

Review

Corticosteroid receptor polymorphisms: Determinants of vulnerability and resilience

Roel H. DeRijk^{*}, E. Ron de Kloet

*Division of Medical Pharmacology, Leiden/Amsterdam Center for Drug Research, Leiden University Medical Center, Leiden University,
PO Box 9502, 2300 RA Leiden, The Netherlands*

Accepted 14 November 2007
Available online 30 January 2008

Abstract

Why some individuals thrive and others break down under similar adverse conditions, is a central question in the neuroendocrinology of stress related psychopathology. The brain mineralocorticoid (MR) and glucocorticoid receptors (GR) operate in balance to coordinate behavioural, autonomic and neuroendocrine response patterns involved in homeostasis and health. Genetic variants of both the MR and GR have been functionally characterized. The four GR-gene single nucleotide polymorphisms (SNPs) (ER22/23EK (allele frequency: 3%), N363S (4%), *BclII* (37%), A3669G (15%)) and the two MR-gene SNPs (−2 G/C (50%), MR-I180V (11%)) showed *in vitro* changes in transactivational capacity, or affect stability of the mRNA (GR exon 9β A3669G). All of these MR-and GR-SNPs change the regulation of the hypothalamus-pituitary-adrenal (HPA) axis at different levels including basal level (−2 G/C), dexamethasone induced negative feedback (ER22/23EK, N363S, *BclII*, 9β A3669G) or following a psychosocial stress test (Trier Social Stress Test (TSST); all of the MR-and GR-SNPs). Importantly, the MR-I180V increased autonomic output and enhanced cortisol secretion during the TSST. Recently, several of these MR-and GR-variants have been found associated with psychopathology (depression, bipolar disorder). These data provide evidence that dysregulation of MR and GR are causative in the pathogenesis of depression and that these MR-and GR-gene variants are part of the genetic make up that determines individual stress-responsivity and coping style, affecting vulnerability to disease.

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Keywords: Vulnerability; Genetic variant; Glucocorticoid receptor; Mineralocorticoid receptor; Stress

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^{*} Corresponding author. Department of Psychiatry, Leiden University Medical Center Gorlaeus laboratories, P.O. Box 9502, 2300 RA Leiden, The Netherlands. Tel.: +31 71 5276229; fax: +31 71 5274715.

E-mail address: r.h.de.rijk@LACDR.Leidenuniv.nl (R.H. DeRijk).

1. Introduction

Glucocorticoids secreted by the adrenals control homeostasis and play a central role in the ability to cope with a challenge (de Kloet et al., 2005). The secretion of glucocorticoids shows an ultradian rhythm of about one pulse per hour (Veldhuis et al., 1989). In humans these pulses have increased amplitude in the morning resulting in peak levels of cortisol. Following any threat to homeostasis, the release of corticotrophin releasing hormone (CRH) from the hypothalamic parvocellular nucleus (PVN) is triggered. CRH activates and coordinates sympathetic outflow and increased release of adrenocorticotropin hormone (ACTH) and subsequently cortisol. The HPA axis is subject to the negative feedback action of cortisol. A rapid activation of the stress–response implies coping with the stressor as long as the stress response is also rapidly terminated. Such a reactive system indicates that the organism is resilient and that homeostatic defense mechanisms operate efficiently.

Glucocorticoids play an important role in both the onset and the termination of the stress–response. Recent data from neurophysiological and behavioural studies (Kruk et al., 2005; Karst et al., 2005) suggest that glucocorticoids actually can enhance in the limbic brain the initial stress reactions, which they prevent subsequently from overshooting (Sapolsky et al., 2000). If either one of these activating or inhibiting influences is dysregulated the stress system becomes imbalanced resulting in an enhanced vulnerability for further insults. Such an imbalance not only is dependent on the amount of circulating hormone, but can also be affected by local tissue-specific conversions of the hormone (Holmes and Seckl, 2006). In addition, also at the effector level dysregulation of corticosteroid signalling may occur which then also contributes to enhance vulnerability or compromises resilience.

