Genetic, Molecular and Clinical Determinants for the Involvement of Aldosterone and Its Receptors in Major Depression

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\textbf{Abstract}

Major depression (MDE) has metabolic and neuroendocrine correlates, which point to a biological overlap between MDE and cardiovascular diseases. Whereas the hypothalamic-pituitary-adrenocortical axis has long been recognized for its involvement in depression, the focus was mostly on cortisol/corticosterone, whereas aldosterone appears to be the ‘forgotten’ stress hormone. Part of the reason for this is that the receptors for aldosterone, the mineralocorticoid receptors (MR), were thought to be occupied by glucocorticoids in most parts of the brain. However, recently it turned out that aldosterone acts selectively in relevant mood-regulating brain areas, without competing with cortisol/corticosterone. These regions are intimately involved in the close relationship between emotional and vegetative symptoms. Genetic analysis supports the role of aldosterone and of MR-related pathways in the pathophysiology of depression. Functional markers for these pathways in animal models as well as in humans are available and allow an indirect assessment of NTS function. They include heart rate variability, baroreceptor reflex sensitivity, blood pressure, salt taste sensitivity and slow-wave sleep. MR activation in the periphery is related to electrolyte regulation. MR overactivity is a risk factor for diabetes mellitus and a trigger of inflammatory processes. These markers can be used not only to assist the development of new treatment compounds, but also for a personalized approach to treat patients with depression and related disorders by individual dose titration with an active medication, which targets this system.

\textbf{Introduction}

Major depression (MDE) does not only exert a profound effect on the quality of life, but also on the physical health of affected patients. An important step to understand the link between MDE and the risk of developing cardiovascular diseases [1, 2] is the investigation of common risk factors [1]. Moreover, MDE in itself appears to be a risk factor for developing cardiovascular disease [3].
Risk factors include metabolic changes such as signs of the metabolic syndrome, increased inflammatory markers as well as neuroendocrine alterations.

Neuroendocrine changes in depression are well documented. In particular, an increase in the plasma concentration of cortisol in patients with melancholic depression has consistently been reported. The increase in cortisol may be interpreted as an overactivity of the stress hormone system, i.e. the hypothalamic-pituitary-adrenocortical (HPA) axis, on the basis of a dysfunctional glucocorticoid receptor (GR) feedback. Besides cortisol, another HPA axis hormone has long been recognized to play a role in stress response, namely aldosterone [4]. The increase of both of these hormones in situations of acute stress is physiologically plausible, as both prepare for a ‘fight-or-flight’ response. Both aldosterone [5, 6] and cortisol [7] increase blood pressure. In addition, cortisol increases catabolism in order to meet the increased energy demand [8]. In healthy individuals these responses fade away when the stress trigger disappears. The fact that certain patients with depression show either elevated cortisol or aldosterone plasma concentrations demonstrates that regulatory mechanisms of these systems may be dysfunctional. It is of importance to note that despite the fact of close interactions in the regulation of these hormones, it appears that they are differentially regulated in different contexts and may differentiate subgroups of patients with depression [9].

Despite the long-known fact that aldosterone is involved in stress response and that depression may be regarded as a stress-related disorder, studies on the involvement of aldosterone, either as a marker or potentially a mediator in the pathophysiology of depression, started rather recently. We are going to summarize what is known about the involvement of the mineralocorticoid receptor (MR) and aldosterone in the pathophysiology and treatment of MDE and highlight in particular molecular and genetic determinants of its activity.

**MR and the Stress Hormone System**

MR are expressed in many organs, including the brain [10]. Within the brain the expression of MR is highly concentrated in the hippocampus, whereas GR are more widely distributed [11]. Of importance, GR and MR interact functionally and often in an antagonistic way [12]. Cortisol, the main glucocorticoid in humans, has affinity for both GR and MR [11]. Due to the fact that the cortisol concentration is about 500-fold higher than the concentration of aldosterone [11], hippocampal MR are widely occupied by cortisol. Functionally the activation of hippocampal MR reduces HPA axis activity, whereas the activation of GR at this structure leads to its activation [11]. With regard to depression it is of interest to note that in animal models hippocampal MR expression is increased following the treatment with the tricyclic antidepressant imipramine, whereas GR expression was unchanged [13]. As hippocampal MR appear to be reduced in suicide victims with depression, the antidepressant-induced MR expression appears to ‘normalize’ this pathology [14]. These findings may be related to observations that hippocampal volume appears to be altered in depression; however, methodological considerations have to be taken into account [15].

