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EXPERIMENTAL MODEL OF SCHIZOPHRENIA AND MORPHOLOGICAL CHANGES IN BRAIN

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ABSTRACT

Schizophrenia is a complex psychiatric mental disorder often characterized by abnormal social behavior and failure to recognize what is real. Common symptoms include false beliefs, unclear or confused thinking, auditory hallucinations, reduced social engagement and emotional expression, and inactivity. Neuroontogenetic theory explains the occurrence of disease events taken place during intrauterine period. Gutamatergic signaling interferes with ethion-pathogenic mechanisms of schizophrenia. The disease is associated with N-methyl-D-aspartate NMDA receptor hypofunction and a number of genes suspected in respect of schizophrenia influence on a NMDA receptor signaling. NRG1 and ErbB4 its receptor involved in the glutamatergic signaling by regulating the NMDA receptor complex. Neuregulins are signaling proteins structurally related to the epidermal growth factor (EGF). The mechanisms underlying the contribution of NRG1/ErbB4 system to schizophrenia pathophysiology remain insignificant, but they reveal only after the complete maturation of the brain. Prenatal stress in rats simulates many of the cognitive and sensory deficits manifest in the disease. Identified in this experimental model deficits in immunochistochemical expression of Neuroregulin1- ErbB4- system directed changes at the molecular level in the hippocampus, a brain structure with a role in the regulation of stress.

Key words: schizophrenia, experimental model, neuroontogenetic theory, neurotrophic factors

Schizophrenia is a complex psychiatric long-term mental disorder of a type involving a breakdown in the relation between thought, emotion, and behaviour, leading to faulty perception, inappropriate actions and feelings, withdrawal from reality and personal relationships into fantasy and delusion, and a sense of mental fragmentation. Studies of schizophrenia show that the most commonly observed pathological manifestations are expressed in cognitive functional deficits, as well as disorders of operative and verbal memory and abstract thinking (1). At present, it is well known that cognitive deficits in patients with schizophrenia (such as difficulties in working memory, attention, and problem solving) are the main characteristics of this disease. (2-4) There is a theory called neuroontogenetic theory that explains the appearance of disease events during intrauterine period. The use of experimental models allows the identification of memory impairment processes in schizophrenia, which is useful in the analysis of these deficits (5). There are models that affect the development of primary brain structures: prenatal stress (6); maternal deprivation (7); growing in isolation (8); maternal malnutrition during pregnancy (9). Prenatal stress in rats simulates many of the cognitive and sensory deficits manifest in the disease. Exertion of stress on experimental pregnant rats results as a reduction in the size of the hippocampal formation and disturbances in the spatial memory of the generation and is associated with increased glucocorticosteroid levels during fetal development (10). Excessive secretion of cortisol can be neurodegenerative and impair neurobiological processes in the hippocamal formation (11, 6). Gutamatergic signaling interferes with ethion-pathogenic mechanisms of schizophrenia. The

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disease is associated with N-methyl-D-aspartate NMDA receptor hypofunction and a number of genes suspected in respect of schizophrenia influence on a NMDA receptor signalling (12-15). To that group belong Neuregulins (NRG)- signaling proteins structurally related to the Epidermal Growth Factor. NRG1 and its receptor ErbB4 are involved in the glutamatergic signaling by regulating the NMDA receptor complex (16).

PURPOSE

The aim of the present study is to determine the immunohistochemical expression of NRG1 and its receptor ErbB-4 in rat hippocampal formation by experimental model of schizophrenia.

MATERIAL AND METHODS

EXPERIMENTAL PROCEDURE: 4 pregnant Wistar rats were obtained at 14 days of gestation and were allocated individually in breeding cages with 24h light/dark cycle and fed ad libidum. 7 days old male Wistar rats (weight 10-15g) were divided into two groups: control and experimental animals (n=10 per group). Experimental rats were under immobilization stress for 2 hours per day for a week. After birth, the offspring were divided into control and prenatally stressed animals in cages 4-8 rats in each. 35 and 56 days old control and stressed animals were anesthetized deeply with 0,2 mg/kg, i.p. Fentanyl and 0,3 mg/kg, i.p, Midazolam and were perfused intracardial through the ascending aorta with 100-150 ml of phosphate- buffered saline (PBS) followed by 100-150 ml of Zamboni's fluid. The brains were removed immediately, cut in slides (4-5 mm thick) and post-fixed in the Zamboni's perfusion solution for 24 h. Then the brain blocks were immersed for cryoprotection in 30% sucrose in 0,1M PBS at 4° C overnight, frozen in liquid nitrogen and prepared for immunohistochemistry and routine morphological analysis.