The classic view of cortisol action is that the glucocorticoid activates intracellular receptors which translocate to the nucleus, bind to specific DNA sequences and modulate mRNA regulation (Schaaf and Cidlowski, 2002; Funder, 1993; Schoneveld et al., 2004; Meijer et al., 2006; Joëls et al., 2006; Joëls, 2006). Two related receptors, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) bind cortisol, with the MR having a 10-fold higher affinity (Reul and de Kloet, 1985). In brain the MR is restricted to limbic areas. Because of the high affinity the occupancy obtained after the pulses is maintained over the one hour pulse intervals under basal condition (Conway-Campbell et al., 2007). The GR is widely expressed and becomes due to its lower affinity progressively occupied only at the ultradian peaks and after a stressful stimulus, while when hormone levels decrease the steroid readily dissociates (de Kloet et al., 2005). The importance of these two receptors in the glucocorticoid regulation of the stress response led us to postulate that their balance determines one's capability to cope with a challenge (de Kloet et al., 2005).

Resilience or vulnerability to develop stress-related disorders has been explained in terms of the so-called “three hit” model. In this model it is proposed that genetic variability in interaction with priming early life influences the response of brain and body following a major challenge in later life (de Kloet et al., 2007). The

genetic variant that potentially provides an unfavourable genetic make up is therefore a central component in this three hit model. In this contribution we focus on the genetic variability in the MR- and GR-genes and their roles in vulnerability and resilience.

1.1. Mechanism of action

GR are near ubiquitous, although their density is regulated to a large extent at the tissue level. Also in the brain, GR are detected in nearly all areas, with high levels observed in the hippocampus (Reul and de Kloet, 1985). The MR is restricted to aldosterone target organs such as sweat glands, distal colon, kidney and salivary glands where these receptors mediate aldosterone effects on the regulation of salt homeostasis (Funder, 2005). In the brain the MR are found in structures involved in maintenance of electrolyte balance such as the organum vasculosum lamina terminalis, the medial amygdala and other periventricular brain regions. Very high levels of MR are found in the hippocampus and other limbic structures, where this receptor is not selective for aldosterone, but also displays high affinity for other steroids such as the naturally occurring glucocorticoids cortisol and corticosterone as well as deoxycorticosterone, aldosterone and progesterone. The MR is actually a promiscuous receptor if not protected by 11β -steroid dehydrogenase type 2 (Holmes et al., 2006).

The classical model for corticosteroid function is based on translocation of the activated receptor to the nucleus where it binds responsive elements in regulatory regions thereby changing expression of the target gene (Pascual-Le Tallec and Lombes, 2005; De Bosscher et al., 2003; Meijer et al., 2006). However, many levels of control of corticosteroid receptor function have been identified. For example, translocation is modulated by chaperone proteins, which have a major impact on receptor conformation. Posttranslational modification of MR and GR changes their transactivational capacity. Transcriptional coactivators (e.g. SRC1) or corepressors (NcoR, SMRT) and the DNA structure (methylation, histone acetylation) confer additional specificity to the corticosteroid signal. In addition, the sequence and compositions of the glucocorticoid responsive element (GRE) will determine gene responsiveness to the MR and or GR (Meijer, 2006). General transcription factors such as NF- κ B and AP-1 interact with the GR (but not with the MR) resulting in mutual modulations (Christ et al., 2005).

Recently, fast non-genomic actions of corticosterone on glutamate transmission in brain were reported that appeared mediated by membrane receptors for the steroids. Surprisingly, the MR involved in non-genomic actions appeared identical to its nuclear congener (Karst et al., 2005), and similar evidence was previously emerging for the GR (Tasker et al., 2006). Importantly, at least in the hippocampal CA1 cell field these MR membrane receptors have a lower affinity/efficacy suggesting that such receptors require stress-levels of corticosteroids (Karst et al., 2005).

2. MR and GR gene structure

The gene for the GR is located on chromosome 5q31-32, and the MR on chromosome 4q31.1. The gene-structure of both

receptors is comparable, with the coding region of the genes being formed by exon 2–9, see Fig. 1 (DeRijk and de Kloet, 2005; de Kloet et al., 2007). The “classic” GR-protein consists of 777 amino acids with a molecular mass of 94 kD, while the “classic” MR-protein is composed of 984 amino acids with a mass of 110 kD. Five prime of exon 2 lies the promoter region, which is found to be highly complex for the GR-gene and recently new data have been added. Much less is known of the MR-gene promoter region.