It has been recognized early that certain brain areas may be selective for aldosterone, and that central MR directly affect kidney function as well as electrolyte and fluid regulation [16, 17], with functionally opposite effects to those of glucocorticoids [12]. With the discovery of defined networks with aldosterone selectivity, an important aspect of network regulation by aldosterone was revealed [18, 19]. The basis for aldosterone selectivity is the co-expression of the MR with an intracellular enzyme, which metabolizes cortisol and therefore allows aldosterone to bind to the MR. This enzyme is called 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2). The anatomical areas where this co-expression occurs are the nucleus of the solitary tract (NTS) and potentially the paraventricular nucleus of the hypothalamus (PVN) and the central nucleus of the amygdala (CeA) [10]. The latter structures have been intensely discussed in the context of stress regulation and the pathogenesis of depression [20, 21]. More recently the NTS has been implicated as a target for antidepressant manipulations, as vagus nerve stimulation, which shows antidepressant effects in therapy-refractory depression, affects the brain via the route of this nucleus [22]. The PVN and the CeA appear not only to be primary targets for aldosterone, but also to receive strong inputs from the NTS [19]. Therefore, a network consisting of the NTS, PVN and CeA is either way a strong candidate for aldosterone’s influence.

These observations suggest that aldosterone itself has a causal role in stress-related disorders and may have a role in depression. Indeed, both in animal models [23, 24] and in humans [25], aldosterone increases during stress. With regard to mood disorders, a close temporal relationship between aldosterone and mood has been reported in bipolar disorder [26]. Further, the suppression of aldosterone with the synthetic glucocorticoid dexamethasone (DEX) differentiated patients with depression from con-
trols [27]. A direct comparison of levels of aldosterone compared with healthy subjects demonstrated a significantly increased plasma concentration of aldosterone during the course of the night [28]. These findings were independently replicated [29] and refined [30–32]; Hallberg et al. [30] demonstrated a positive correlation between aldosterone levels and the severity of depression. However, the same study found reduced aldosterone levels in suicidal patients. The significance of the latter finding has to be further explored. Häfner et al. [31, 32] demonstrated specific factors for an increased level of aldosterone in depression, in particular a co-occurrence with arterial hypertension and with social isolation. Further support of a causal role of aldosterone comes from observations that patients with primary hyperaldosteronism frequently show symptoms of depression, anxiety and somatization and a reduced quality of life [33–35]. A direct causal involvement of aldosterone is also suggested by animal data: subchronic administration of aldosterone with a minipump led to depression- and anxiety-like behavior in rats [24]. However, given the clinical and biological heterogeneity of depression, a closer look into the activity of the MR in the CNS and its manipulations is necessary.

**MR Activation and Its Manipulation**

MR expression appears to be altered in patients with depression, as observed in postmortem studies [14]. Moreover, MR expression appears to be changed by antidepressants [13]. Despite the evidence for a causal relationship between aldosterone and depressive symptoms, other mediating factors cannot be neglected. It is therefore important to explore biological factors contributing to this relationship. In the context of a neuroendocrine hypothesis, the first features to look into are changes of neuroendocrine parameters. Later on, additional functional markers of MR activity will be reported, including effects on heart rate variability (HRV), blood pressure, baroreceptor reflex sensitivity and sleep EEG parameters.

Neuroendocrine manipulations with the purpose of gaining information about the involved systems are commonly performed (see below), but difficulties in linking these results to biological disturbances remain. The main reason for these difficulties is the fact that compounds which are used for these tests exert both peripheral and central effects [36]. For example, the impact of a given compound on CNS activity is uncertain, due to its variable penetrance through the blood-brain barrier (BBB). Therefore, the direct stimulation/inhibition with a given compound must be differentiated from the consequences of peripheral neuroendocrine changes. Three frequently used tests are highlighted in the following paragraphs.

