Mineralocorticoid receptor in human brain : a key player in resilience

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Summary

Glucocorticoids secreted by the adrenals during stress have a profound influence on the brain and behavior. The effects of the stress hormone are mediated by two related receptors that have a distinct localization in limbic brain areas involved in stress regulation. The two types of receptors, the mineralocorticoid (MR) and the glucocorticoid receptor (GR), function as gene transcription factors regulating the expression of target genes underlying coping with stress and behavioral adaptation. Recently, however, a membrane-bound variant of the MR was discovered to mediate rapid effects on neuronal excitability.

The MR and GR mediate glucocorticoid (corticosterone in rodents, cortisol in humans) effects in a complementary fashion. In the limbic brain the MR is involved in the initial stress reactions, while via the GR the hormone prevents these initial stress reactions from overshooting and makes energy available for recovery from the stressor. The present thesis is focused on the MR, in particular on the common gene variants of the *MR* that may provide a genetic basis for individual differences in coping with stress.

Research has shown that the MR plays an important role in cognitive and behavioral flexibility while dealing with challenging (stressful) conditions. Therefore, variants of the *MR* gene may relate to individuals' differential susceptibility to stress, with possible implications for the pathophysiology of stress-related disorders such as depression. Hence, therapeutic interventions targeting the MR may provide relief of clinical symptoms and indeed an MR agonist has been shown to enhance the efficacy of a commonly prescribed selective serotonin reuptake inhibitor (SSRI). However, until today no clear data are available on the relation between *MR* gene variants and susceptibility to stress and depression.

The **aim** of this thesis was to assess the influence of variations in the *MR* gene on neuroendocrine regulation and psychological functioning. To achieve this goal we have identified and characterized common *MR* gene variants and have subsequently examined the significance of these gene variants in genetic association studies. We tested association with cortisol regulation in depressed patients. In addition we tested association with variability in psychological traits that predict the risk of depression and with depression diagnosis itself.

The studies were inspired by an influential hypothesis proposing that aberrant MR vs. GR expression in the brain may be fundamental for the susceptibility to stress-related disorders including depression. Only a few comparisons of receptor expression between *postmortem* brains of depressed patients and non-depressed controls have been reported. We assessed MR mRNA expression, including the two splice variants MRα and MRβ, in five limbic brain structures using *postmortem* brain tissue from six non-depressed subjects and six patients diagnosed with a major depressive disorder **(Chapter 2)**. The highest MR expression level was detected in the hippocampus (where it was even higher than the GR expression), while 20 – 100 times lower MR expression levels were detected in the amygdala, cingulate gyrus, inferior frontal gyrus and nucleus accumbens. In all regions MRβ expression was lower compared to MRα. Strikingly, central MR expression was significantly lower in the brains of

depressed patients, specifically in the hippocampus, inferior frontal gyrus and cingulate gyrus. Also GR expression was slightly but significantly decreased in the depressed brain. Together the differences in MR and GR expression did, however, not result in a significant change in the MR/GR balance. Decreased MR expression could be implicated in the disturbances in neuroendocrine regulation, emotions and cognitive flexibility often observed in depressed patients.

Previously, two variants of the *MR* gene (so-called single nucleotide polymorphisms, SNPs; the MR -2G/C and I180V SNPs in exon 2) were identified that influence MR functioning. Here we determined whether additional functional SNPs exist in the *MR* gene promoter region, which potentially can modulate MR expression **(Chapter 3)**. Along a sequence of almost 4000 basepairs eight SNPs were identified. The SNPs were highly linked to the two functional SNPs in exon 2. Together the SNPs result in three common SNP combinations, so-called haplotypes. The promoter SNPs were found to modulate promoter activity in human neuroblastoma cells; with haplotype 2 (freq. ~0.38) resulting in a higher *MR* gene transcription compared to haplotypes 1 and 3. Together with the previous data regarding exon 2 this means that haplotype 2 results in a higher (1) gene transcription (more MR mRNA), (2) translation (more MR protein) and (3) transactivation (expression) of its target genes. Whether haplotype 2 also results in higher MR expression in the brain needs further investigation. However, the data do indicate that these common *MR* gene variants potentially influence MR functioning. Therefore these SNPs and haplotypes can be used for studying the relationship of genetic variation in the *MR* with psycho-neuroendocrine regulations and the risk of depression.

