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Research Article

Histological Changes in the Neurons of the Frontal Cortex of One and Twenty-Day-Old Rats After Prenatal Administration of L-Name

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Abstract

Endothelial dysfunction is one of the causes of gestosis. Inhibition of the vasodilator properties of the endothelium of blood vessels is the cause of impaired uteroplacental circulation and intrauterine development of the offspring. The main mechanism of endothelial dysfunction is impaired nitric oxide (NO) production. the administration of a non-selective NOS inhibitor L-NAME to female rats during placenta caused morphological changes in the frontal cortex neurons of rat offspring on day 1 of postnatal development, which were manifested by an increase in cell size and a proportion of hypochromic neurons. In 20-day-old rats, the effects of L-NAME administration were manifested by a decrease in neuronal size and an increase in the proportion of hyperchromic neurons as well as by the appearance of hyperchromic wrinkled neurons.

Keywords: Ingibitor; NO synthase; pregnancy; portex; rat

Introduction

Endothelium is actively involved in the regulation of vascular tone, and hence in the regulation of blood circulation, including placental circulation. Preeclampsia is a major cause of preterm birth, intrauterine retardation, and perinatal mortality. In developed countries it accounts for about 16-18% of maternal deaths and up to 40% of fetal and neonatal deaths. Endothelial dysfunction is one of the causes of gestosis [1]. Inhibition of the vasodilator properties of the endothelium of blood vessels is the cause of impaired uteroplacental circulation and intrauterine development of the offspring [2]. The main mechanism of endothelial dysfunction is impaired nitric oxide (NO) production [3]. NO in the brain is known to be formed in neuronal and extra-neuronal sources forming a 'nitrergic system' [4]. In the nervous system, NO takes part in synaptic connections as a neurotransmitter, ensuring the efficiency of synaptic transmission (synaptic plasticity), plays a role in the regulation of synaptogenesis during nervous system formation and cerebral blood flow, and provides antigen homeostasis [5]. The use of a non-selective NO synthase inhibitor (NOS), Nω-nitro-L-Arginine Methyl Ester (L-NAME) is a model of experimental pre-eclampsia [6]. Adverse effects of L-NAME on the cardiovascular system, decreased perfusion of the uterine-placental bed, reduced placental weight and offspring weight are known [7]. However, changes in neuronal structure in the brain of the offspring under conditions of experimental NOS inhibition have not been sufficiently studied.

Objective

To study the morphological features of neurons of the cerebral cortex of newborn rats at day 20 of postnatal development after administration of nonselective NO synthase inhibitor (L-NAME) during pregnancy.

Methods of investigation

The experiments were carried out on 12 female outbred albino rats weighing 300±20 g and their offspring (n=24), kept according to the European Parliament and Council Directive 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) administered 0.9% NaCl solution once intramuscularly; the experimental group, rats, received L-NAME (25 mg/kg, once, i.m.) on day 11 of pregnancy (n=6). Brain sampling of rats was

performed on the 1st day of postnatal development. After the rats were decapitated, the brain was quickly extracted, and pieces of the anterior cortex of the large hemispheres were fixed in Carnua fluid. Serial paraffin sections were prepared and stained with 0.1% toluidine blue according to the Nissl method [8]. Histological preparations were studied, microphotographed, and neuronal morphometry was performed using an Axioscop 2 plus microscope (Zeiss, Germany), a digital video camera (LeicaDFC 320, Germany), and Image Warp image analysis software (Bitflow, USA). The location of the frontal cortex in histological preparations of rat brains was determined using a stereotactic atlas [9]. At least 30 neurons were evaluated in each animal, and 150 neurons of the fifth cortical layer in each experimental group, which provided a sufficient sample size for subsequent analysis. Changes in neuronal area (mkm^2), shape (elongation factor, shape factor) and degree of cytoplasm chromatophilia (normochromic, hypochromic, hyperchromic and hyperchromic wrinkled) [10]. Statistical processing was performed using non-parametric statistical methods.