The DEX suppression test and its extension, the DEX-CRH test, is frequently applied in psychiatric research to characterize HPA axis activity [37]. The primary action of DEX is to activate pituitary GR, which leads to a reduction of ACTH release. As ACTH releases both cortisol and aldosterone at the adrenal cortex, the DEX-induced suppression of ACTH leads to an acute reduction of both cortisol and aldosterone. DEX does not easily cross the BBB at relevant concentrations [11]; therefore, a direct central GR activation can be ruled out. Due to the reduction of peripheral cortisol and aldosterone, a reduced activation of both central GR and MR is the most likely effect in case of DEX-induced suppression of cortisol [36].

Spironolactone, an inhibitor of the classic MR (we will cover non-classic membrane MR later), appears to cross the BBB reasonably well and therefore appears to inhibit central MR activity [9]. In addition to blocking the effect of aldosterone in the areas discussed above, spironolactone also inhibits the depression-inducing effect of the glucocorticoid corticosterone on rat behavior as well as the corticosterone-induced downregulation of MR expression in the hippocampus and hypothalamus [38]. This demonstrates that glucocorticoids, which are known to have a high affinity for MR (see above), can provide additional MR activation in anatomical areas in which MR are generally considered to be fully occupied at all times [11]. Further, the aldosterone selectivity in areas which co-express MR and 11β-HSD 2 is not absolute, i.e. glucocorticoids may still contribute to the MR activation in these areas.

Fludrocortisone is an MR agonist with some GR activity. It only crosses the BBB to a limited extent and has a short half-life in the brain [39]. It leads to reduced plasma concentrations of cortisol, but not aldosterone, in healthy subjects [40], and numerically reduces cortisol and aldosterone in subjects with depression [41]. Its effect is mediated by MR activation at the level of the pituitary and potentially adrenal cortex [42, 43] to reduce the release of cortisol and aldosterone. From a CNS perspective, administration of fludrocortisone thus leads to reduced central GR and MR activation in all related neuroanatomical areas. Therefore, interpretations of interventions with these compounds need to be done with care, as their CNS effects may be opposite to their peripheral effects.

The heterogeneity of depression, both in symptomatology and physiological signs, has been attributed in part to differences in MR sensitivity [9]: MR activation is
involved in reducing HPA axis activity as well as in supporting slow-wave sleep (SWS). Therefore, a characterization of patients on the basis of these parameters may be related to differential MR activity [9]. The use of challenge tests for the MR may therefore provide insights in different patient populations. Nonhospitalized patients who were free of medication for at least 3 months and had depression of moderate severity and no signs of therapy refractoriness were studied for the effect of spironolactone [44]. Spironolactone administration led to an increase in plasma cortisol concentration in these patients, exceeding that in healthy volunteers. This demonstrated that the MR in these subjects was rather supersensitive in comparison to that in controls. In contrast to healthy volunteers, in patients with therapy-refractory depression administration of spironolactone was without effect [45]. This observation points to a desensitization of MR in therapy-refractory depression. Combined, this could support the role of MR desensitization in refractory depression. As spironolactone crosses the BBB [9], it cannot be determined whether these effects are peripherally, i.e. at the level of the pituitary, or centrally mediated.

In a recent study [46], markers of MR activity were collected in patients with depression who were hospitalized, i.e. included a higher rate of treatment nonresponders. In order to clarify the role of MR-related activity with response to antidepressant treatment, aldosterone and central and peripheral markers of MR activation were assessed. Aldosterone and cortisol were determined at the time of awakening by collecting salivary samples. Plasma Na⁺, K⁺ and Mg²⁺ were regarded as peripheral markers as aldosterone leads to an increased uptake of Na⁺ at the kidney, whereas K⁺ and Mg²⁺ are excreted by its action. Blood pressure regulation by aldosterone has central as well as peripheral components [10]. The central activity of MR function was assessed by determining HRV, SWS and salivary cortisol and salt liking [46]. 32 subjects were assessed briefly after they were hospitalized and again 2 and 6 weeks later. In case patients were released earlier, the second assessment was done briefly before discharge. Pooled over all visits, a significant correlation between awakening aldosterone in saliva and severity of depression was revealed. A high ratio of aldosterone/cortisol at baseline predicted impaired improvement. Reduction of cortisol and aldosterone after 2 weeks predicted response at outcome. High HRV and high SWS were associated with worse outcome in male subjects. These data are in line with a role of central MR activation in therapy refractoriness possibly mediated via NTS regulation.