For the human MR-gene, two different promoter regions have been described, designated P1 and P2 located just upstream of exon 1 α and 1 β , respectively (Zennaro et al., 1995; Listwak et al., 1996; Zennaro et al., 1996). P1 has been shown to be sensitive to corticosterone while P2 is sensitive to aldosterone. In addition, estrogens decrease expression, while progesterone increases expression of the MR, as previously was shown in the rat (Castren et al., 1995; Carey et al., 1995; Kalman and Spencer, 2002). In this species specific regulation of exon 1 α , β and γ have been observed in hippocampal subregions (Vazquez et al., 1998). Conditions such as stress, ageing and antenatal corticosteroid treatment are known to affect hippocampal MR mRNA expression (Noorlander et al., 2006; Schmidt et al., 2003; van Eekelen et al., 1991; Gesing et al., 2001). In the coding region of the MR-gene, a splice variant skipping exon 5 and 6, resulting in a protein of 75 kDa, was found to be a ligand independent transactivator capable of recruiting coactivators (Zennaro et al., 2001). A 12 bp insert just after exon 3, resulting from alternative splicing, did not affect DNA binding and its effect is currently unknown. Translational variants, resulting from alternative usage of the different ATG start codons in exon 2, give rise to MR-A and MR-B proteins with different transcriptional activities (Pascual-Le Tallec et al., 2004).

The human GR-gene 5' promoter region consists of at least 7 exon 1's showing additional splicing variability (Russcher et al.,

2007; Presul et al., 2007; Turner and Muller, 2005). These exon 1's are thought to be involved in the regulation of expression of the GR (Russcher et al., 2007). GR-expression is highly variable and is thought to underly differences in effects of corticosteroids on leukemia, metabolism, immune regulation and stress-reactivity. Tissue-specific regulation of GR during different stages of development has been shown. Interestingly, these differences during development appear permanent and seem to originate from the regulation of multiple alternative first exons or promoters of the GR gene (Meaney et al., 2007). GR regulation is also altered during the ageing process (Galeeva et al., 2006; Peiffer et al., 1991). Among the many factors that seem to influence GR-expression are corticosteroids, neurotransmitters and cytokines (Holmes et al., 1997; Webster et al., 2001; Nyirenda et al., 1998; Herman and Spencer, 1998; Mitchell et al., 1990). Changes in regulation of the GR-gene are thought to predispose for vulnerability to disease, particularly with respect to stress-related metabolic, cardiovascular and brain diseases.

In the GR-gene several splice variants, including the GR-P, GR- γ and GR β , have been tested for functionality. The GR-P (or GR-d) resulted from a splicing event in which exon 8 is replaced by intron G, giving rise to a truncated protein (676 amino acids). This GR-P is present in several freshly isolated haematological tumor cells or circulating lymphocytes from different donors, suggestive of widespread expression (de Lange et al., 2001; Gaitan et al., 1995; Parks et al., 2001; Hagendorf et al., 2005). Ligand binding is absent in this variant due to lack of exon 8–9, however, this variant could confer decreased corticosteroid-sensitivity, possibly important in tumor cells. The insertion of an additional codon, GTA (Arg), between exon 3 and 4 at AA 452, resulted in the so-called GR- γ (Rivers et al., 1999). Although hGR- γ seems rather ubiquitously expressed its function is presently unknown. The GR β is a variant of the 3' UTR of exon 9, in which an A to G change at the first A in the

