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Disruption of glucocorticoid receptors in the noradrenergic system leads to BDNF up-regulation and altered serotonergic transmission associated with a depressive-like phenotype in female GR^{DBHCre} mice

Piotr Chmielarz [a,1], Grzegorz Kreiner [a,1], Marta Kot [b], Agnieszka Zelek-Molik [a], Marta Kowalska [a], Monika Bagińska [a], Władysława Anna Daniel [b], Irena Nalepa [a,*]

[a] Department of Brain Biochemistry, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland
[b] Department of Pharmacokinetics and Drug Metabolism, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

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ABSTRACT

Recently, we have demonstrated that conditional inactivation of glucocorticoid receptors (GRs) in the noradrenergic system, may evoke depressive-like behavior in female but not male mutant mice (GR^{DBHCre} mice). The aim of the current study was to dissect how selective ablation of glucocorticoid signaling in the noradrenergic system influences the previously reported depressive-like phenotype and whether it might be linked to neurotrophic alterations or secondary changes in the serotonergic system. We demonstrated that selective depletion of GRs enhances brain derived neurotrophic factor (BDNF) expression in female but not male GR^{DBHCre} mice on both the mRNA and protein levels. The possible impact of the mutation on brain noradrenergic and serotonergic systems was addressed by investigating the tissue neurotransmitter levels under basal conditions and after acute restraint stress. The findings indicated a stress-provoked differential response in tissue noradrenaline content in the GR^{DBHCre} female but not male mutant mice. An analogous gender-specific effect was identified in the diminished content of 5-hydroxyindoleacetic acid, the main metabolite of serotonin, in the prefrontal cortex, which suggests down-regulation of this monoamine system in female GR^{DBHCre} mice. The lack of GR also resulted in an up-regulation of alpha2-adrenergic receptor (α_2-AR) density in the female but not male mutants in the locus coeruleus. We have also confirmed the utility of the investigated model in pharmacological studies, which demonstrates that the depressive-like phenotype of GR^{DBHCre} female mice can be reversed by antidepressant treatment with desipramine or fluoxetine, with the latter drug evoking more pronounced effects. Overall, our study validates the use of female GR^{DBHCre} mice as an interesting and novel genetic tool for the investigation of the cross-connected mechanisms of depression that is not only based on behavioral phenotypes.

1. Introduction

Serotonergic and noradrenergic deficits in the central nervous system are crucial components of the monoaminergic hypothesis that underlies the pathophysiological basis of depression (Delgado, 2000). This observation is supported by the well-known mechanism of the primary action of antidepressants, which enhance the levels of these neurotransmitters in the brain while alleviating depression symptoms (Ressler and Nemeroff, 2000; Vetulani and Nalepa, 2000). However, the greatest shortcoming of this assumption may be the fact that it fails to explain the observed delay in the clinical effects of antidepressants and the profound rate of treatment resistant patients. Other prominent hypotheses associate depression with chronic stress exposure and glucocorticoid signaling dysregulation (Holsboer, 2000) or with changes in neurotrophins, particularly brain derived neurotrophic factor (BDNF) (Duman et al., 1997; Autry and Monteggia, 2012), which can influence synaptic plasticity and adult neurogenesis (Duman and Monteggia, 2006).

These hypotheses are not mutually exclusive, and there is considerable evidence for interactions among the monoaminergic system, neurotrophins and glucocorticoids (Vinet et al., 2004), as well as their crosstalk at the intracellular protein level (Nalepa, 1994; Nalepa and Sulser, 2004). Moreover, there have been several attempts to identify a protein that could serve as a convergence point for antidepressant treatment; the prominent example is cyclic AMP response element binding protein (CREB) transcription factor, which is known to be

Abbreviations: 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; AR, adrenergic receptors; BDNF, brain derived neurotrophic factor; CREB, cyclic AMP response element binding protein; DG, dentate gyrus; FST, forced swimming test; GRs, glucocorticoid receptors; HPA, hypothalamic–pituitary–adrenal; HPLC, high-performance liquid chromatography; LC, locus coeruleus; NA, noradrenaline; PFC, prefrontal cortex; TST, tail suspension test.