Genetic Aspects of the MR System in MDE

MDE is a heritable disease; however, the polygenic mode of inheritance and the complex phenotype of the disease render the identification of genetic factors rather difficult [47]. Genetic association studies of candidate genes and the whole genome have revealed candidate regions with some contribution to MDE, but this goes beyond the scope of this review. Here we will focus on parameters with potential physiological relevance to our topic. Recent reviews [48, 49] list a number of markers of relevance to the discussed system. Relatively strong associations between depression risk and single-nucleotide polymorphisms have been reported for genes involved in the regulation of aldosterone or its downstream effects. Polymorphisms of the gene for angiotensin-converting enzyme (ACE) as well as for the angiotensin II (ATII) receptor with an increased risk for MDE for the more active form have been reported [50–52], i.e. a higher stimulatory signal from the RAAS. Polymorphism of the 5-HT₂A receptor and FKBP5 have been confirmed as well [53, 54]. The 5-HT₂A receptor is related to the activity of the both the HPA axis and the RAAS [55]. Of interest, polymorphisms of the 5-HT₂A receptor are related to somatization, i.e. ‘medically unexplained physical complaints’, which are also related to therapy refractoriness in patients with depression [56]. Both again point to a role of MR activation in these syndromes as well as in therapy response. FKBP5 is a co-chaperone for both the GR as well as the MR, i.e. it regulates their functional activities [57, 58]. Furthermore, aldosterone induces FKBP5 expression in several tissues [59]. As ACTH is an activating factor for aldosterone release, polymorphisms of the CRH receptor are also of relevance. A polymorphism of the CRH1 receptor has been linked to anxiety [60], which in itself is a negative predictor of the efficacy of antidepressant treatment [61]. Further, polymorphisms of the MDRI gene, which leads to the production of the protein for P-glycoprotein (P-gp), has been implicated in therapy refractoriness [62]. P-gp is a transport protein which is responsible for extruding ‘xenobiotics’ from target organs, including the brain. Importantly, P-gp also transports cortisol and aldosterone [63] and via this mechanism modulates the activity of the HPA axis [64]. Further, P-gp activity is related to the release of aldosterone from adenocortical cells [65] and affects the aldosterone plasma concentration [66], pointing to a link between P-gp and aldosterone action. The results of these approaches to target ‘candidate genes’ show that a sizable number of identified genes are related to the release of aldosterone or the sensitivity of the MR (fig. 1). Additional insights can be
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Molecular Aspects of MR Sensitivity and Function: Downstream Effects

MR in their balance with GR have extensively been studied in CNS cortical areas, in particular the hippocampus and prefrontal cortex as well as areas more directly involved with endocrine regulation, such as the hypothalamus [76]. In these anatomical areas the classic intracellular MR appears to be occupied most of the time with corticosterone/cortisol due to their high affinity with this receptor. Only high concentrations of cortisol/corti-

costerone lead to an occupancy of GR, which partially reverse the action of MR activation [11], for example on the electrical activity in the hippocampus and on HPA axis activation. In addition, activation of a nonclassic membrane MR in the hippocampus leads to an increased synaptic 
glutamate release and increases AMPA receptor localization into the synapse via lateral diffusion and, as a consequence, an increase in long-term potentiation [76]. As these membrane MR have a relatively low affinity to corti-
costerone, aldosterone may contribute to their activation [77]. Via GR activation at higher concentrations of corticosterone, different types of AMPA receptors containing GluA2 subunits are incorporated into the synap-
ase, which then lead to long-term depression [78].
changes are integrally involved in memory processes. A second link between aldosterone/MR activity and glutamatergic transmission is provided by the MR-induced activation of serum glucocorticoid kinase-1 (Sgk1). Sgk1 is regulated by both GR and MR [78] and affects brain function via several pathways, including the induction of NMDA and other glutamate receptors as well as glutamate transporters [79, 80]. Via this mechanism it is involved in stress-induced memory impairment [80]. Furthermore, it is activated by insulin and is involved in blood pressure and electrolyte regulation [79]. The involvement of Sgk1 in depression and hippocampal volume has recently been demonstrated [81].

