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The reduced level of growth factors in an animal model of depression is accompanied by regulated necrosis in the frontal cortex but not in the hippocampus

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ABSTRACT

In the present study, we asked if the different types of stress alter neuronal plasticity markers distinctively in the frontal cortex (FCx) and in the hippocampus (Hp). To do so, we implemented various stress regimens to analyze changes evoked in these rat brain structures.

We utilized several molecular techniques, including western blot, ELISA, quantitative RT-PCR, and various biochemical assays, to examine a range of proteins and subjected rats to behavioral tests to evaluate potential maladaptive alterations. A decrease in the level of growth factors in the FCx was accompanied by changes suggesting damage of this structure in the manner of regulated necrosis, while the Hp appeared to be protected. The observed changes in the brain region-specific alterations in neurotrophin processing may also depend on the period of life, in which an animal experiences stress and the duration of the stressful stimuli.

We conclude that chronic stress during pregnancy can result in serious alterations in the functioning of the FCx of the progeny, facilitating the development of depressive behavior later in life. We also suggest that the altered energy metabolism may redirect pro-NGF/p75^{NTR}/ATF2 signaling in the cortical neurons towards cellular death resembling regulated necrosis, rather than apoptosis.

1. Introduction

Stress is an important advancing factor in affective disorders. In particular, the chronicity of stressful stimuli is often linked to the pathogenesis of depression. Several theories can explain the molecular basis of the development of depression, often reporting stress-related stimulation as a sensitizing factor. Particular attention has been paid to disturbances in the hypothalamus-pituitary-adrenal (HPA) axis and altered brain monoamine levels, primarily serotonin and norepinephrine. Neuroinflammation and increased levels of several cytokines gave rise to the 'immunological hypothesis' of depression. Post-mortem analysis of depressive patients' brains revealed significant reductions in the mRNA levels of brain-derived neurotrophic factor (BDNF), leading to the formation of the 'neurotrophic hypothesis' of depression. BDNF levels are also depleted after prolonged stress, further linking stress with depression (Chen et al., 2001; Shimizu et al., 2003).

Shifting from transcriptome analysis to proteomic data, many reports have indicated that the reduced level of brain neurotrophins is an important factor in the pathogenesis of depression. In contrast to BDNF, the participation of other neurotrophins, such as nerve growth factor (NGF), in the pathogenesis of depression remains poorly understood, which is most likely because the synthesis of NGF is controlled by numerous factors, including glucocorticoids, cytokines, the thyroid and thymus hormones, the levels of which are altered in depression (Aloe et al., 2002).

We previously evaluated the rat's hypothalamic NGF levels and showed that only chronic mild stress (CMS), but not prenatal stress (PS) or acute stress (AS), affects NGF synthesis and maturation in this structure (Kucharczyk et al., 2015). The mature forms of NGF and BDNF are produced from their precursors (pro-neurotrophins), and their biological effects are often opposite (Kichev et al., 2009; Kucharczyk et al., 2015; Lee et al., 2001; Yeh et al., 2012). Current research on the participation of these growth factors in the pathogenesis of depression has revealed that an increase in the level of pro- and the reduction of

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mature forms of NGF and BDNF may be the main cause of characteristic for depression disturbances in synaptic plasticity. In fact, in the brains from different animal models of depression, preventing the proteolytic conversion of pro- to the mature form or enhanced proteolysis of the mature form correlated with alterations in the tissue plasminogen activator and matrix metalloproteinases, respectively (Kucharczyk et al., 2015; Yeh et al., 2012).

Given that both the frontal cortex (FCx) and hippocampus (Hp) are highly interconnected with the hypothalamus and both actively regulate HPA axis functioning and are significantly altered in mood disorders (Chen et al., 2001; Detka et al., 2015; First et al., 2013; Głombik et al., 2015; Harro et al., 2014; Sowa et al., 2015; Yeh et al., 2012), we analyzed these structures upon various stress regimens.

In current research, we applied two most commonly used and well-validated animal models of depression, i.e. PS and CMS. In PS model, the increased immobility time in forced swim test, the induction of anhedonia (i.e. loss of interest in normally rewarding stimuli, i.e. reduction of sucrose consumption), disturbances in sleep and cognitive functions, decreases in sexual behavior, neurogenesis inhibition in the dentate gyrus of the hippocampus, enhanced corticosterone concentration in light-dark cycle and after stress have been observed (Głombik et al., 2015; Koehl et al., 1999; Lemaire et al., 2000; Morley-Fletcher et al., 2004, 2003; Rao et al., 1999; Rhees et al., 1999; Szymańska et al., 2009). Moreover, changes observed in PS, in contrast to those present after chronic stress applied to adult animals, are long lasting. In turn in CMS model adult rats are exposure to continuous, unpredictable variety of mild stressors that lead to the development of behavioral and neurobiological alterations, such as induction of anhedonia, decrease in reactivity to rewards, the increased immobility time in forced swim test, decreased sexual behavior and self-care and changes in sleep architecture (Willner, 2005, 1997). Moreover, the changes observed in PS and CMS that parallel symptoms of depression can generally be reversed upon chronic antidepressant treatment. However, it should be noted that in these models of depression, like in other models of this disease based on stress procedure, not all animals, despite the use of identical stressors, develop depression-like behavioral changes and a few even develop reverse effects (Willner, 2005). So despite the limitations of all available animal models of depression and fact that they may not accurately reflect the symptoms of depression in humans, PS and CMS are used in most studies as the best approximation for the studies of depression pathogenesis.

