increases in aldosterone for the development of high blood pressure [113].

### 4.1 Sympathetic nervous system in primary aldosteronism

The sympathetic nervous system has been shown to be a major mediator of experimental mineralocorticoid hypertension in several species [66,114]; however reports of its role in primary aldosteronism in humans have been equivocal. Potential reasons are that many studies were acute and used indirect arm cuff blood pressures and inappropriate control groups [115,116]. A study in which intra-arterial pressure was monitored continuously and plasma was sampled hourly for 24 hrs to provide data for regression curves that reflected dynamic changes in blood pressure and norepinephrine levels demonstrated that sympathetic drive was a greater influence on blood pressure in primary aldosterone patients before, compared to after, removal of the aldosterone producing adenoma [117]. In another study significantly greater muscle sympathetic nerve activity was found in patients with primary aldosteronism and essential hypertension compared to non-hypertensive subjects and the sympathetic activity was decreased in the primary aldosteronism patients after surgical resolution of their disease [118]. In a study of 26 normal human volunteers who served as their own controls, treatment to induce a 4-fold increase in plasma aldosterone, which is in the pathophysiologic range for primary aldosteronism, increased muscle sympathetic nerve activity and impaired baroreflex responses, similarly to results from experimental animal studies [119].

### 4.2 Aldosterone excess and depression

Some of the neuronal pathways influenced by the NTS neurons co-expressing MR and11β-HSD2 are also involved in motivational and reward behavior [35,36,120].

Chronic mineralocorticoid excess and sodium deprivation which leads to high aldosterone levels are both associated with signs of depression in humans and behavior in experimental animals, for example anhedonia, thought to approximate human depression [35,121,122]. This may explain the difficulty patients have in complying with a truly low salt diet, as well as reports of the amelioration of depression and cognition in patients with primary aldosteronism after resolution of their disease [123,124]. Given the prevalence of Primary Aldosteronism, between 4–12% of persons with hypertension [61], the affective aspect of inappropriate MR activation in the brain may be significant and under appreciated. These effects should not be surprising given that, in addition to dense expression in the hippocampus, MR are found in a significant number of neurons of the cerebral cortex where binding by aldosterone appears to occur in preference to glucocorticoids [62–64].

The human MR polymorphism of a valine substitution for isoleucine at amino acid 180 is associated with a higher risk of depression and of deficient stress-induced reward learning in individuals with normal affect [125]. The MR is very important in the feedback mechanism regulating the HPA axis, as well as serotonergic physiology of the hippocampus. The relatively common c.-2G>C polymorphism of human MR involving the kozac consensus sequence and associated with greater activation of the RAAS and mild hypertension, is also associated with an abnormal cortisol regulation in individuals taking selective serotonin reuptake inhibitors [22,126].

Several studies have reported that quality of life indicators were significantly better in heart failure patients treated with MR antagonists, sometimes despite lack of significant effect on cardiac function [127,128] The addition of a MR antagonists to the treatment was shown to increase performance on a test of cognitive function in hypertensive patients [122]. Taken together, these data suggest that even if there are no functional connections between MR-expressing neurons that mediate blood pressure control and those associated with cognition and affect, physiological and pharmacological perturbations of the MR may involve both.

### 4.3 Brain MR and cardiac remodeling

The vascular and myocardial hypertrophy and fibrosis leading to eventual heart failure associated with hypertension was once thought to be a simple response to increased force and shear stress. However, compared with patients with essential hypertension of similar severity and duration, patients with primary aldosteronism have increased left ventricular hypertrophy, demonstrating the importance of elevated aldosterone levels in addition to blood pressure (reviewed in [61,77]). The initiation of cardiovascular and renovascular remodeling produced by chronic mineralocorticoid or angiotensin II excess in experimental animals is independent of blood pressure, preceded by increases in markers of oxidative stress and inflammation, and mitigated by MR antagonists (reviewed in [77,129,130]). Pathology was assumed to be due to inappropriate activation of MR in these tissues. However, in experimental heart failure models and clinical studies in the RALES trial [131] and subsequent similar clinical studies [132], inappropriate activation of MRs contribute to cardiovascular dysfunction in conditions of normal or low circulating levels of aldosterone. Though 11β-HSD2 is expressed along with MR in vessels, it is not expressed in cardiomyocytes, so MR in cardiomyocytes are assumed to be activated by glucocorticoids [133,134].