Chapter 4 describes the results of a first genetic association study that we performed with respect to psychological functioning in individuals selected from the general population. The *MR* haplotypes 1 till 3 were tested for association with variability in dispositional optimism. Dispositional optimism indicates to what extent people hold generalized favorable expectancies for their future. It is associated with successful coping behavior, less distress, a longer life expectancy and a lower risk of depression. We found in this first study consisting of an elderly population that specifically the *MR* haplotype 2 was associated with heightened dispositional optimism in women, but not in men.

Chapter 5 describes the results of a second study among students that was performed as a follow-up on the one described in **Chapter 4**. The *MR* haplotypes 1 till 3 were this time tested for association with variability in cognitive reactivity to sad mood, with a special emphasis on thoughts of hopelessness. Hopelessness is by definition inversely related to optimism and predicts a higher risk of depression. In line with the results described in **Chapter 4**, *MR* haplotype 2 was associated with fewer thoughts of hopelessness, again only among women and not among men. In addition, a first indication was found for an association between haplotype 2 and a lower risk of depression.

Previous studies by our group and others have demonstrated that the common and functional *MR* SNPs and haplotypes differentially affect cortisol secretion in healthy individuals. In **Chapter 6** we describe the results of a study aimed to test the role of *MR*

gene variants in the variability of cortisol secretion observed in depressed patients. We found no relationship of the *MR* gene variants with the cortisol awakening rise (CAR) in the total group of depressives. However, a clear association was found between the -2G/C SNP and the CAR in the patients frequently using selective serotonin reuptake inhibitors (SSRIs). Patients with the -2G/G genotype showed a heightened CAR while carriers of the -2 C-allele displayed a low or even flattened CAR. The *MR* I180V SNP had no significant effect on the CAR. With respect to the haplotypes, particularly the *MR* haplotype 2 was associated with a lower CAR. Also haplotype 3 was significantly associated with a lower CAR but this effect was smaller. The question rises whether the *MR* gene variants also affect clinical response to antidepressants. Longitudinal studies are warranted to verify this

The study described in **Chapter 7** was performed in order to replicate the results described in **Chapter 5** regarding the association found with depression, among a large group of depressed patients and healthy controls. Again, *MR* haplotype 2 was associated with a lower risk of depression and again only among women rather than men. Importantly, the association was found in the women that were aged 51 years or younger (the mean age for menopause) and particularly among the women that were younger than 41 years. In the women aged older than 51 years no association of *MR* haplotype 2 with depression was found. To what extent sex hormones play a role in the association between the *MR* genotype and the risk of depression remains to be investigated.

Concept of brain MR and depression

The data show that MR expression was lower in the limbic brain of depressed patients, while common and functional *MR* gene variants modulate the regulation of cortisol secretion and an individual's personality with possible consequences for the risk of depression. Indeed, the *MR* gene variants were found to associate with the risk of depression in a study among a large group of depressed patients and healthy controls. Together the data identify *MR* haplotype 2 (freq. ~0.38) as a protective factor against depression. The associations were particularly valid for women and not for men, which is interesting considering the twice higher prevalence of depression among women compared to men. Moreover, the *MR* genotype was found to modulate the effect of commonly used antidepressants (SSRIs) on the cortisol awakening response.

Conclusion

We postulate that the MR in the limbic brain mediates the central position cortisol has in determining an individual's initial neuroendocrine and psychological responses to challenging (stressful) conditions. The functioning of the MR, which is in part determined by *MR* gene variants, likely is implicated in the individual's susceptibility to stress and stress-related disorders. We propose therefore that the *MR* genotype may provide an excellent opportunity for personalized therapeutic intervention of stress-related mental disorders such as depression and may be an important marker for prevention.