* Corresponding author at: Department of Brain Biochemistry, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Kraków, Poland.
E-mail address: nfnalepa@cyf-kr.edu.pl (I. Nalepa).
[1] These authors contributed equally to this work.

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up-regulated by chronic treatment with some antidepressants (Blendy, 2006). It has been demonstrated that the level of BDNF, for which transcription is regulated by a CREB-dependent mechanism, is increased both by noradrenergic and serotonergic (including dual-action) antidepressants, whereas exposure to stress downregulates BDNF expression (Duman and Monteggia, 2006), and this downregulation can be prevented by antidepressant treatment (Russo-Neustadt et al., 2001; Tsankova et al., 2006). However, BDNF can regulate serotonin (5-HT) signaling because its main receptor, TrkB, is also present on serotonergic neurons (Madhav et al., 2001). It has also been proven that BDNF application can affect 5-HT reuptake (Deltheil et al., 2008).

The monoaminergic systems can influence each other. The serotonergic system may be inhibited by noradrenaline (NA) through actions on α_1- and β-adrenergic receptors on serotonergic neurons in the raphe nuclei; however, serotonergic projections can inhibit the activity of the locus coeruleus (LC), a major source of noradrenergic projections (Haddjeri et al., 1997; Ressler and Nemeroff, 2000). Furthermore, glucocorticoid receptors (GRs), which are present on both noradrenergic and serotonergic neurons (Harfstrand et al., 1986; Czyrak and Chocyk, 2001), are involved in the regulation of the biosynthesis of both 5-HT (Clark et al., 2008) and NA (Makino et al., 2002), an additional player that influences the action of antidepressants (Heydendael and Jacobson, 2009).

Considering the interactions between monoamines, neurotrophins and glucocorticoids, as well as their ubiquitous presence in the limbic structures that regulate central integrative systems, such as the hypothalamic–pituitary–adrenal (HPA) axis (Erdmann et al., 2008; Kvetnansky et al., 2009), it is conceivable that initial alterations in any of these compounds may ultimately lead to abnormalities in all compounds, which may result in a depressive-like state. Nevertheless, it remains unclear which of these observed abnormalities are primary or secondary.

We have recently demonstrated that female but not male mice that harbor selective ablation of GR in the noradrenergic system (GR^{DBHCre} mice) exhibit depressive- and anxiety-like behaviors (Chmielarz et al., 2013). Moreover, the male GR^{DBHCre} mutants not only did not exhibit changes in basal behavior but were resistant to chronic restraint stress, which clearly indicates the gender-dependent response to this particular mutation. The gender differences in our model are particularly interesting because depression is more prevalent in women (Seedat et al., 2009), and this issue has not been properly addressed in most descriptions of transgenic and pharmacological models of this illness (Beery and Zucker, 2011; Kreiner et al., 2013). Interestingly, several studies performed with transgenic mouse models with differences in male and female behavioral responses have reported gender-specific differences in depressive-like behavior after modification of the serotonergic system (Bhatnagar et al., 2004; Jones and Lucki, 2005), HPA axis elements (Chen et al., 2006; Solomon et al., 2012) and BDNF expression (Monteggia et al., 2007).

In this study, we attempted to dissect how selective ablation of glucocorticoid signaling in the noradrenergic system of GR^{DBHCre} mice influences depressive-like behavior in females previously reported by our group (Chmielarz et al., 2013) and whether this behavior might be linked to neurotrophic alterations or changes in the serotonergic system.