The Role of the NTS

The involvement of aldosterone as a predictor of treatment response described above highlights the importance of aldosterone-specific anatomical areas, which include the NTS [19]. Markers of MR activity at the NTS are HRV, blood pressure, salt taste sensitivity and SWS [9]. Earlier studies already pointed to this nucleus for its involvement in stress reactions [82, 83]. Evolutionary and neuroanatomical observations also placed the NTS at the center of a network regulating feelings, including pleasure, disgust, pain [84], and interoception, which is often disturbed in patients with depression, in particular those presenting symptoms of somatization [85]. Hypothetically accepting the NTS as a core region for feelings and interoception makes it an interesting target for drug development for therapy-refractory depression. The fact that vagus nerve stimulation affects the brain via the NTS and affects therapy-refractory depression has already been mentioned [22]. Its specific pharmacology can be explored. Indeed, direct blockade of the MR with spironolactone [86] or drospirenone [87] demonstrated the efficacy of MR antagonism in depression, anxiety, irritability, and somatization, the latter in the context of premenstrual syndrome. At first glance apparently paradoxical, there is some evidence that the administration of fludrocortisone, an MR agonist, may speed up the improvement of the anti-depressant eplerenone to increase the release of the empathy-enhancing peptide oxytocin [89]. Furthermore, there is clinical evidence that patients with chronic fatigue syndrome, i.e. a disorder belonging to the atypical spectrum of depression [90], benefit from fludrocortisone [91]. Therefore, both MR antagonists like spironolactone and drospirenone as well as the peripheral MR agonist fludrocortisone may exert beneficial effects on mood. Interestingly, the effects of fludrocortisone and spironolactone on anxiety in an animal model appear to be similar [92], in line with the suggestion that fludrocortisone leads to a central MR deactivation. However, whether similar or different mechanisms are in place for these treatments needs to be further investigated.

The NTS is a pharmacologically rich region, therefore, apart from direct manipulation of MR activity, alternative pharmacological interventions may act on its function. A recently approved antidepressant, vortioxetine, has, besides other properties, 5-HT3 antagonist properties, which was assessed by its capability to modify the Bezold-Jarisch reflex [93], which is mediated via the NTS [94]. 5-HT1 receptors have earlier been demonstrated to have beneficial effects on patients with depressive disorders and somatization [90]. Similarly, NMDA antagonism inhibits this reflex [95]. NMDA receptor antagonists, in particular ketamine, received considerable attention because of their rapid antidepressant activity in patients with treatment-refractory depression [96, 97]. The role of the NTS for the antidepressive action of ketamine has, however, not yet been studied, but deserves attention. Further, vasopressin antagonists have been implied in the treatment of depression. Vasopressin influences NTS activity via V1a receptors [98] (not V1b receptors, which are involved in ACTH release); therefore V1a antagonists may have importance in the pathophysiology of depression. Given that NTS-related parameters of MR activity like the baroreceptor reflex [99], HRV, blood pressure and potentially salt taste sensitivity can be assessed relatively easily, personalized approaches to treating depression are at hand.

Conclusion

We characterized functional systems which are involved in the release of aldosterone and the sensitivity of MR in relevant CNS circuits. Genetic analysis supports the relevance of many of the involved receptor systems for depression. These include already targeted ones, like the 5-HT2A, the MR and more recently NMDA and 5-HT3 re-
Further potentially relevant systems include endothelin receptors, P-gp, and vasopressin V1a receptors. An area of importance, which was recently reviewed, is the regulation of electrolytes, in particular of Mg²⁺ [97], and is not covered in detail here. Some of the identified signal pathways appear to be related to clinical characteristics of therapy refractoriness of depression, for example higher expression of anxiety and somatization. The identification of the NTS circuit points to a system which should be further studied in this context. Its easy accessibility and interesting pharmacology may make this structure a worthwhile target for rational drug development.


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