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Results of Using the Method of Studying the Degree of Chromatophilia of Brain Neurons in Animals Under Prenatal Administration Of L-NAME

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Abstract

Endothelial dysfunction during pregnancy underlies many complications of the gestational process [5]. The main mechanism for the development of endothelial dysfunction is a change in the metabolism of nitric oxide. The introduction of a non-selective NO–synthase inhibitor - L-NAME during placentation caused the occurrence of morphological disorders of neurons in the frontal cortex of the offspring of rats.

Keywords: *chromatophilia; neuron; rat; cerebral cortex; L-NAME*

Introduction

Endothelial dysfunction during pregnancy underlies many complications of the gestational process [5]. The main mechanism for the development of endothelial dysfunction is a change in the metabolism of nitric oxide (NO) [3]. It is known that NO in the brain is formed in neuronal and extra-neuronal sources forming a "nitrergic system" [8]. In the nervous system, NO participates in synaptic connections as a neurotransmitter, ensuring the effectiveness of synaptic transmission (synaptic plasticity), plays a role in the regulation of synaptogenesis during the formation of the nervous system and cerebral blood flow, provides antigenic homeostasis [2]. The use of a non-selective NO-synthase inhibitor (NOS) Nw--nitro-L-Agdine Methyl Ester (L-NAME) is a well-established model of experimental hypertension, cardiovascular, renal diseases, ADMA-like (asymmetric dimethylarginine) preeclampsia, etc. [4,7,12]. L-NAME inhibits nitric oxide synthase (NOS), which leads to a decrease in NO production. It is known about the adverse effects of L-NAME on the cardiovascular system: increased maternal blood pressure, loss of vascular refractoriness to vasopressor stimuli, decreased perfusion of the uteroplacental bed, as well as the placenta, decreased placenta weight and offspring weight [6,8]. However, changes in the structure of neurons and the degree of their chromatophilia in the brain of offspring under experimental NOS inhibition have not been studied sufficiently.

The previously existing method of counting neurons of varying degrees of chromatophilia of the cytoplasm of neurons is imperfect due to the high density of cells in one field of view, especially when counting neurons in the structures of the brain of newborn animals.

Goal

To study changes in the chromatophilia of the cytoplasm of neurons in the cerebral cortex of rat offspring using the proposed method under prenatal administration of a non-selective NO-synthase blocker L-NAME.

Materials and methods

The experiments were performed on 12 female mongrel white rats weighing 300 ± 20 g and their offspring (n=24), kept on a standard vivarium diet in compliance with the requirements of Directive of the European Parliament and of the Council No. 2010/63/EU of 22.09.2010 on the protection of animals used for scientific purposes. The control group consisted of pregnant animals (n=6) who received 0.9% NaCl solution once intramuscularly, the experimental group consisted of rats who received L–NAME at a dose of 25 mg/kg once intramuscularly on the 11th day of pregnancy (n=6). The brain sampling of baby rats was carried out on the 1st and 20th days of postnatal development. After decapitation, the brain was quickly extracted, pieces of the anterior cortex of the large hemispheres were

fixed in Carnois fluid. Serial paraffin sections were prepared, which were stained with 0.1% toluidine blue according to the Nissl method [10].

Histological preparations, their microphotography, and neuron morphometry were studied using an Axioscop 2 plus microscope (Zeiss, Germany), a digital video camera (Leica DFC 320, Germany), and an Image Warp image analysis program (Bit flow, USA). The location of the frontal cortex in the histological preparations of the rat brain was determined using a stereotactic atlas [11]. At least 30 neurons were evaluated in each animal, and 150 neurons of the fifth layer of the cerebral cortex in each experimental group, which provided a sufficient sample size for subsequent analysis.

The average digital data obtained for each animal were analyzed using nonparametric statistics using the Statistica 10.0 program for Windows (Stat Soft, Inc., USA). The results are presented as the median value (Me) and the percentile boundary (from 25 to 75) [1]. The differences between the control and experimental groups were considered significant at $p<0.05$ (Mann- Whitney U-test).

With the help of Image Warp software (Bit flow, USA) installed on a personal computer, as well as a camera located on a microscope (lens 40, eyepiece 10), a section of histological preparation corresponding to the field of view of the microscope was photographed. In order to more accurately count neurons of varying degrees of cytoplasmic chromatophilia in a photo of a histological preparation, a grid was modeled in Microsoft Word with its subsequent superimposition on a photo of the studied brain region (Fig. 1).

