Psychoneuroendocrinology 70 (2016) 33–37

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Short communication

Chronic stress does not further exacerbate the abnormal psychoneuroendocrine phenotype of Cbg-deficient male mice

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ARTICLE INFO

Article history:
Received 15 February 2016
Received in revised form 24 March 2016
Accepted 18 April 2016

Keywords:
CBG
Transcortin
Corticosterone
Chronic stress
Social defeat

ABSTRACT

Chronic stress leads to a dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis which can constitute a base for pathophysiological consequences. Using mice totally deficient in Corticosteroid binding globulin (CBG), we have previously demonstrated the important role of CBG in eliciting an adequate response to an acute stressor. Here, we have studied its role in chronic stress situations. We have submitted Cbg ko and wild-type (WT) male mice to two different chronic stress paradigms – the unpredictable chronic mild stress and the social defeat. Then, their impact on neuroendocrine function – through corticosterone and CBG measurement – and behavioral responses – via anxiety and despair-like behavioral tests – was evaluated. Both chronic stress paradigms increased the display of despair-like behavior in WT mice, while that from Cbg ko mice – which was already high – was not aggravated. We have also found that control and defeated (stressed) Cbg ko mice show no difference in the social interaction test, while defeated WT mice reduce their interaction time when compared to unstressed WT mice. Interestingly, the same pattern was observed for corticosterone levels, where both chronic stress paradigms lowered the corticosterone levels of WT mice, while those from Cbg ko mice remained low and unaltered. Plasma CBG binding capacity remained unaltered in WT mice regardless of the stress paradigm. Through the use of the Cbg ko mice, which only differs genetically from WT mice by the absence of CBG, we demonstrated that CBG is crucial in modulating the effects of stress on plasma corticosterone levels and consequently on behavior. In conclusion, individuals with CBG deficiency, whether genetically or environmentally-induced, are vulnerable to acute stress but do not have their abnormal psychoneuroendocrine phenotype further affected by chronic stress.

1. Introduction

While an appropriate acute response to stress is crucial for adaptation, intensified and prolonged responses have been suggested to cause or exacerbate several ailments—from common cold to cancer and various psychiatric conditions (de Kloet et al., 2005; McEwen et al., 2015). Given the amount of chronic stressors that individuals living in modern societies are exposed to, the study of its impact on health is of great importance. The hypothalamic-pituitary-adrenal (HPA) axis is the major neuroendocrine system involved in the stress response, and regulates the secretion of glucocorticoids. Protracted stressor exposure, called chronic stress, leads to a dysregulation (hyper- or hypo-activity) of the HPA axis which can induce detrimental effects on the whole body and constitute a base for pathophysiological consequences (Chrousos, 2009; de Kloet et al., 2005; McEwen, 2007).

Evidence shows that Corticosteroid binding globulin (CBG) – a plasma binding protein with high affinity for glucocorticoids – is particularly important for an appropriate response to stress. Our team has developed a model of HPA axis hyporesponse due to CBG deficiency, the Cbg ko mice. These animals display altered emotional reactivity and memory function after acute stress, which is associated with a blunted rise of glucocorticoids. We have reported that male Cbg ko mice display more despair-like behaviors than wild-type (WT) mice. This was observed in several depression models that involve acute stressors, such as the forced swim test, the tail suspension test and the learned helplessness paradigm (Richard et al., 2010). These results were confirmed in female Cbg ko mice, although estrogens-glucocorticoids crosstalks take place in WT ani-

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http://dx.doi.org/10.1016/j.psyneuen.2016.04.014
0306-4530/

mals (Minni et al., 2014). We have also demonstrated that CBG, through its role in regulating GC delivery to the brain, is indirectly involved in memory processes. Indeed, Cbg ko mice are insensitive to the impairments in memory retrieval induced by an acute stress, due to their lower rise of GC levels in the hippocampus (Minni et al., 2012).

In the light of the important role of CBG for an adequate response to an acute stressor, we decided to study its role in chronic stress situations. For that, we have chosen two different paradigms that can be compared to chronic stressors to which humans are regularly submitted to: the unpredictable chronic mild stress (UCMS) – representing the chronic accumulation of daily mild stressors; and the social defeat – as a parallel to more intense and stressful situations promoted by social interactions. Cbg ko mice were submitted to both chronic stress paradigms, and their impact in some neuroendocrine and behavioral responses was then evaluated.