IMMUNOHISTOCHEMISTRY On cryostat coronal sections (20 μm thick, cut at -20° C) from brain blocks, in which the hippocampus is located immune reactions for NRG1 (primary polyclonal anti- Neuroregulin1 antibody, Santa Cruz, USA, 1:1000) and ErbB4 (primary polyclonal anti- ErbB4 antibody, Santa Cruz, USA, 1:1000) were performed using Vectastain® Elite ABC detection kit (Vector, USA). The peroxidase activity was then developed by means of the Peroxidase Substrate kit (3, 3'-diamonobenzidine tetrahydrochloride, DAB), (Vector, USA). Free-floating sections were used to perform the immunohistochemical reactions for the antigens under study. As controls, sections were used in which PBS replaced the primary or secondary antibodies and only the peroxidase activity was visualized. Hematoxylineosin staining and impregnation with silver nitrate were performed to examine the routine histology of the sections. Microphotographs were made on Nikon Microphot SA and NGR1 and ErbB4 expression pattern was analyzed on image software – Olympus DP-Soft (version 4,1 for Windows).

RESULTS AND CONCLUSION

Prenatal stress in rats simulates many of the cognitive and sensory deficits manifest in the disease. There are molecular-level changes occurred in prenatal stressed rats, mainly affecting the reorganization of genes responsible for receptor-encoded transmission and neurotransmitters in the frontal lobe. Immunohistochemical analysis was performed using cryostat and paraffin sections, both from brain fragments containing hippocampal formation and from fragments of the hippocampus itsself, in 35 and 56 day-old control rats and after prenatal stress. In both age groups of control animals, NRG1 was expressed with a moderate-intensity immune response (with cytoplasmic localization) primarily in pyramidal neurons of the CA3 hippocampal field **(Figure 1).** Positive, but less pronounced, immune response rates are also CA1 and CA2 cells, as well as those in the granular layer of the dentate gyrus **(Figure 1, Figure 2).** ErbB4 positive signals in the control groups were observed both in CA3 hippocampal field neurons and in cells in the region of the dentate gyrus (**Figure 3, Figure 4).** Identified by the present experimental model deficits in immunochistochemical expression of NRG1- ErbB4- system directed changes at the molecular level in the hippocampus, a brain structure with a key role in the regulation of stress.

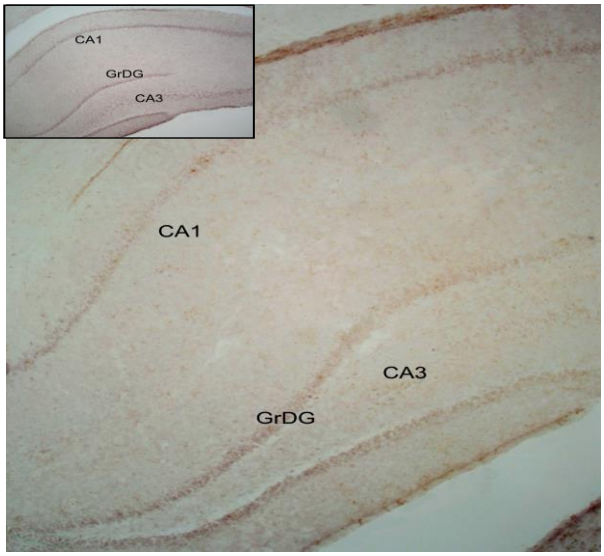

Figure 1. 35 days rats with prenatal stress and controls (in the box). NRG1 immunoreactivity in CA1, CA2 and CA3 hippocampal fields; a polymorphic layer (PoDG) and a granular layer of dentate gyrus (GrDG). x 400.

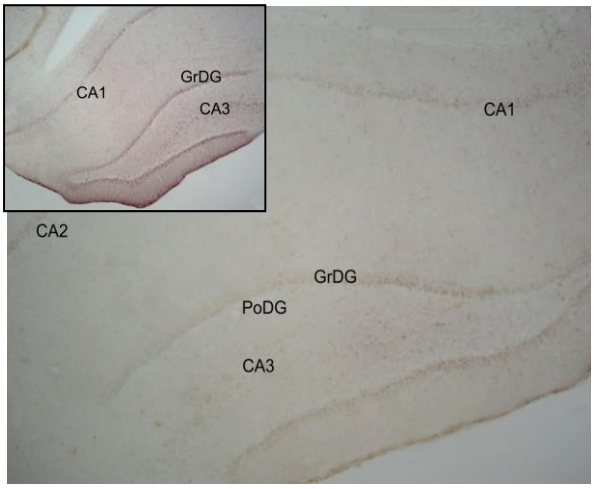

Figure 2. 56 day old rats with prenatal stress and controls (in the box). NRG1 immunoreactivity in CA1, CA2 and CA3 hippocampal fields; a polymorphic layer (PoDG), and a granular layer of dentate gyrus (GrDG). x 400.