### 4.4 Inflammation, hypothalamic MR and sympathoexcitation

It has been assumed that pathological changes in the injured heart mediated by the prolonged and inappropriate activation of MR are due to MR within the heart. Addition of a relatively low dose of spironolactone to a standard heart failure treatment regimen for humans that included an ang II receptor antagonist and loop diuretic, decreased cardiac sympathetic drive as well as increased left ventricular function [135].

Recent studies suggest that brain MR also have a significant role. Myocardial infarction (MI) produced by coronary ligation in rats is associated with significantly increased tumor necrosis factor (TNF)-α and interleukin (IL)-1β concentrations in the blood and heart, neuronal activity and TNF-α and IL-1β synthesis within the PVN, and sympathetic drive to the heart (reviewed in [136,137]). Structural damage and the progression of cardiac dysfunction to heart failure in this MI model are mitigated by MR antagonists targeted to the AV3V area and PVN. MR antagonist treatment significantly reduced plasma norepinephrine, TNF-α, IL-1β, and IL-6, cerebrospinal fluid prostaglandin E, and hypothalamic COX-2 and CRH protein expression. Inhibiting the MR also decreased immunohistochemical staining for TNF-α, IL-1β, IL-6 and CRH in PVN neurons in these rats [136–138]. MR antagonist treatment had a similar anti-inflammatory effect in the hypothalamus in experimental MI as that of the anti-cytokine drugs etanercept and pentoxifylline [138].

Blood-born proinflammatory cytokines do not pass through the blood brain barrier, but act on neurons indirectly by inducing cyclooxygenase (COX-2) activity and synthesis of prostaglandin E2 (PGE2) within perivascular macrophages in the brain. PGE2 does penetrate the barrier. Circulating TNF-α induces PGE2 production around hypothalamic microvessels that activates CRH neurons of the PVN, increasing ACTH and glucocorticoid synthesis. Similarly, PGE2 release induced by circulating IL-1β causes excitation of neurons within the AV3V area that project to the medial preoptic area and PVN [139]. The central administration of a lipopolysaccharide increases the expression of COX-2, TNF-α, and NAD(P)H oxidase in the PVN and PGE2 and TNF-α concentrations in the CSF, as well as sympathetic nervous system activity. This is prevented by the icv administration of a COX-2 inhibitor, confirming the association between inflammation and sympathoexcitation [140].

The icv infusion of a macrophage toxin clodronate one day after coronary ligation eliminated perivascular macrophages and COX-2 immunoreactivity within the PVN and significantly reduced PVN excitation and decreased cerebrospinal fluid PGE2 and norepinephrine, without reducing the increased levels of TNF-α and IL-1β in plasma or PVN produced by the myocardial infarction. Macrophage depletion also decreased blood pressure, renal sympathetic activity, and heart rate in response to the injection of TNF-α into the carotid artery [141].

In the icv infusion of polysaccharide and the heart injury models, inflammation occurred first, leading to NAD(P)H oxidase production and sympathoexcitation through a MR-mediated pathway [140]. This pathway is also activated by systemic aldosterone-salt excess and can be blocked by antagonists, including the siRNA for the MR, of the MR and of the generation and accumulation of reactive oxygen species [80]. Inflammatory reactions are an adaptive immediate response to injury [142] and augmentation of sympathetic drive to the heart is an essential homeostatic function. MR antagonist treatment significantly benefited the outcome in experimental MI when initiated a day or more after coronary ligation, however, starting treatment before or at the time of the ligation decreased survival of rats [143]. Thus, while MR antagonists have had clear benefits in patients with established disease, aggressive preemptive treatment may limit adaptive responses to injury.

