Pituitary glucocorticoid receptor deletion reduces vulnerability to chronic stress

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Summary   The incidence of chronic stress is frequently related to the development of psychiatric disorders like depression. The hypothalamic-pituitary—adrenal (HPA) axis is a major physiological system that mediates the stress response. Tight HPA axis regulation through negative feedback mechanisms is essential for health and environmental adaptation. This feedback regulation acts in part through the glucocorticoid receptor (GR) on several organizational levels, including the pituitary, the hypothalamus and the hippocampus. However, the precise role of the different anatomical structures, specifically the pituitary, in HPA axis regulation is yet largely unknown. Here, we show that a conditional pituitary GR knockout is not necessarily detrimental for the animal's ability to cope with chronic stress situations. Mice with a deletion of the GR at the pituitary (GR^{POMCCre}) were subjected to 3 weeks of chronic social defeat stress. We analyzed both the behavioral and neuroendocrine phenotype as well as the central nervous system expression of genes involved in HPA axis function in these animals. Our results show a more resilient phenotype of GR^{POMCCre} mice with respect to anxiety-related behavior and neuroendocrine parameters compared to stressed wild type animals. In light of the previously reported high corticosterone levels during postnatal development in GR^{POMCCre} mice, our findings suggest that adverse early life events may have beneficial developmental effects on the organism to improve stress coping later in life.

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1. Introduction

In modern societies, stressful challenges, e.g. at the workplace, are prevalently encountered. Acute, short-term stressful situations can have predominantly beneficial effects that increase the individual's capability to cope with the increased environmental demands. On the other hand, chronic exposure to stress may lead to an allostatic load and has been consistently described to be a major risk factor for the development of psychiatric diseases, such as depression (McEwen, 2003, 2004). Etiological indications of a causal link between chronic stress and depression (Tennant, 2001) are further supported by findings from animal experiments showing a depression-like phenotype after application of

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chronic stress paradigms (Willner, 2005). However, while chronic stress is widely accepted as a major risk factor for the emergence of psychiatric diseases, the molecular basis of this interaction is not yet understood.

One of the main physiological systems that mediate the stress response is the hypothalamic-pituitary—adrenal (HPA) axis. Glucocorticoids (mainly cortisol in humans, corticosterone in rodents) are the hormonal endpoint of the HPA axis. Next to their manifold functions in the body, glucocorticoids also regulate the activity of the HPA axis through negative feedback via two steroid receptor subtypes, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) (de Kloet et al., 1998). Negative feedback has been shown to occur at different hierarchical levels of the HPA axis, including the hippocampus, the paraventricular nucleus of the hypothalamus and the pituitary (Sapolsky et al., 2000; Schmidt et al., 2005; Herman et al., 2005). Interestingly, one of the most consistent findings in depressed patients is an impairment of negative feedback in the dexamethasone/corticotropin-releasing hormone (CRH) challenge test (Holsboer, 2000; Ising et al., 2005). However, the particular contribution of the different brain circuits in the physiology and pathophysiology of negative feedback regulation is still unclear.

Studies in animal models were able to show that chronic stress has pronounced and lasting effects on the physiology and function of the HPA axis (Joëls et al., 2007; Ulrich-Lai and Herman, 2009). One of the best established models for chronic social stress in rodents is the chronic social defeat stress (CSDS) paradigm, which constitutes a potent stressor, based on social confrontation with a conspecific, over a prolonged period of time (Keeney and Hogg, 1999; Bartolomucci et al., 2004; Keeney et al., 2006). Kudryavtseva and Avgustinovich showed marked behavioral alterations in mice following CSDS such as decreased exploratory activity and increased anxiety-like behavior (Kudryavtseva et al., 1991; Kudryavtseva and Avgustinovich, 1998). It was also shown that CSDS influences the brain expression of core HPA axis regulators like arginine-vasopressin (AVP) and CRH (Keeney and Hogg, 1999; Keeney et al., 2006).

Human studies clearly demonstrate that chronic stress exposure is not detrimental for every individual and actually most people show a remarkable degree of resilience against chronic stress (Feder et al., 2009). As stress-associated psychiatric diseases also have a substantial heritable proportion, it is generally thought that specific genetic risk factors need to be combined with environmental challenges to result in disease (Kendler et al., 1995; Schmidt et al., 2010). Therefore, combining a chronic social stress model with a certain genetic risk factor may contribute further insights to the particular involvement of the specific genes or structures in disease vulnerability.

Recently, we developed a novel conditional knockout mouse line (GR[POMCCre]), which lacks the GR in all Pro-opiomelanocortin (POMC) expressing cells, thus mainly in the pituitary and in some neural structures as the arcuate nucleus (Schmidt et al., 2009). These animals show a very high HPA axis activity and elevated corticosterone levels during postnatal development due to the lack of negative feedback at the pituitary level. Remarkably, this excess of postnatal glucocorticoid exposure had only moderate effects on the adult phenotype of these mice. In light of these findings, we hypothesized that these mice may show an altered risk for chronic stress exposure during adulthood. To test this hypothesis, we analyzed the response of these animals to a CSDS at the behavioral, neuroendocrine and molecular level.

2. Methods

2.1. Animals and animal housing

Generation, breeding and genotyping of GR[POMCCre] mice have been described previously (Schmidt et al., 2009). In short, mice with a homozygous mutation of the GR gene, in which exon three is flanked by two loxP sites, were crossed with an effector mouse line, which expresses the Cre-recombinase (Cre) in a region- and cell-specific manner under the control of the Pro-opiomelanocortin (POMC) promoter (Akagi et al., 1997). This leads to a deletion of the GR in all POMC-expressing cells, including POMC-expressing cells in the pituitary. Genotyping was performed by PCR analysis of tail DNA. Only male mice at the age of 11—13 weeks were used for the experiment. These were single housed at least 2 weeks before onset of the experiment, held under a 12 h light, 12h-dark cycle (lights on at 06:00 h) and constant temperature ($23 \pm 2\,°C$) conditions. Food and water were provided ad libitum.

Male CD1 mice (16—18 weeks of age) served as resident mice, which were held under the conditions described above. They were allowed to habituate to the social defeat cage for 2 weeks prior to the experiment. The experiments were performed in accordance with European Communities Council Directive 86/609/EEC. All efforts were made to minimize animal suffering during the experiments. The protocols were approved by the committee for the Care and Use of Laboratory Animals of the Government of Upper Bavaria, Germany.

2.2. Chronic social defeat stress paradigm

The CSDS procedure has been extensively described and validated before (Berton et al., 2006; Kinsey et al., 2007; Haenisch et al., 2009). Briefly, animals were introduced to a physically superior resident mouse until defeat was achieved. Subsequently the animals spent 24 h in the same cage (45 cm × 25 cm), divided by a holed metal partition to allow for sensory, but not for physical contact. Stressed animals were introduced to a new resident cage every day. The rotation schedule was set to exclude a repeated encounter with the same resident throughout the experiment. Experimental mice were always defeated by the resident males along the entire experimental phase. Control mice remained in their home cages for the course of the experiment. All mice were handled daily, weight and fur status were assessed every 3—4 days.

The evaluation of the fur state was carried out as described previously (Mineur et al., 2003). Briefly, furs were rated on a scale from one to four by an experienced investigator, where 1 represents a perfect, clean fur, while 4 stands for a dishevelled, scruffy fur, often including wounds and scurf. Ratings of 2 and 3 represent intermediate fur states, respectively.