The main goal of the present work was to prove that different types of stress distinctively alter brain regions with respect to neuronal plasticity markers. As we published previously, examination of the hypothalamus in rats challenged with either chronic mild stress (CMS) or prenatal stress (PS) resulted only in the former in the altered processing of NGF, leading to a decrease in its mature form and an increase in its precursor form (Kucharczyk et al., 2015). BDNF levels in the hypothalamus were unaffected. Here, we analyzed two other brain regions, the FCx and Hp, often linked to the pathogenesis of depression from rats subjected to PS and CMS and evaluated an acute response to the stressful experience as well as the combination of such events on the PS background.

We first verified our models in the behavioral tests. Next we analyzed the transcript levels for NGF, BDNF and their receptors (RT-qPCR) followed by respective protein's levels quantification by Western Blotting. The stressful conditions that revealed the most robust changes in the neurotrophin levels were further investigated in these groups by analyzing the downstream signaling from respective receptors by Western Blotting and ELISA. To evaluate the potential activation of cellular stress mechanisms, analyzed tissues were subjected to various biochemical tests aiming to examine markers of oxidative stress and to dissect cell death type.

2. Materials and methods

2.1. Chronic mild stress experiment

Male Wistar rats (Charles River Laboratories, Germany) were singly housed with free access to food and water and were maintained on a 12-h light/dark cycle (lights on at 06:00 am) under a constant temperature (22 ± 2 °C) and humidity (50 ± 5%). The behavioral experiments were conducted between 09:00 am and 3:00 pm All procedures used in this study were conducted in compliance with the rules and principles of the EU directive 2010/63/EU, and were approved by the Bioethical Committee of the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

2.1.1. Chronic mild stress and sucrose consumption test

CMS procedure was conducted as described previously (Kucharczyk et al., 2015), with minor modifications. Briefly, animals were trained to the consumption of a 1% sucrose solution over 8 weekly 1-h baseline tests following 14 h of food and water deprivation. Based on their sucrose intake in the final baseline test, the animals (320 ± 10 g) were divided into two matched groups: controls and to-be-stressed. Stress was administered for a total of 2 weeks and consisted of variety of unpredictable stressors (Kucharczyk et al., 2015). All stressors lasted for 10–14 h and were applied individually and continuously, day and night. Both control and CMS rats were tested in 1-h-long sucrose consumption tests after one and two weeks from the beginning of the CMS procedure.

2.2. Prenatal and acute stress experiment

Sprague-Dawley rats (Charles River Laboratories, Hamburg, Germany) initially weighed 200–250 g were maintained with food and water available ad libitum, and housed in groups of five per cage under standard conditions. All experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Local Ethics Committee in Krakow, Poland.

Prenatal stress was performed as previously described (Kucharczyk et al., 2015; Morley-Fletcher et al., 2003). Half of the 3-month-old male offspring from the control and PS groups were subjected to acute immobilization stress, by placement in plastic cylinders (7/19 cm) and exposal to bright light (2 × 150 W bulbs) for 60 min.

2.2.1. Forced swim test

Three-month-old PS and control rats were individually subjected to two trials in which they were forced to swim in a cylinder (44 cm high, 22.5 cm in diameter) filled with water (23 °C) up to a height of 35 cm. There was a 24-h interval between the first and second trials. The first trial lasted for 15 min, and the second trial lasted for 5 min. The total duration of immobility and climbing time were measured throughout the second trial (Porsolt et al., 1978).

2.3. Tissue collection

All efforts were made to implement the 3Rs rule (replacement, reduction, and refinement) both to reduce the number of animals used and suffering during the experiments. In the case of CMS, the animals were killed under non-stress conditions by rapid decapitation 24 h after the last sucrose consumption test. Animals from the PS group were sacrificed 3 h after the AS procedure. The brains were rapidly removed and dissected on ice-cold glass plates. Tissues were frozen on dry ice and stored at −80 °C.

2.4. Western blotting

Western blotting procedure was conducted exactly as described previously (Kucharczyk et al., 2015). Blotted membranes were probed