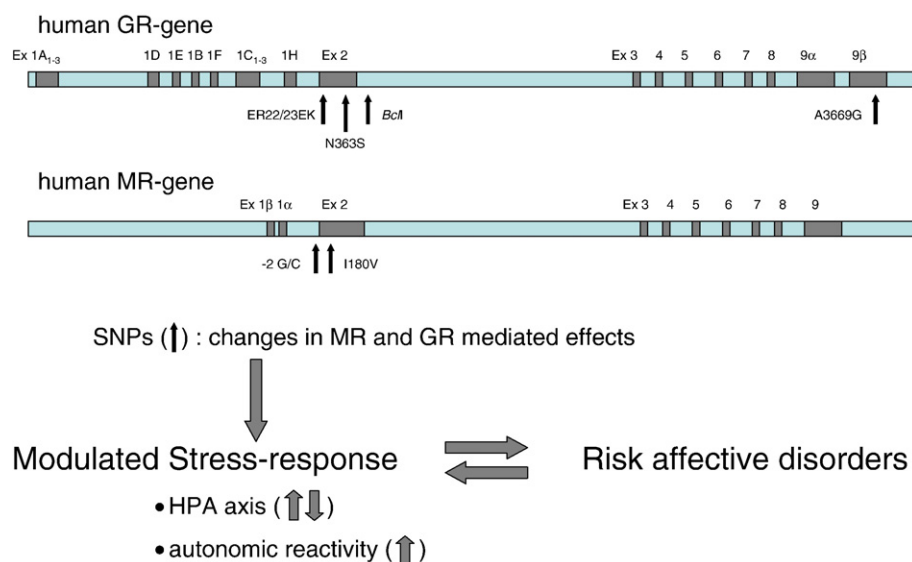


Fig. 1. Human MR-and GR-gene variants. Translation starts in the beginning of exon 2, and ends in exon 9, followed by an untranslated region (UTR). The GR-gene shows alternative splicing with two exon 9's which both can join exon 8, giving rise to GR α or GR β . Indicated are functional SNPs leading to amino acid changes GR: ER22/23EK, N363S; MR: I180V, involved in mRNA stabilization GR: A3669G, or molecular mechanisms still unknown GR: BclI-site; MR-2 G/C. These SNPs modulate the regulation of the stress-response as measured by cortisol and autonomic responses following the Trier Social Stress Test. GR and MR SNPs have been found to associate with depression, indicating that the GR and MR determine vulnerability to disease, such as affective disorders.

ATTTA sequence resulted *in vitro* in stabilization of GR β -mRNA and increased GR β protein expression (DeRijk et al., 2001). The expression of the GR β -variant in human brain tissue was found to be very low, although several days of exposure of immune cells to cytokines increases the GR β /GR α ratio to levels in which the presumed dominant activity of the GR β over the GR α could become important (Webster et al., 2001).

The complexity of the GR and MR-gene expression allows for extensive tissue and context specific regulation of corticosteroid-sensitivity. This could explain the numerous and diverse physiological responses elicited by corticosteroid hormones.

2.1. Mutations

Variations in nucleotide sequence can result in amino acid changes (non-synonymous SNPs) if they are located in a codon. If not in a coding region, they can influence gene regulation by effects on gene-splicing, transcription efficacy, promoter activity, translation efficacy or by introducing a stop codon (DeRijk et al., 2002).

Mutations, associated with a severe clinical phenotype, often consist of premature stop codons, deletions, inserts, abnormal splicing or amino acids changes. Mutations in the human MR-gene resulting in decreased function are associated with hypotension or pseudohypoaldosteronism (PHA1), a rare form of mineralocorticoid resistance characterized by salt loss, dehydration, vomiting and failure to thrive. The phenotype can be rather heterogeneous, ranging from severe forms with poor clinical outcome to milder forms in which treatment can be discontinued. In contrast to MR loss of function, activating MR mutations also exist, which can result for example in hypertension (see below; the S810L). Mutations within the GR-gene are also often not compatible with life. The corticosteroid-resistance syndrome is characterized by hypertension, excess androgens and increased plasma cortisol concentrations in the absence of the stigmata of Cushing's syndrome. Furthermore, several GR-gene mutations have been found in human malignancies including Cushing's disease and leukemia's.

2.2. Single nucleotide polymorphisms in the GR

Single nucleotide polymorphisms (SNPs) are one kind of variations in DNA sequences that occur with a frequency of at least 1%. These changes in nucleotide sequences give rise to different forms of the gene, which are called alleles. SNPs often appear in combinations, especially when they have a high frequency. Combinations of SNPs or alleles are named haplotypes. These combinations can have different effects compared to "isolated" SNPs. In genes often blocks of combinations of several SNPs are found, giving rise to several haplotypes in a gene. The construction of the haplotype map for the human genome is currently performed within the HapMap project. An example of GR gene haplotypes in a Dutch population is provided by the group of Koper (van den Akker et al., 2006a).

