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Neurotrophic factors and schizophrenia

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Neurotrophins have an essential role in neuronal development, synaptogenesis, and response to stress/anxious stimuli. Furthermore, these agents are neuromodulators of monoaminergic, GABAergic, and cholinergic systems. There is a growing interest of the developmental neurobiology of schizophrenia, as well as the tendency for progressive brain changes and abnormalities in the expression of neurotrophins in schizophrenia. The role of neurotrophins and their precursor, pro-neurotrophins in the pathophysiology of several CNS disorders, including depression and schizophrenia is derived from current genetic, neurochemical and therapeutic research. The present research aims to demonstrate immunoreactivity for the neurotrophic factor neurotrophin-3 (NT-3) in brain structures of rats in an experimental model of schizophrenia by neonatal lesion with *i*- butenic acid and to attempt connection between obtained results and disontogenetic hypothesis of the origination of schizophrenia.

Key words: NT-3, schizophrenia, experimental model, rat

Introduction

Schizophrenia is a complex disease characterized by disturbances of thinking, emotional disorders and severe functional disabilities. There is a theory, called neuroontogenetic theory, which explains the emergence of disease during intra-uterine period. It leads to brain structural and functional alterations, expressed in appearance of typical disease symptoms decades later. Morphological damage of brain structure is caused by abnormal expression of factors regulating the proliferation and differentiation of neuroectodermal cells and chronology of these processes (7). This pathological process translates into a reduction in the amount of neurons and glial cells and functional connections between different neuronal phenotypes therefore functional disorders can reveal at a large stage in the mature nervous system (1,11).

Hypothesis of neurotrophic factors

Abnormalities in fetal neurogenesis result from pathological changes of genetic code of certain neurotrophic factors (NTF) and their corresponding receptors. These changes consist in primary and secondary violations of biologically active substances, neurotrophic factors, under the influence exogenous effects. Developmental role of NTF during embryogenesis and in adult isn't well studied. Data for these factors are insufficient in the context of schizophrenia accompanying structural and functional abnormalities

in certain brain structures which occur only after complete maturation of the brain. Our research aims to:

- To demonstrate immunoreactivity for the neurotrophic factor neurotrophin-3 (NT-3) in brain structures of rats in an experimental model of schizophrenia by neonatal lesion with *i*- butenic acid.
- To attempt connection between obtained results and disontogenetic hypothesis of the origination of schizophrenia.

Materials and methods:

Experimental model of neonatal hippocampal lesion by Lipska et. al., (5)
7-day old Wistar rats (n=20) are divided into control and experimental groups. After hypothermic anesthesia using Hamilton needle and infusion pump 0.3 μl of 10 μg / μl solution of *i*- butenic acid (experimental animals) or artificial spinal liquor (control animals) was injected bilaterally in the area of hippocampal formation (AP – 3.0 mm, ML +_ 3.5 mm, VD – 5.0 mm, from bregma) at rate of 0.15 μl/min.

35 and 56 days rats (control and with neonatal lesion) after deep anesthesia were perfused intracardial, through the ascending aorta with Zamboni fixative. After the experiment, the brains are removed and cut into 4-5mm thick fragments of the frontal cortex and hippocampal formation. Brain fragments are placed in cryoprotective solution at 4° C overnight, frozen in liquid nitrogen and prepared for routine histological (staining with hematoxylin-eosin and impregnation techniques) and immunohistochemical analyses. Immunohistochemical techniques, amplified by the combination of ABC and PAP methods on cryostat and paraffin slices of rat brain (frontal cortex and hippocampal formation) were applied using polyclonal anti- NT-3 antibody, Santa Cruz, USA, 1:1000 and kits for detection and visualization of the immunochistochemical reactions-Vectastain® *Elite* ABC detection kit (Vector, USA) and DAB Peroxidase Substrate kit, (Vector, USA).

Results

NT- 3 immunoreactivity is visualized in hippocampal pyramidal cells (CA-1, CA-2 and CA-3 fields), cells of granular layer of the dentate gyrus cortex (GrDG) of 35 and 56 day injured rats. Reaction intensity is reduced compared with control animals (**Fig.1**). Declination in the immune reactivity of NT-3 is more pronounced on 56 day injured experimental animals, especially in the cells from CA-3, GrDG and cells in the hilus of gyrus dentatus (**Fig.2**).

