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Research Report

Expression of neurogranin in hippocampus of rat offspring exposed to restraint stress and pulsed magnetic fields

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ABSTRACT

Stressor acting upon the organism during pregnancy can produce distinct and long lasting effects on the offspring. However, the essential mechanism remains unclear. Neurogranin (Ng) is a postsynaptic brain-specific protein involved in the regulation of calcium signaling and neuronal plasticity. Our purpose was to investigate whether Ng plays a regulating role in the effects of prenatal restraint stress (PS) and prenatal pulsed magnetic fields (PMFs) on the hippocampus of rat offspring. Sprague Dawley female rats at gestational days 14–20 were given restraint stress or pulsed magnetic fields. The male and female offspring rats were sacrificed at the age of 1 month. The expression of Ng in the offspring hippocampus was determined using immunohistochemistry and western blotting. The results showed that PS induces a significantly inhibitory effect on the expression of Ng, especially in female offspring. The 0.11 T of prenatal PMFs could increase the expression of Ng in offspring hippocampus. There was no significant difference between female and male offspring in PMFs group. The prenatal restraint stress-induced decrease in Ng expression in offspring hippocampus might be associated with the deficit in spatial learning and memory reported

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previously. The 0.11 T of prenatal PMFs induced a significant stimulatory effect on protein expression of Ng. It was believed that PMFs stress might enhance the synaptic growth and remodeling.

1. Introduction

Pregnant dams exposed to stressors can cause changes in neurotransmitter metabolism, brain morphology, ability to cope with cognitive and emotional tasks in offspring juvenile and even in adult life (Bock et al., 2011; Mychasiuk et al., 2012). Accumulative researches show that pregnant women exposed to environmental factors alter the neuroendocrine and behavior in their offspring. One of the environmental factors is extremely low frequency magnetic fields (ELF-MF, <300 Hz), which derive from everyday used electrical appliances. It has been shown that ELF-MF modulated different steps of synaptic transmission in the brain, as well as motor behavior in physiological and pathophysiological conditions (Cook et al., 2006; Rauš et al., 2012). Pulsed magnetic fields (PMFs) are kind of extremely low frequency magnetic fields and are widely used in clinical applications because of their efficacy, generally related to the rapid raise of the magnetic field resulting in higher induced currents (Capone et al., 2009). PMFs have been shown to alter animal and human behaviors, such as directional orientation, learning, pain perception and anxiety-related behaviors (Sartucci et al., 1997; Thomas et al., 2001). In the rodent hippocampal tissue as the research object, some results show that PMFs amplify and attenuate the release and uptake of glutamate respectively, and the glutamergic synapses are the target of magnetic fields action (Wieraszko et al., 2005). PMFs amplify the evoked potentials recorded (Wieraszko, 2004) and induce the increase of excitability in hippocampal neurons (Ahmed and Wieraszko, 2008). The hippocampus, which has the highest density of glucocorticoid (GC) receptors in the brain, is involved in the regulation of the hypothalamic–pituitary–adrenal (HPA) and the behavioral responses to stress. However, there have been few papers about the effects of prenatal PMFs on the offspring hippocampus.

In addition, increasing evidences show that prenatal stress (PS) can lead to severe impairments in the offspring's development, in human as well as in animals. PS animals show developmental delays, together with alterations in physiological and behavioral responses to stress (Lee et al., 2007; Pivina et al., 2007). Human studies indicate that PS children develop more mood illnesses, developmental lags, and demonstrate behavioral disturbances such as aggression and hyperactivity (Meijer, 1985). They also exhibit elevated neuroendocrine responses to stress, including altered regulation of the HPA axis, elevated secretion of adrenocorticotropin releasing hormone (ACTH) and corticosterone, and increased activation of the sympathetic nervous system (Weinberg et al., 2008; Weinstock et al., 1998). In previous studies, we found that PS affected the capability of spatial learning in the offspring rat, especially in female exposed to later stage prenatal restraint stress (Li et al., 2008). PS caused an increase in intracellular Ca^{2+} and reactive oxygen species (ROS) in the hippocampal CA3 region in female offspring (Zhu et al., 2004). Especially, PS increased Ca^{2+} channels peak current amplitude, current density, and integral current of neurons in hippocampal CA3 region of female offspring (Cai et al., 2007). The gender difference is generally considered to be related to the low activity of placenta 11β-HSD in female fetus (Montano et al., 1993) and to the sexually dimorphic HPA axis responded to stress (McCormick et al., 1995). The levels of corticosterone and 8-OH-dG in hippocampal CA3 region were increased and nuclear factor-κB was activated in response to PS (Li et al., 2008). We also observed that pulsed magnetic fields could increase the number and proliferative capability of the neural stem cells in offspring, resulting in a compensatory response to neuron injury (Xia et al., 2010).

Ng, a marker protein of the structural plasticity, is a postsynaptic brain-specific protein. Ng expresses abundantly in the cerebral cortex, hippocampus, amygdala, and striatum that are stress-related brain regions of emotion and behavior (Watson et al., 1990). Ng is also a Ca^{2+}-sensitive calmodulin (CaM) reservoir and a protein kinase C (PKC) substrate (Díez-Guerra, 2010), binds CaM in the absence, or at low levels of Ca^{2+} and releases at micromolar or higher concentrations of Ca^{2+}. Ng phosphorylation by PKC prevents its binding from CaM. Ng is believed to play a role in adjusting the availability of free CaM, augmenting the Ca^{2+} transients critical for the enhancement of synaptic plasticity and learning and memory (Kubota et al., 2007; Zhong et al., 2009).

In the present research, our target was to investigate the effects of PS and PMFs on the changes of Ng protein levels in offspring hippocampal neurons using immunohistochemistry and western blotting, hopefully to reveal the effects of prenatal-stage environment on the developmental hippocampus.

2. Results

2.1. *Effects of PS on expression of Ng in offspring hippocampus*

2.1.1. *Effects of PS on Ng-positive cells in sub-regions of offspring hippocampus*

Immunohistochemistry demonstrated Ng immunoreactive cells in all principal neuronal populations of the hippocampus, namely pyramidal neurons in hippocampus subfields 1–4 (CA1–4) and granule cells in the dense cell layer of the dentate gyrus (DG). Ng-positive cells showed brown cytoplasm and transparent nuclei (Fig. 1). Fig. 1(B and C) shows representative photomicrographs of Ng staining in hippocampus of CON groups. Fig. 1(F and G) shows representative photomicrographs