Several SNPs in the GR-gene have been tested for functionality. In intron 2, between the first and second ATG, the codons 22 and 23 of exon 2 (GAG AGG [GluArg or ER] to GAA AAG [GlyLys or

EK]; rs6189/rs6190) show linked polymorphisms (haplotype) originally detected by Koper et al. (1997). *In vitro*, the presence of this haplotype, that is the GAA AAG form having a frequency of approximately 4%, resulted in increased expression of GR-A, maybe as a result of changed secondary structure of the mRNA (Russcher et al., 2005b). The GR-A was found to be transcriptionally less active, resulting in an overall mild corticosteroid-resistance (Russcher et al., 2005a). After transfection in COS cells, the ER22/23EK displayed decreased transactivation, but no difference compared to wild type GR was seen on the NF- κ B transrepression (Russcher et al., 2005a). Importantly, ER22/23EK polymorphisms show strong linkage with the functional SNP in exon 9 β , the rs6198 (discussed below) (van den Akker et al., 2006a), leading to a possible interaction and modulation of each others effect *in vivo* (Kumsta et al., 2007).

At codon 363 (AAT $\rightarrow$ AGT, rs6195) in exon 2 of the GR-gene a non-synonymous change from Asp to Ser was initially identified in a Dutch kindred with hypercortisolism (Karl et al., 1993). Only recently functionality of this N363S was inferred using peripheral blood mononuclear lymphocytes and dexamethasone induced GILZ/decreased IL-2 production. Homozygote carriers of the N363S showed not only enhanced induction of GILZ, in line with the previously described increased corticosteroid-sensitivity, but also decreased inhibition of IL-2 production (van den Akker et al., 2006b). Using a whole blood assay, van Winsen et al. found a decreased sensitivity for dexamethasone using inhibition of lipopolysaccharide-induced TNF α production (van Winsen et al., 2005). The extent of potential N363S effects was further tested in both transiently and stable transfected cell lines (Jewell and Cidlowski, 2007). Although hardly any differences were observed in the transiently transfected cell, many marked changes in gene-expression, using a microarray, were seen in stable transfected cells even without incubation with ligand. These contrasting results, increased vs decreased activity of dexamethasone mediated effects, indicate that the effects of GR, and even more GR-N363S, are complex, modulating several regulatory systems at a time. For example not only suppression of NF- κ B action but also induction of inhibitory I- κ B α is possible, thereby interfering with IL-2 suppression (Russcher et al., 2005a).

The 3' UTR of the human GR-exon 9 harbors several ATTTA motifs, known to destabilize mRNA and thus providing a regulatory mechanism. Consequently, the A to G change (A3669G; rs6198) at the first A in the ATTTA sequence resulted *in vitro* in stabilization of GR β -mRNA and subsequently in an increased GR β expression (DeRijk et al., 2001). More recently, in leukocytes of carriers (without ER22/23EK) a modestly decreased transrepression of IL-2 mRNA was observed, although no effect was seen on transactivation of GILZ (van den Akker et al., 2006b).

Two more SNPs in the GR-gene have been used in numerous association studies, the so-called *BclI* site (rs 41423247), located 647 bp inside intron B, 3' of exon 2. For this *BclI* site, the wild type is a 5'-TGATCA-3' sequence and the variant is a 5'-TGATGA-3' sequence, the latter not being cut by *BclI* (Fleury et al., 2003). In the wild type, the *BclI* enzyme cuts and generates a 2.5 kD fragment, while the variant is not sensitive to *BclI* generating a fragment of 4.5 kD. In addition, Stevens et al. (2004), found the *BclI*-site to be in linkage disequilibrium with

two other polymorphism both located downstream, showing an allele spanning almost the entire intron B (between exon 2 and exon 3; ~80 kB). In the promoter region of the GR-gene, a polymorphic *Tth1111* site is found upstream of exon 1c, 3807 bp upstream of the first translation start site in exon 2 (van Rossum and Lamberts, 2004). To date the functionality of the *BclI* and *Tth1111* variants *in vitro* has not been reported.

2.3. Single nucleotide polymorphisms in the MR

In the MR-gene, a SNP in codon 810, changing serine to leucine, changed the effect of progesterone from being an antagonist for cortisol and aldosterone to an agonist at low concentrations. Also other steroids lacking the C–21–OH group became agonists. However, the frequency of this SNP in the general population seems to be very low (<1%), making the impact for common disorders unclear (Geller et al., 2000).

