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Mineralocorticoid receptors in control of emotional arousal and fear memory

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ABSTRACT

The stress hormone corticosterone acts via two receptor types in the brain: the mineralocorticoid (MR) and the glucocorticoid receptor (GR). Both receptors are involved in processing of stressful events. A disbalance of MR:GR functions is thought to promote stress-related disorders. Here we studied the effect of stress on emotional and cognitive behaviors in mice with forebrain-specific inactivation of the MR gene ($MR^{CaMKCre}$, 4 months old; and control littermates). $MR^{CaMKCre}$ mice responded to prior stress (5 min of restraint) with higher arousal and less locomotor activity in an exploration task. A fear conditioning paradigm allowed assessing in one experimental procedure both context- and cue-related fear. During conditioning, $MR^{CaMKCre}$ mice expressed more cue-related freezing. During memory test, contextual freezing remained potentiated, while control mice distinguished between cue (more freezing) and context episodes (less freezing) in the second memory test. At this time, plasma corticosterone levels of $MR^{CaMKCre}$ mice were 40% higher than in controls. We conclude that control of emotional arousal and adaptive behaviors is lost in the absence of forebrain MR, and thus, anxiety-related responses are and remain augmented. We propose that such a disbalance in MR:GR functions in $MR^{CaMKCre}$ mice provides the conditions for an animal model for anxiety-related disorders.

Introduction

The involvement of the glucocorticoid stress system in control of emotional arousal and cognitive performance is well established (de Kloet et al., 2005; Lupien et al., 2007; Taylor and Liberzon, 2007). The major glucocorticoid hormones, corticosterone in rodents and cortisol in humans, bind to two steroid receptor types in the brain: the high affinity mineralocorticoid (MR) and the tenfold lower affinity glucocorticoid receptor (GR). Both receptors are located in forebrain areas involved in emotional regulation, learning and memory processes (Oitzl and de Kloet, 1992; de Kloet et al., 1999; Berger et al., 2006). Altered functionality of MR and GR is considered as vulnerability factor for stress-related disorders such as depression, generalized anxiety and post traumatic stress disorder (Xing et al., 2004; van West et al., 2006; de Kloet et al., 2007; Tronel and Alberini, 2007; Kellner and Wiedemann, 2008). Genetic changes in the MR:GR system of mice provide the base for animal models for such disorders (Gass et al., 2001; Urani and Gass, 2003; Berger et al., 2006).

Recently, Berger et al. (2006) generated mice with ablations of MR in forebrain brain areas that showed a weak behavioral phenotype. Since the effects of corticosterone are conditional, thus, depending on time and contextual factors (de Kloet et al., 2005; Joels et al., 2006), we expected that MR functions will become prominent under stress. Therefore, we will identify the emotional and cognitive consequences of a disbalanced MR:GR function in stressful conditions.

MR and GR act as transcription factors and mediate complementary but also in part overlapping actions of corticosterone in endocrine and behavioral functions. Corticosterone facilitates the recovery from stress by a negative feedback action via GR (Ratka et al., 1989; van Haarst et al., 1997; Feldman and Weidenfeld, 1999) and in parallel, facilitates memory consolidation (Oitzl and de Kloet, 1992; Oitzl et al., 2001; Donley et al., 2005). MR mediates the regulation of basal corticosterone levels and the initial corticosterone secretion during the ultradian rhythm and after stress (Ratka et al., 1989; Oitzl et al., 1995; Pace and Spencer, 2005; Atkinson et al., 2008). MR is involved in behavioral responses in novel situations, the so-called behavioral strategy (Oitzl and de Kloet, 1992; Oitzl et al., 1994; Berger et al., 2006; Lai et al., 2007) and coordinates most likely together with GR, subsequent memory processes (Berger et al., 2006; Lai et al., 2007; Rozeboom et al., 2007).

Mice with forebrain ablation of MR ($MR^{CaMKCre}$; Berger et al., 2006) had been subjected to a variety of standardized behavioral paradigms which revealed rather subtle behavioral changes such as delayed learning of the water-maze task, some deficits in working memory on the radial maze. Anxiety-related behavior and exploration patterns in the zero maze and open field, light-dark-box, elevated 0-maze were comparable to control mice. Also the performance in a two-way avoidance task was not affected by the lack of forebrain MR. Some hyperreactivity was observed when a novel object was introduced in a

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familiar environment. Secretion of corticosterone in the morning and evening and in response to restraint appeared to be comparable in $MR^{CaMKCre}$ and control mice. In fact, surprisingly minor effects were reported given the loss of forebrain MR.

We expected that MR functions will become more prominent under stress. Behavioral tests for unconditioned behavior are usually short-lasting (5–10 min). When performed under low(non)-stress conditions, MR functions might not be revealed given the conditional action of corticosterone. Therefore, we acutely stressed $MR^{CaMKCre}$ and control mice and tested for unconditioned behavior in an exploratory task which is sensitive to differential MR and GR activation (Brinks et al., 2007a). In a second experiment we subjected $MR^{CaMKCre}$ and control mice to a fear conditioning protocol that allows to test acquisition, consolidation, retrieval and extinction of fear memories for both context and cue in one procedure (Brinks et al., 2008, 2009). Based on the proposed function of MR on the appraisal of novel situations, we expected changes in the acquisition and extinction of fear memories to context and cues. In both experiments, we measured plasma corticosterone concentrations before and in response to the tasks, as index for endocrine corticosteroid receptor function.

