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Chronic mild stress influences nerve growth factor through a matrix metalloproteinase-dependent mechanism

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ABSTRACT

Stress is generally a beneficial experience that motivates an organism to action to overcome the stressful challenge. In particular situations, when stress becomes chronic might be harmful and devastating. The hypothalamus is a critical coordinator of stress and the metabolic response; therefore, disruptions in this structure may be a significant cause of the hormonal and metabolic disturbances observed in depression. Chronic stress induces adverse changes in the morphology of neural cells that are often associated with a deficiency of neurotrophic factors (NTFs); additionally, many studies indicate that insufficient NTF synthesis may participate in the pathogenesis of depression.

The aim of the present study was to determine the expression of the nerve growth factor (NGF) in the hypothalamus of male rats subjected to chronic mild stress (CMS) or to prenatal stress (PS) and to PS in combination with an acute stress event (AS).

It has been found that chronic mild stress, but not prenatal stress, acute stress or a combination of PS with AS, decreased the concentration of the mature form of NGF (m-NGF) in the rat hypothalamus. A discrepancy between an increase in the *Ngf* mRNA and a decrease in the m-NGF levels suggested that chronic mild stress inhibited NGF maturation or enhanced the degradation of this factor. We have shown that NGF degradation in the hypothalamus of rats subjected to chronic mild stress is matrix metalloproteinase-dependent and related to an increase in the active forms of some metalloproteinases (MMP), including MMP2, MMP3, MMP9 and MMP13, while the NGF maturation process does not seem to be changed. We suggested that activated MMP2 and MMP9 potently cleave the mature but not the pro- form of NGF into biologically inactive products, which is the reason for m-NGF decomposition. In turn, the enhanced expression of *Ngf* in the hypothalamus of these rats is an attempt to overcome the reduced levels of m-NGF. Additionally, the decreased level of m-NGF together with the increased level of pro-NGF can decrease TrkA-mediated neuronal survival signalling and enhance the action of pro-NGF on the $p75^{NTR}$ receptor, respectively, to evoke pro-apoptotic signalling. This hypothesis is supported by elevated levels of the caspase-3 mRNA in the hypothalamus of rats subjected to chronic mild stress.

1. Introduction

Stress is a physiological condition that defines an organism's response to a stressful stimulus. In general, stress is a beneficial experience that motivates an organism to action to overcome the stressful challenge. However, particular situations, when stress becomes chronic might be harmful and devastating. Furthermore, different stress factors, both those experienced prenatally as well as in adulthood, could significantly influence neuronal plasticity and survival (Elizalde et al., 2008; Koehl et al., 1999). Many clinical and experimental studies indicate that prolonged stress is an important factor in the pathogenesis of depression (Alfonso et al., 2005; Hall et al., 2015). The increased glucocorticoid and glutamate levels resulting from chronic stress disturb synaptic plasticity and can even lead to loss of neurons and astrocytes (Timmermans et al., 2013). Glucocorticoids are known to induce dendritic atrophy in the hippocampal CA3 region and prefrontal cortex, inhibit neurogenesis in the dentate gyrus of the hippocampus, increase the

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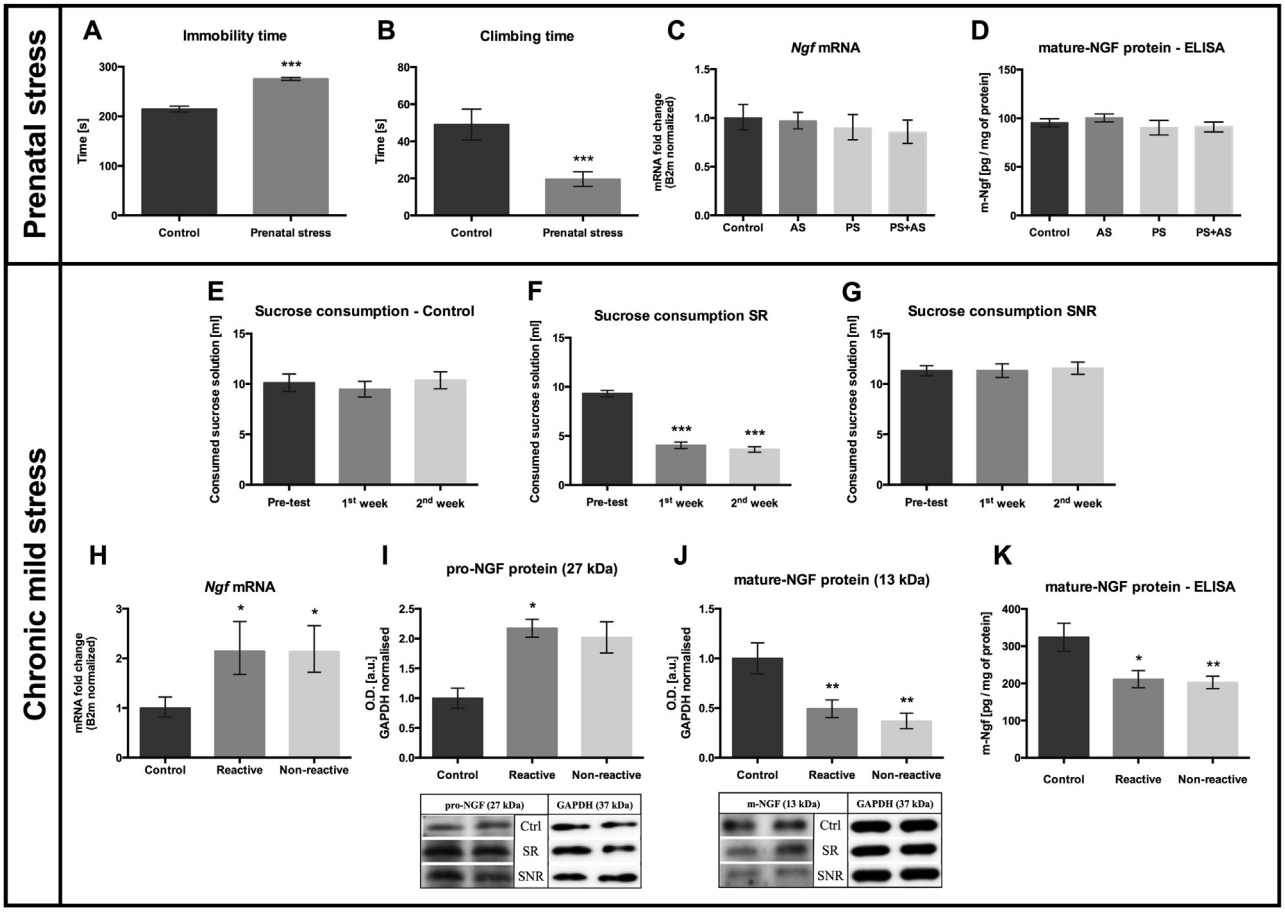