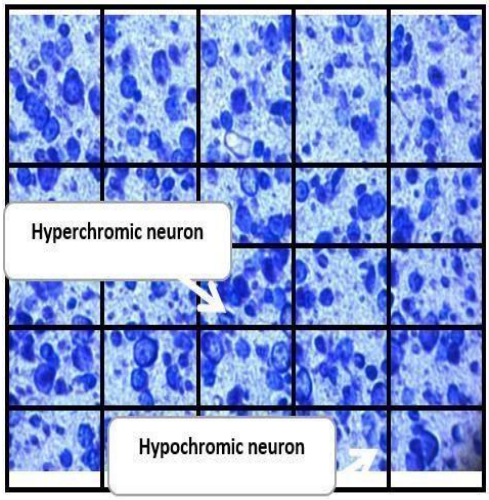


Fig 1: Neurons of the frontal cortex of the rat brain. Digital micrography. Painting by the Nissl method. Uv about x 40, ok x10

The grid is a table corresponding to the size of the resulting image (123mm x 165mm), with 10 equal cells measuring 24.7mm x 33.0mm. In all cells of the grid corresponding to the area of the histological preparation, taking into account the magnification of the microscope equal to 0.00746 mm2, neurons of different degrees of cytoplasm icchromatophilia (normochromic, hypochromic, hyperchromic, hyperchromic wrinkled) were counted, followed by determination of their percentage to the total number of neurons in the grid or the absolute number per 1 mm2. To calculate the percentage of each of the types of neurons, a proportion was

A – 100%

B – X %, where

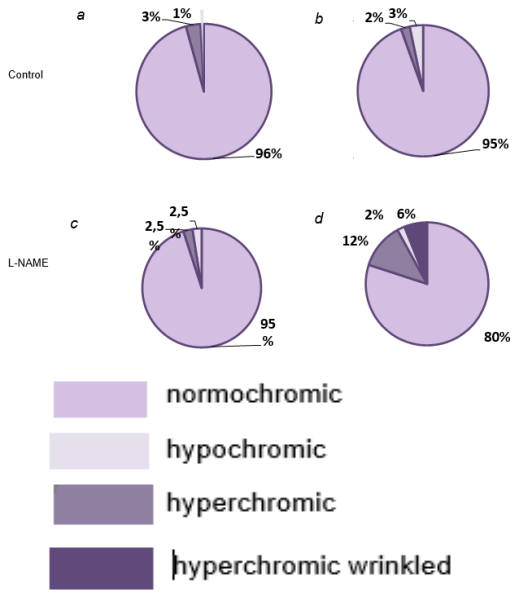
A is the total number of neurons in the grid,

B is the number of each of the types of neurons (for example, hyperchromic),

Results and their discussion

In 1- and 20-day-old control group rats, normochromic neurons predominate in the inner pyramidal layer of the frontal cortex of the brain (Fig.2).

Fig 2: The ratio of neurons with varying degrees of chromatophilia of the cytoplasm of the fifth layer of the frontal cortex



a – control 1st day,

b – control 20th day

c – L-NAME 1st day,

d – L-NAME 20th day.

Hyperchromic shrunken neurons were not observed in control group rats. On the 1st day of postnatal development, the number of hypochromic neurons in the experimental group increased by 68% ($p<0.05$).

In rats born to females who received L-NAME, on the

20th day of postnatal development, the number of hyperchromic neurons increased by 82% compared to the control group ($p<0.05$), and hyperchromic shrunken neurons appeared in the amount of 336/mm2 ($p<0.001$).

Using the proposed method for assessing the degree of chromatophilia of the cytoplasm of neurons, neurons of different degrees of chromatophilia of the cytoplasm of neurons in the cerebral cortex of control group rats and baby rats born under the conditions of L-NAME administration during pregnancy were counted.

Conclusion

Thus, the introduction of a non-selective NO–synthase inhibitor - L-NAME during placentation caused the occurrence of morphological disorders of neurons in the frontal cortex of the offspring of rats. An increase in the number of hypochromic neurons on the 1st day of postnatal development, as well as the number of hyperchromic neurons and the appearance of hyperchromic shrunken neurons on the 20th day of postnatal development, was revealed. This effect with the introduction of L-NAME may be due to a decrease in the formation of NO in the neurons of the cortex and in the endothelium of the cerebral vessels. The appearance of hyperchromic shrunken neurons may be a sign of oxygen deficiency caused by a decrease in cerebral blood flow and, as a consequence, energy deficiency in the cell.

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