2. Material and methods

2.1. Animals, treatment and tissue dissection

All experiments were conducted using separate cohorts of male and female animals, which originated from our own transgenic mouse colonies maintained in the C57BL/6N background. Transgenic animals with selective ablation of GR in the noradrenergic system (GR^{DBHCre}) were obtained by crossing animals that hosted Cre recombinase under the dopamine beta-hydroxylase (DBH) promoter with animals that harbored a floxed GR gene as previously described (Parlato et al., 2007, 2009). The animals were maintained with their control (Cre-negative) littermates of the same sex in self-ventilated cages under standard laboratory conditions (12 h light/dark cycle, food and water ad libitum) until 12 weeks of age. Desipramine (20 mg/kg, Sigma-Aldrich, USA) and fluoxetine (10 mg/kg, Tocris, USA) were injected intraperitoneally 30 min prior to the test. The control groups received 0.9% NaCl. Restraint stress was performed by placing the mice in 50 ml disposable centrifuge tubes that were adapted for this purpose by drilling holes to permit air circulation for 30 min. For biochemical and molecular analyses, the animals were killed by cervical dislocation 2 h after the start of the light phase or 30 min after the restraint stress procedure. Tissues from selected brain areas were dissected and frozen on dry ice for protein extraction or high-performance liquid chromatography (HPLC) assay or refrigerated in RNA stabilizer (RNA-later, Ambion, USA) for subsequent RNA extraction.

All experimental procedures conducted on living animals were conducted in strict accordance with the recommendations included in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocols were approved by the Animal Ethical Committee at the Institute of Pharmacology, Polish Academy of Sciences (Permit Number: 789, issued: Sept 30, 2010). The GR^{DBHCre} animal colony was maintained with the permission of the Ministry of Environment (Permit Number: 01-4/2009, issued: March 13, 2009).

2.2. Tail suspension test (TST)

The TST was performed on female control and mutant mice as previously described by our group (Chmielarz et al., 2013). Briefly, the animals were suspended by the tail for 6 min, and the total time spent immobile was scored by the automatic video tracking software EthoVision XT8 (Noldus, the Netherlands).

2.3. Western blot

Each sample was homogenized in 4 volumes of ice cold RIPA buffer (Sigma-Aldrich, USA, #R0278) that contained protease inhibitor cocktail (Sigma-Aldrich, #P8340), agitated for 2 h in 4 °C and centrifuged at 8000 ×*g*. The proteins from the supernatant were subsequently precipitated by methanol/chloroform. The protein concentration was measured with a BCA protein assay kit (Sigma, USA, #BCA1ANDB9643); 25 μg of protein for each sample was run on a polyacrylamide gel and transferred to a nitrocellulose membrane. The membranes were blocked by 5% Bailey's Irish Cream liqueur (Bailey's, Ireland) in TBST; the blots were then incubated overnight with primary antibodies against BDNF (1:1000, ab72439, Abcam, UK) and beta-actin (1:10,000, A5441, Sigma, USA), followed by incubation with the proper secondary antibody linked to horseradish peroxidase. The Western blot signal was visualized with a Lumi-Light Plus WB substrate (Roche, Germany, #12015196001), recorded with a CCD camera on a FujiLas1000 Imager (Fujifilm, Japan) and subjected to densitometric analysis.

2.4. Real time polymerase chain reaction (RT-PCR)

Total RNA was extracted from the samples using an RNeasy Mini kit (Qiagen, Germany) according to the manufacturer's instructions. The concentration was assessed spectrophotometrically (NanoPhotometer, Implen, Germany) and reverse transcribed (1000 ng/sample of total RNA) using Oligo d(T)16 primers and MultiScribe Reverse Transcriptase (Applied Biosystems, Germany) according to the manufacturer's protocol. RT-PCR was performed in a final volume of 20 μl using the Chromo4 Platform (Biorad, USA) with commercially available TaqMan Gene Expression Assays (Applied Biosystems, Germany). Each RT–PCR mixture contained 50 ng of cDNA template, 10 μl of 2× TaqMan Universal PCR Master Mix (Applied Biosystems, Germany) and 1 μl of 20× TaqMan Gene Expression Assay. Hypoxanthine-phosphoribosyl