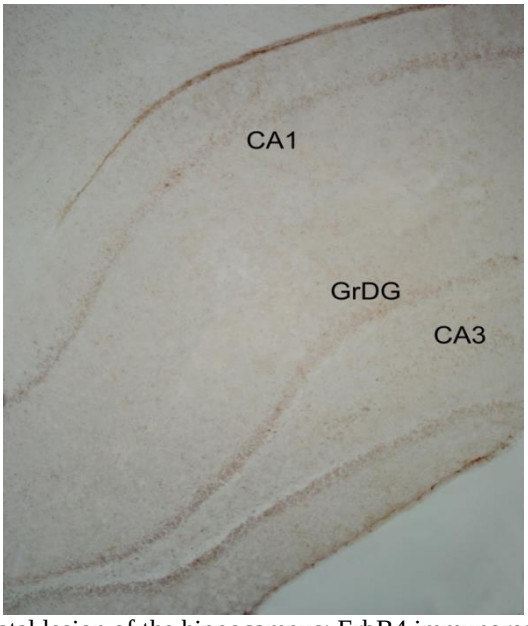

Figure 3. 35 day rats with neonatal lesion of the hippocampus; ErbB4 immunoreactivity in CA1 and CA3 hippocampal fields; a polymorphic layer (PoDG) and a granular layer of dentate gyrus (GrDG). x 400.

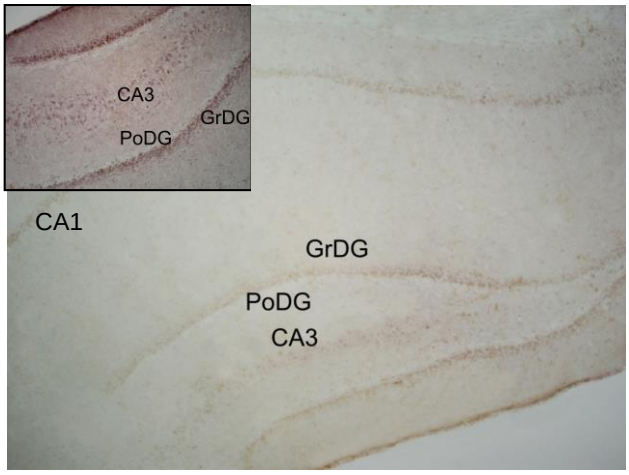

Figure 4. 56 day old rats with neonatal lesion of the hippocampus and controls (in the field); ErbB4 immunoreactivity in CA1 and CA3 hippocampal fields; a polymorphic layer (PoDG) and granular layer of dentate gyrus (GrDG). x 400.

In the analysis of NRG1 expression in hippocampal formation of rats, decreased expression of NRG1 was observed in stressed 35 day experimental animals compared to 35 day controls (P <0.001). At 56 days, this difference is observed in stressed animals and is less expressed than control groups (P <0.001). We don't find statistically significant differences in NRG1 expression between 35 days and 56 days, both in the control group and in the stressed ones (P> 0.05). The analysis of immunohistochemical expression of ErbB4 in hippocampal formation of rats, reveals increased receptor expression in the control 35 day experimental animals compared to the stressed 35 day animals (P <0.001). Receptor expression was decreased in 56 daily stressed rats compared with 56 daily controls (P <0.001). We found no difference in the expression of NRG1 and its receptor at day 35 compared with day 56, both in the control group and in the stressed ones (P> 0.05).

Our results are in accordance with previously reported apical dendritic atrophy of pyramidal neurons in the hippocampus (CA3 layer) by prenatal stress in rats and changes in expression of NMDA and glutamate levels in the hippocampal formation. On the other hand, it should be noted the established role of NRG1-ErbB4 system in the regulation of glutamatergic and GABA-ergic neurotransmission synaptic level. Hypofunction of NRG1-ErbB4 system leads to a decrease in the efficacy of glutamate and GABA-mediated synaptic transmission manifesting as deficits in working memory and other cognitive and psychotic symptoms in schizophrenia. In conlusion, the observed deficiencies in the immunohistochemical expression of the NRG1-ErbB4-system revealed molecular-level changes in the hippocampus, a brain structure with an important role in stress regulation.

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