In exon 2, at codon 180, a GTT to ATT change resulted in an isoleucine to valine change. As with the S810L, this SNP modifies ligand specific activity of the MR. We and others showed that the MR-I180V shows *in vitro* loss of function using cortisol as a ligand, while aldosterone induced transactivation was not affected (Arai et al., 2003; DeRijk et al., 2006). Finally, at position-2, that is two nucleotides before the first ATG start codon, a G/C SNP (rs2070951) changes *in vitro* transactivational activity (Arai et al., 2003). The MR-I180V has an allele frequency of 11% resulting in 20% carriers, while the MR-2 G/C has an allele frequency of around 50%, making them both potential important risk factors.

Establishing functionality of a genetic variant *in vitro* indicates that the variant has a potential effect *in vivo*. This helps to formulate a *a priori* hypothesis, although this should be done with care, since *in vitro* often highly artificial systems are used bearing little resemblance with *in vivo* situations. An additional argument to strengthen associations is support from whole genome wide analysis. In case of the GR several studies have indicated that the 5q31-32, region where the GR is located, is associated with immune disorders such as Chron's disease, coeliac disease and asthma. Acute leukemias and other cancers often show deletion of the 5q region. Bipolar disease and schizophrenia have also been associated with the 5q region. The MR, located on 4q31, has been found in several studies to associate with mental disorders such as depression.

3. HPA axis regulation

Regulation of HPA-axis responsiveness is highly complex and dynamic with stress and context dependent responses (Goldstein, 2003; Dickerson and Kemeny, 2005; de Kloet et al., 2005). Indeed many different tests and paradigms have been developed testing these specific aspects of the HPA-axis.

Under basal non-challenge conditions the GR-gene *Tth1111* variant located in the promoter region of the GR-gene was reported to be associated with higher total and evening cortisol concentrations (Rosmond et al., 2000). However this could not be confirmed by others and it has been suggested that linkage with other GR-SNPs could interfere with the findings (van Rossum

et al., 2004). Recently, using 6 haplotype markers of the GR-gene, one particular haplotype was associated with higher cortisol during the day (Rautanen et al., 2006). Unfortunately it is not completely clear which functional GR-gene SNPs constitute this haplotype although the *BclI* G-variant was included. The MR has been implicated in tonic inhibition of the HPA axis under basal non stress conditions (Ratka et al., 1989). Consistent with this finding is that C-allele carriers of the MR G/C at position-2 of the MR-gene had lower cortisol levels than non carriers (Kuningas et al., 2006).

Using dexamethasone (1 and 0.25 mg) to test for negative feedback inhibition at the level of the pituitary, both the GR-gene *BclI* polymorphism and the N363S were found to be associated with increased suppression of cortisol in the morning (Stevens et al., 2004; Panarelli et al., 1998; van Rossum and Lamberts, 2004). In contrast, the GR-gene ER22/23EK showed the opposite effect: a decreased sensitivity to dexamethasone and thus a higher post-dexamethasone cortisol level (van Rossum and Lamberts, 2004). However, not all other studies could confirm these associations, although the *in vitro* tests of the GR-gene ER22/23EK and the N363S are supportive (van den Akker et al., 2006b; Russcher et al., 2005a). As also discussed below, the GR-gene 9 β A3669G was associated with significantly higher levels of post dexamethasone (0.25 mg) plasma ACTH and cortisol in males, but with lowest levels in females (Kumsta et al., 2007). No significant effect of the other GR-SNPs (ER22/23EK, N363S or *BclI*), was observed in this study.

The combined dexamethasone–CRH challenge is a pharmacological test of reactivity of the HPA axis (Heuser et al., 1994). Both GR and MR activity has been proposed to be involved (Holsboer, 2000). In a German cohort of depressed subject ($n=342$) the ER22/23EK, N363S and *BclI* were tested with respect to cortisol and ACTH responses to dexamethasone suppression (1.5 mg) and the subsequent stimulation with CRH (van Rossum et al., 2006b). However, no significant genotype effect was found in these patients.