Fig 1. Immobility time (A) and climbing time (B) in the Porsolt test for the control and prenatally stressed rats. *Ngf* mRNA levels (C) and mature NGF levels (ELISA) (D) in the hypothalamus of the control and rats subjected to acute stress (AS), prenatal stress (PS) and the combination of acute and prenatal stress (AS + PS). Sucrose consumption test for the control (E), stress reactive (SR) (F) and stress non-reactive (SNR) (G) group in 1st and 2nd week after the total of 2-week chronic mild stress (CMS) procedure. *Ngf* mRNA levels (H) in the hypothalamus of chronic stress reactive (Reactive) and stress non-reactive (Non-reactive) animals. Western blot analysis of the pro (pro-NGF) (I) and mature (m-NGF) forms of NGF (J) in the hypothalamus of CMS rats. ELISA results for the mature NGF (K) in the hypothalamus of CMS rats. The data are presented as the means ± SEM. The groups included 16 animals for the Porsolt test, 12 for the sucrose consumption test, 8–11 for ELISA, 6–8 for Western blotting and 6–8 for RT-qPCR. Two representative animals were shown from one Western Blotting membrane. The animals subjected for 2 week of chronic mild stress procedure were sacrificed 24 h after the last sucrose consumption test. Rats from the prenatal stress group were sacrificed three hours after the acute stress procedure (see more details in material and method section). Statistical analyses of the changes were performed using the appropriate ANOVA followed by Tukey's post-hoc-test. Values with $P < 0.05$ were considered significant (* denotes differences vs. the control).

adverse effects of glutamate and inhibit the synthesis of trophic factors (Wellman, 2001). To date, these detrimental effects of stress have been reported mainly in the hippocampus and less often in the frontal cortex; however, the limited data indicate that stress or glucocorticoids affect cellular plasticity and exert neurodegenerative effects in the hypothalamus as well (Marco et al., 2015; Viveros et al., 2010). The hypothalamus is a critical coordinator of the stress and metabolic responses; therefore, disruptions in this structure may be a significant cause of the hormonal and metabolic disturbances observed in depression (Flak et al., 2012). Chronic stress induces adverse changes in the morphology of neural cells that are often associated with a deficiency of neurotrophic factors (NTFs), and many studies indicate that insufficient NTF synthesis may participate in the pathogenesis of depression (Chen et al., 2015; Yulug et al., 2009).

Neurotrophins (NTs) are growth factors that are present in both the central and peripheral nervous system. They play a crucial role in neuronal crest cell survival and differentiation as well as maintaining the synaptic plasticity and development of neuronal circuits (Allard et al., 2012; Bibel and Barde, 2000). The best-described NTs are Nerve Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF), and neurotrophins-3, -4, and -5 (NT-3, -4, -5). Changes in the NT levels have been demonstrated in different types of stress as well as in animal models of depression. Most of the existing data indicate reduced BDNF levels in the hippocampus and frontal cortex in animal models of depression as well as in the blood of patients with depression (Chen et al., 2015). The inhibitory action of glucocorticoids on BDNF synthesis in the brain following a stressful event is already well-documented (Filho et al., 2015). Current research with animal models of depression also indicates decreased levels and/or reduced activity of insulin-like growth factor (IGF-1) (Basta-Kaim et al., 2014). Concerning the hypothalamus, in the maternal deprivation model of depression in male and female rats, alterations in BDNF and IGF-1 are associated with changes in cellular proliferation and maturation (Viveros et al., 2010). Moreover, the stress-induced decrease in growth factor concentrations is considered to be responsible for the disturbances in neuronal plasticity and the attenuation of neuronal viability. However, not all research supports this tendency, particularly in the case of NGF there is still much controversy. Decreased NGF levels are primarily observed in the hippocampus in some animal models of depression. However, there were no changes in the NGF concentrations in the hippocampus and frontal cortex in the Flinders Sensitive Line rat model of depression (Angelucci et al., 2003; Della et al., 2013;