Discussion

In this research is used an animal developmental model obtained by bilateral infusion of the glutamatergic agonist *i*-botenic acid. This is a molecular trigger of the excitotoxic cascade in the ventral hippocampus of 7 days old male rats (5). Fundamental features of schizophrenia are wide spectrum of structural and physical abnormalities and they are very similar to that caused by neonatal damage of the hippocampal formation with *i*-botenic acid (4,5). Our routine morphological analysis does not show any damages in the hippocampal cytoarchitectonic pattern of the lesioned rats compared to rats in the control group. A possible explanation of this fact is the low dose of *i*-botenic acid

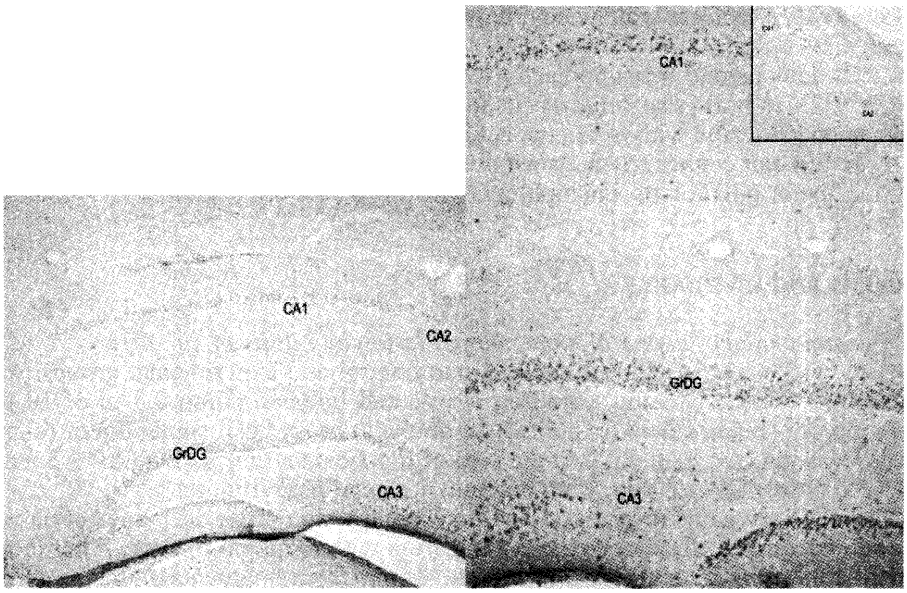

Fig.1. 35 days rats. Group of lesioned animals. NT-3 expression in hippocampus and dentate gyrus. x 200; x 400.

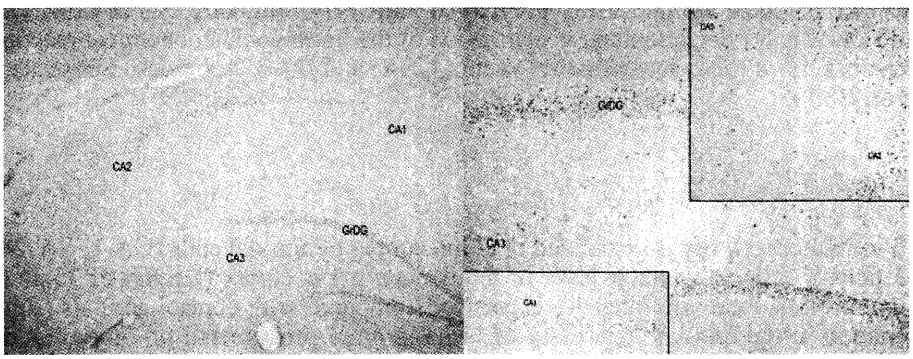

Fig. 2. 56 days old rats. Group of lesioned animals. Immunostaining for NT-3 in hippocampus and dentate gyrus. x 200; x 400.

used in the experimental procedure. Our opinion that disturbances of cytoarchitectonic depend on the used dose of *i*-botenic acid confirmed previously reported results (8). The highest levels of NT-3 expression were detected in the cerebellum and hippocampus of adult brains (6). Our findings are related with data which demonstrate that the expression of NT-3 in hippocampal formation prevails largely in the vast of dentate granule cells as well as in subpopulations of pyramidal neurons located in CA1 and CA2 (2). Low levels of NT-3 mRNA have also been detected in CA3 and CA4. In the current study, immunohistochemical staining for NT-3 shows that the NT-3 immuno-

reactivity localized in the hippocampal pyramidal cells (CA1, CA2 and CA3 fields), dentate granule cell layer (GrDG) and cells of the polymorph layer of the dentate gyrus (PoDG) of the 35 and 56 days old lesioned rats is with markedly reduced intensity than that of the control animals. The decrease in NT-3 immunoreactivity is more obvious in the hippocampal formation of the 56 days lesioned animals, especially in the cells of CA3, PoDG and dentate hilar cells. This fact suggests a change or a kind of alteration in regulatory mechanism of NT-3 expression as a consequence of neonatal hippocampal lession. The hippocampal dentate gyrus is one of the few areas of the rat brain that continues to generate neurons postnatally whereas the neuronal progenitor cells divide at the border between the hilus and inner and outer blade of the GrDG (3,9). The current data reveal reduced NT-3 expression in the hippocampal formation of the lesioned animals, especially in the cells of dentate gyrus and indicate that the dysfunction of the neurotrophins may contributes to impaired brain development, neuroplasticity and synaptic neurotransmitter system leading to the schizophrenic syndrome (10).

Conclusion

The present study reveals additional evidence in support of the neurodevelopmental model and neurotrophic factor hypothesis for schizophrenia. Our data prove the role of neurotrophins in normal brain development and their possible relevance in the neuropathology and neuropharmacology of schizophrenia.

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