The Trier Social Stress Test (TSST) involves a public speaking and mental arithmetic task in front of an audience and camera (Dickerson and Kemeny, 2005). The subsequent variability in cortisol responses can at least in part be explained by GR and MR-genotype. Wüst et al showed strong genetic effects of the N363S and the *BclI* variant on HPA-axis responsivity in a male twin cohort (Wüst et al., 2004). Carriers of the N363S showed higher saliva and plasma cortisol responses, after exposure to the TSST. Also following ACTH administration higher cortisol levels as compared to “wt-type” subjects were observed. *BclI* heterozygotes, had less high responses, both for cortisol and ACTH following the psychological challenge than the N363S carriers, but they were still higher than in “wt-type” individuals. *BclI* homozygotes however had lower cortisol responses as compared to controls, both following the psychological stressor and ACTH administration. The same cohort was also genotyped for the MR-I180V variant. It was found that the MR-I180V variant is not only associated with increased saliva and plasma cortisol responses, but also with increased autonomic output as measured by heart beat (DeRijk et al., 2006). The effects of the MR-I180V were not confounded by the GR-SNPs although the cohort was too small to

test for GR $\times$ MR interaction. In a follow-up study subjects were recruited based on their GR-genotype (Kumsta et al., 2007). In these males similar effects of the GR-genotypes *BclI* and N363S were found as in the male twin cohort. Interestingly, the previously not tested GR-gene 9 β A3669G was associated with even higher levels, both plasma cortisol and ACTH, but not for saliva cortisol (Kumsta et al., 2007). In women (all oral anticonception users), the GR-gene *BclI* G/G genotype showed highest levels of cortisol, while man with the same genotype had the lowest plasma cortisol level. Moreover, female GR-gene 363S carriers displayed an almost blunted salivary cortisol response, while this was highest in males.

Clearly, MR and GR gene variants are associated with the observed variability in HPA axis responses, with strong gender effects appearing. The MR seems to be involved in additional regulation of autonomic function during stress.

4. Disease

Two recent studies tested for associations between mental performance and GR and MR-gene SNPs in elderly cohorts since in old age cognitive decline and dementia is more prevalent. Based on the protective effect of the GR-gene ER22/23EK on metabolisms, the relationship between this allele and the risk of dementia and cognitive performance was tested in the so-called Rotterdam Study (van Rossum et al., 2006a). Indeed a protective effect of the ER22/23EK was suggested for dementia, although the effects were modest. Furthermore, psychomotor speed was improved in carriers, while no statistical significant effect was seen on global cognitive function and memory performance. White matter lesions in periventricular and subcortical areas (but not in the amygdala or hippocampus) were less prevalent in carriers, while the progression of the subcortical lesions was 70% lower. In a prospective study of subjects aged 85–90 years (Leiden 85-plus Study) a decline in several measures of cognitive function was observed as expected. No association with the MR-2 G/C or MR-I180V or the GR-gene ER22/23EK, N363S or *BclI* was found (Kuningas et al., 2006). High morning cortisol levels ($47 \geq \mu\text{mol/L}$) however, did predict lower attention and slower processing speed, while C-allele carriers of the MR-2 G/C had lower cortisol levels than non-carriers. No interaction between MR and GR-gene SNPs was found. Finally, carriers of the MR-I180V had higher values on the Geriatric Depression Scale (GDS-15) as compared to non-carriers. These findings provide some evidence that GR and MR gene variants interfere with cognitive function and brain integrity.

In patients suffering from Post Traumatic Stress Disorder (PTSD), low basal cortisol levels, increased reactivity of the HPA axis and increased corticosteroid-sensitivity has been inferred (Yehuda, 2002). Frequency of the GR-gene N363S or *BclI* was however not different in 118 Vietnam War veterans compared to control subjects ($n=42$) (Bachmann et al., 2005). However, also no differences were observed in corticosteroid-sensitivity as assessed using the 0.25 mg DST as would be expected from previous research.

Chronic fatigue syndrome is of unknown etiology although studies suggest HPA axis and autonomic dysregulation, with

possibly immune factors involved. In a relative small cohort ($n=140$), Rajeevan et al. found an association between different SNPs and with haplotypes (constructed with these SNPs) in the GR-gene and several scores of fatigue (Rajeevan et al., 2007; Smith et al., 2006). Interestingly, the extent of linkage disequilibrium varied with respect to the clinical status, indicative of effects of heterozygosity within the GR-gene on pathology. No functionality of the haplotypes was however provided.