## 5. Obesity-induced hypertension, metabolic syndrome, aldosterone, and the brain MR

An increase in aldosterone, as well as blood pressure, is associated with obesity and metabolic syndrome and the use of MR antagonists significantly reduces the associated inflammation and cardiovascular and renal pathology [144–147]. Higher levels of aldosterone predict the development of impaired glucose tolerance and diabetes [148,149]. There is evidence that an as yet unidentified aldosterone secretagogue is produced in adipocytes, particularly in obesity [150–152]. Such a factor might stimulate extra-adrenal synthesis of aldosterone in the brain as well as the adrenal glomerulosa cell. Inappropriately high levels aldosterone cause insulin resistance through both MR-independent, via reactive oxygen species generation, and MR-mediated mechanisms at the level of the pancreatic β-cell and insulin target cells [146,153]. As in aldosterone excess, activation of sympathoexcitatory neurons of the PVN is a major effector of obesity-induced hypertension, however the melanocortin receptors (MC3R and MC4R), in addition to, MR, are involved [154,155]. Insulin activates a melanocortin-dependent pathway to the PVN that increases glutamatergic drive to the rostral ventrolateral medulla and alters cardiovascular function. Inhibition of central central melanocortin receptors MC3R and MC4R decreases sympathoactivation and hypertension in various animal models of obesity and insulin resistance [154].

## Conclusion

MR are widely expressed throughout the brain and are often, if not always, expressed in the same neuron as are glucocorticoid receptors with which they may interact at the transcription and functional levels. Most MR in the brain are occupied by glucocorticoids which circulate in concentrations that greatly exceed those of aldosterone. Nonetheless, aldosterone acts through MR neurons of the NTS and amygdala to stimulate sodium appetite and neurons of the hypothalamus and circumventricular organs to mediate hemodynamic control through sympathetic nervous system activation, release of vasopressin and dampening of the baroreflex. The precise molecular role of NADP(H) oxidase and reactive oxygen species is still unclear, as there is evidence that they are involved both in MR-dependent excitation of neurons mediating blood pressure control, as well as inflammation

and repair in the heart, vessels and kidneys, as well as MR-independent effects of aldosterone through the GPR30 g-protein. Aldosterone and angiotensin II act synergistically through the MR and AT1R, probably at the level of the PVN, to increase blood pressure and sympathoexcitation. Obesity is associated with both an increase in aldosterone and sympathetic drive leading to hypertension, the etiology of which is complex, involving hypothalamic melanocortin receptors, as well as MR. MR antagonists are useful in the treatment of cardiovascular and renovascular disease. However, because the preponderance of MR in the brain have functions unrelated to cardiovascular homeostasis or inflammation, it is crucial to discover downstream effectors of the pathological effects initiated by MR activation to provide specific targets for therapy.

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## Table 1
### Expression of MR and 11β-HSD2 in the rat brain

Expression of MR in the adult rat brain is relatively wide spread [42,156,157]; that of 11β-HSD2 is much more limited in distribution and quantity [26,27,29–32]. 11β-HSD2 expression is more wide spread and in greatest quantity in the fetal brain. Most of the information for 11β-HSD2 expression in the adult rat brain is derived from RT-PCR, *in situ* hybridization and enzymatic activity.

| Rat Brain (adult) | MR | 11β-HSD2 fetus | 11β-HSD neonate | 11β-HSD2 adult |
|---|---|---|---|---|
| cortex | x | xx | | |
| hippocampus | xx | xx | | |
| hypothalamus | | | | |
| PVN | x | xx | x | x |
| VMN | x | xx | x | x |
| amygdala | x | xx | x | x |
| thalamus | x | | xx | |
| cerebellum | x | | xx | |
| brain stem | | | | |
| NTS | x | xx | x | x |
| locus | x | xx | x | x |
| coeruleus | x | xx | x | x |
| med vestibular | | | | |
| nuc | | | | |
| subcommissural organ | x | xx | x | x |
| choroid plexus | xx | | | |