Materials and methods

Animals

$MR^{CaMKCre}$ mice (female, 4 months) and female control ($MR^{flox/flox}$) littermates ($n=8$ per group) were obtained from the German Cancer Research Center (Heidelberg, Germany). As described elsewhere the conditional MR allele (MR^{flox}) was generated in 129Ola embryonic stem cells and the CaMKCre transgene was injected into the male pronucleus of FVB/N zygotes (Berger et al., 2006). The MR^{flox} allele and the Cre transgene have been backcrossed into C57BL/6J for more than 12 generations. The female $MR^{CaMKCre}$ mice and their control sisters were obtained by breeding $MR^{flox/flox}$ with $MR^{flox/wtCaMKCre}$ mice. The eight mutant and control animals originated from 7 litters from 7 different breeding pairs containing at least one control and one mutant animal. In an additional experiment, C57BL/6J mice (female, 4 months) were used to assess the effect of stress conditions on the outcome of the holeboard test (Charles River, Maastricht, The Netherlands; with specific attention to the BL/6J from this supplier). After arrival, mice were housed individually in the experimental room in Macrolon cages (translucent plastic: $44\times22\times17$ cm) with sawdust bedding, a tissue for nest building, water and food *ad libitum*, at 20 °C with controlled humidity under a 12 h: 12 h light/dark cycle (lights on at 08.00 h) for 1 week. Experiments were performed between 09.00 and 13.30 h (during the non-active phase of the mice) and were approved by the committee on Animal Health and Care from Leiden University, The Netherlands, in compliance with the EC Council Directive of November 1986 (86/609/EEC) for the care and use of laboratory animals.

Experiment 1: stress-induced unconditioned behavior in the modified holeboard

Apparatus

The modified holeboard consisted of a grey PVC box ($50\times50\times50$ cm) with a grey PVC centerboard (37×20 cm) on which ten dark grey cylinders were staggered (4 cm height; with a grid at the bottom; (Ohl et al., 2003; Brinks et al., 2007b). During testing, a camera was placed above the setting to allow later pathway reconstruction from video tape. Light intensity of the experimental room was set at 80 lx and a 20 dB background noise originating from a radio.

Experimental procedure

To induce a stress response, mice were subjected to 5 min of restraint in an adjacent room. Mice were fixed in a narrow cylinder that still allowed breathing but no further movement (transparent Plexiglas; diameter: 2.5 cm, 8 cm long). This method has been shown to activate the HPA-axis and enhance corticosterone concentrations in mice (Dong et al., 2004; Bowers et al., 2008). Immediately after restraint, the mice were placed in the holeboard for 5 min. All mice started in the same corner, facing the wall. The setup was cleaned with normal tap water between trials.

Behavioral observations

We used a semi-automatic system (The Observer 4.1, Noldus, Wageningen, The Netherlands) and scored the total number of rearing, sitting and walking events, as well as the time on the centerboard, sitting, walking and grooming. Walking patterns, distance moved and velocity were analyzed from video tapes (EthoVision, Noldus, Wageningen, The Netherlands). Thigmotaxic was defined as time spent in the rim area: 6.5 cm width along the walls.

Experiment 2: conditioned responses — fear conditioning to cue and context

Apparatus

Combined cue and contextual fear conditioning was performed in a conditioning chamber (black Plexiglas walls; $25\times25\times35$ cm high). The top was fitted with a 3 cm transparent rim. A speaker was positioned in one of the walls (25 cm high) connected to a tone generator (70 dB). Stainless steel bars on the bottom of the chamber ($n=37$; 5 mm diameter, spaced 5 mm) were connected to a shock generator (0.4 mA). Tissues were placed in a tray under the bars to collect faeces and urine of the mice. A white light source (260 lx) and a camera were placed 20 cm above the conditioning chamber. A radio on the other side of the experimental room produced 20 dB of background noise. The light intensity of the experimental room was 90 lx. After each animal, the chamber was cleaned with tap water and the tissues were replaced.

Experimental procedure

The fear conditioning experiment started 1 week after holeboard testing. The paradigm allowed to test the ability of mice to express their fear-related behavior during alternating cue-on and context (cue-off) episodes in the same setting. We recently found that BALB/c mice show strong fear-responses to context and cue (i.e., generalization), while C57BL/6J mice display specific fear memory towards the predictive conditioned stimulus, the cue (Brinks et al., 2008, 2009). Conditioning (training; day 1) included 3 min of baseline recording, followed by 6 light/tone (CS)+ shock (US) pairings with a 1 min cue-off interval, i.e., the context episode. Light and tone were paired for 20 s and an electric footshock was administered during the last 2 s. Two minutes after the last pairing, mice returned to their home cage. At 48 and 72 h after conditioning (days 3 and 4 respectively), the same procedure was repeated without shocks to test for memory and extinction of the conditioned fear response. The procedure lasted 12 min per mouse per day and was performed between 09:00 and 13.00 h.

Behavioral observations

Freezing behavior was recorded as main expression of fear behavior. Freezing is defined as immobility of the body including the head, devoid of any interaction with the environment. We also scored jumping, rearing, sitting, walking, scanning, grooming and stretched-attends, to determine possible strain differences in unconditioned responses to the apparatus before fear conditioning started, as well as on all days of the fear conditioning experiment. Behaviors were scored from video tape using a semi-automatic scoring program (The Observer 4.1, Noldus, Wageningen, The Netherlands). Walking patterns during first exposure to the fear conditioning apparatus were reconstructed from the video tape using EthoVision (Noldus,