Dysregulation of HPA axis reactivity in healthy family members of patients with affective disorders are documented, suggesting a genetic contribution through cortisol regulation (Holsboer et al., 1995; Ellenbogen et al., 2004). Several GR-gene SNPs have been tested for association. van West et al. (2006) tested the ER22/23EK, N363S and the N766N plus a “new” SNPs (designated NR3C1-1) in the promoter region between exon 1B and 1C in both a Belgium and a Swedish cohort. In the Belgium cohort an association was found with the SNP in the promoter region, while in the Swedish cohort the ER22/23EK associated with major depression using the DSM-IV criteria. In a German sample of depressed patients the ER22/23EK and the *BclI* were found to have significantly higher frequencies (van Rossum et al., 2006b). Also higher frequencies were found for the N363S, but this failed to reach significance. Frequencies of all three SNPs went up if the patients were additionally subdivided in recurrent unipolar depression. Response to antidepressant treatment was improved in carriers of the ER22/23EK. In a Dutch cohort, carriers of the *BclI* showing high ACTH responses during a combined dexamethasone–CRH challenge test had significant lower treatment response rates than non-carriers (Brouwer et al., 2006).

These data are indicative of causal effects of the MR and GR in cognition and psychopathology. Regulation of GR-expression seems also to be important, since haplotypes extending into the promoter region of the GR also associated with psychopathology (van West et al., 2006; Rajeevan et al., 2007). Finally, GR- and MR-SNPs could also contribute to the co-morbidity often observed in psychopathology, including cardiovascular diseases and autonomic activation (anxiety), metabolic syndrome (depression) and immune disturbances (bipolar diseases) (DeRijk and de Kloet, 2005).

5. Conclusions

Identification of MR- and GR-variants has important implications for understanding the role of corticosteroids in the maintenance of health and homeostasis. As summarized above functional GR-variants associate with changes in HPA axis regulation and behaviour, crucial systems for health and ageing (Brakefield et al., 2005). Recent evidence is accumulating that MR- and GR-variants underly psychopathology. MR- and GR-gene variants explain a large part of the variability in HPA axis (and autonomic) responses, indicating the importance of these corticosteroid receptors in centrally mediated stress-regulation. This is crucial since regulation of the stress-response will affect the whole body including e.g. the metabolism, cardiovascular systems and immune functions in which these MR- and GR-variants have effects in their own right. Indeed, associations of the four GR-SNPs have been described with metabolic parameters,

immune function and cardiovascular control (Rosmond et al., 2006; DeRijk et al., 2002; DeRijk and de Kloet, 2005).

Current evidence suggests that the MR activates signalling pathways aimed to prevent disturbance of homeostasis by facilitating the selection of an appropriate response to deal with a challenge (de Kloet et al., 2005). The GR represents a mechanism to recover from stress. A picture emerges that corticosteroid action is indispensable for optimal coping with a challenge and that their action is aimed at improving the (specific) system handling the challenge (Wilckens and DeRijk, 1997; Joëls et al., 2006). Unfortunately, as a trade-off effect, other systems are temporarily downregulated, enhancing vulnerability to other challenges. Moreover, efficacy of corticosteroid action is highly dependent on the context with determinants such as activity of the target system, timing of their effect, where they act, corticosteroid concentration and corticosteroid receptor activity. This complex mechanism of action probably underlies the large phenotypic differences in reaction to a challenge observed in a population. It is in this context that genetic variants of corticosteroid receptors can be protecting or vulnerability factors.

Diversity of corticosteroid action is explained at several levels (Joëls et al., 2006; Pascual-Le Tallec and Lombes, 2005; Schaaf and Cidlowski, 2002; Wilckens and DeRijk, 1997), with an important level being the dynamics of the MR-and GR-gene expression. This is provided not only by SNPs in the coding region of the gene, but also at the level of the expression of the gene with promoter region variants and splicing variants. Flexibility in expression of variants provides the gene with a mechanism to interact extensively with the environment, which effect can hardly be underestimated (van Praag et al., 2004), and provides the organism with a mechanism of phenotypic plasticity. Furthermore, HPA axis activity leading to the secretion of corticosteroids, is a resultant of central integration of peripheral signals threatening homeostasis. By regulating the stress-response the brain becomes a crucial factor determining the success of coping with a challenge.

Taken together, the data provide evidence that indeed MR and GR increase the risk for stress-related disorders such as depression and that these MR- and GR-gene variants are part of the genetic make up that determines individual coping style, affecting vulnerability to disease.

Acknowledgements

The research in this review was supported by de Hersenstichting Nederland (project No: 10F02(2).37: Single Nucleotide Polymorphism in the HPA-axis and in corticosteroid-responsive genes: implications for depression) and the Royal Netherlands Academy of Arts and Sciences.

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