2. Materials and methods

2.1. Animals

Two groups of 6–7 months old mice (MGI ID: 4833641, Serpina6tm1·1Mmp, created in our laboratory in Bordeaux—France)—were used in this experiment. Cbg ko and WT mice were littermates, obtained by breeding males and females heterozygous for the CBG mutation, and genotyped from a tail biopsy. As 1 out of 8 pups on average has the male sex and genotype Cbg−/− (ko) or Cbg+/+ (WT), and the litter size was of 2–12 pups (mean of 6.5 pups), we used 1 pup per litter per genotype per group. Our mice have a C57BL/6J genetic background above 95%. Animals were maintained, from birth and throughout the whole experiment, in an animal facility with constant temperature (23 °C), under a 12 h light/dark cycle (lights on at 0700 h) and with *ad libitum* access to food and water. All the experiments were conducted in strict compliance with current European Conventions (86/609/CEE) and approved by Institutional Regional Committee for animal experimentation (agreement# 5012050-A).

2.2. Unpredicted chronic mild stress

A first set of 36 mice, 8–10 mice/group, was evaluated in this experiment. Half of the animals from each genotype were placed into individual cages and submitted to a 4-week protocol of UCMS (adapted from Schweizer et al., 2009) while the other half were kept four mice per cage and served as the control group. The UCMS consisted of random exposure to several mild stressors, twice a day. The stressors included wet cage, tilted cage, no sawdust bedding, lights-on overnight, cage exchange with another mouse and facial air puff. After the four weeks of UCMS, mice were submitted to behavioral tests and sacrificed four weeks after the end of the UCMS protocol.

2.3. Social defeat

A second set of 24 mice, 6 mice/group, was submitted to a social defeat protocol as described in Berton et al., 2006 and modified according to Larrieu et al., 2014. Half of the mice were exposed to a CD1 mouse (aggressor) for 10 days, 5 min a day; while the other half served as the control group. Social defeat was followed by 3 h of protected sensory contact with the aggressor. Different aggressors were used on each day to prevent any habituation, and animals from the same cage were tested simultaneously. During the 3 h of protected sensory contact, mice from the control groups were submitted to a similar display, but in the absence of other mouse. Animals were sacrificed one week after the end of the social defeat protocol.

2.4. Behavioral tests after chronic stress

Mice from the first (after UCMS) and second (social defeat) experiments had their despair-like behavior (assessed through the forced swim test) and anxiety-related behavior (assessed through the open field test after the UCMS and through the elevated plus maze after the social defeat) analyzed. All three tests were performed as previously described (Minni et al., 2014; Richard et al., 2010). All behavioral sessions were recorded and analyzed by a trained observer blind to the animals' genotypes.

Twenty-four hours after the end of the social defeat protocol, both control and defeated mice were submitted to the social interaction test (Berton et al., 2006; Larrieu et al., 2014). During the first 5 min of test, the mouse was placed in an open field arena containing an empty wire mesh cage in one of the corners. Then, a CD1 mouse was introduced into the wire mesh cage and active investigatory behavior towards the intruder was recorded for another 5 min to assess social interaction. Approach and avoidance zones were defined and the number of visits and time spent in each of these zones were quantified by a trained experimenter blind to the animals' genotypes.

2.5. Sacrifice

Mice from both experiments were anesthetized with isoflurane (Aerrane, Baxter SA, Maurepas, France) and blood samples were obtained by cardiac puncture. Blood samples obtained in the morning were centrifuged at 10,000 rpm for 10 min, and the supernatant (plasma) was stored at −80 °C.

2.6. Total corticosterone and CBG measures

Total corticosterone was measured by an in-house radio immunoassay (RIA) from plasma samples previously extracted with ethanol. This assay is based on the competition for a specific anti-corticosterone antibody, between endogenous plasma corticosterone and increasing concentrations of a radio-labeled corticosterone (^{3}H) (see Richard et al., 2010 for details). Anti-corticosterone antibody was provided by Dr. H. Vaudry (University of Rouen, France). CBG *B*max in WT animals was estimated by adding increasing concentrations of radio-labeled corticosterone (^{3}H) (from 0.5 nM up to 32 nM) to plasma that had their endogenous steroids previously removed with charcoal-dextran. Through linear regression we obtained a saturation curve that allowed us to determine CBG's maximum binding capacity (Richard et al., 2010).

2.7. Statistics

All results are expressed as mean ± S.E.M. Statistical values were calculated with the software Statistica v.6.1. Unpaired Student's *t*-test was used for CBG binding capacity assay results. Two-way analysis of variance (ANOVA) was used to detect genotype and chronic stressor effects, followed by Duncan post hoc tests when necessary. A two-way repeated measures ANOVA was used to analyze weight change throughout the chronic stress paradigms, also followed by Duncan post hoc tests when necessary. The level of statistical significance was set as p values <0.05 for all tests. Outliers were detected and removed from analyses following Grubbs test.

3. Results

Mice submitted to both types of stress lost weight when compared to unstressed groups [main effect of UCMS, $F(1,32) = 9.72$,