Results and discussion

Normochromic neurons predominate in the internal pyramidal layer of the frontal cortex in the control group rats of the 1st and 20th days of postnatal development (Figure 1). In newborn rats in the experimental group, along with a decrease in the number of normochromic (8%, p<0,05) and hyperchromic neurons (33%, p<0,05), there was an increase in hypochromic neurons by 68% (p<0,05). There was a 33% (p<0.05) increase in neuronal area in newborn rats from the experimental group, while the shape parameters of neuronal pericarions - shape factor and elongation factor - did not change (p>0.05) (Table 1). The observed change in the size of neurons in the form of an increase in their area along with hypochromia may be a consequence of cell swelling caused by energy deficiency with the development of electrolyte imbalance. In 20-day-old rats born to L-NAME-treated females, the number of hyperchromic neurons in the cerebral cortex increased by 82% compared with the control group (p<0.05), and hyperchromic wrinkled neurons appeared in the number of 336/which were absent in control rats (p<0.001) (Figure 2). Twenty-day-old experimental rats showed a tendency to a decrease in neuronal area (by 22%, p>0.05), while the shape of neuronal pericarions did not change (p>0.05). Size and shape of pericaryon neurons of the fifth layer of the frontal cortex of rats under conditions of Nω-nitro-L-Arginine Methyl Ester (L-NAME) administration during pregnancy (Me, LQ; UQ).

Table 1: Size and Shape of Pericaryon Neurons of the Fifth Layer of the Frontal Cortex of Rats Under Conditions of Nω-Nitro-L-Arginine Methyl Ester (L-Name) Administration During Pregnancy (Me, Lq; Uq).

Group	1 day	20 days
Area, mkm^2		
Control	26,97 (23,07; 27,62)	111,5 (94,6; 118,9)
L-NAME	40,30 (34,30; 44,20)*	87,2 (73,1; 112,2)
Elongation factor		
Control	1,20 (1,20; 1,24)	1,19 (1,19; 1,23)
L-NAME	1,24 (1,21; 1,30)	1,51 (1,35; 1,69)
Form Factor		
Control	0,84 (0,82; 0,86)	0,87 (0,85; 0,88)
L-NAME	0,87 (0,81; 0,88)	0,77 (0,78; 0,84)

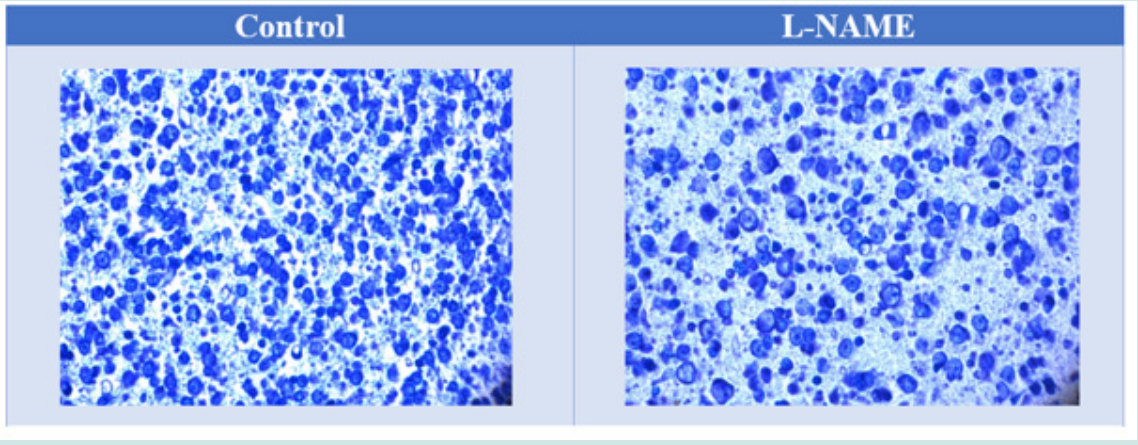

Figure 1: Neurons in the Frontal Cortex of Newburn rats. Digital Micrograph. Nissl Staining. Magnification x 40.

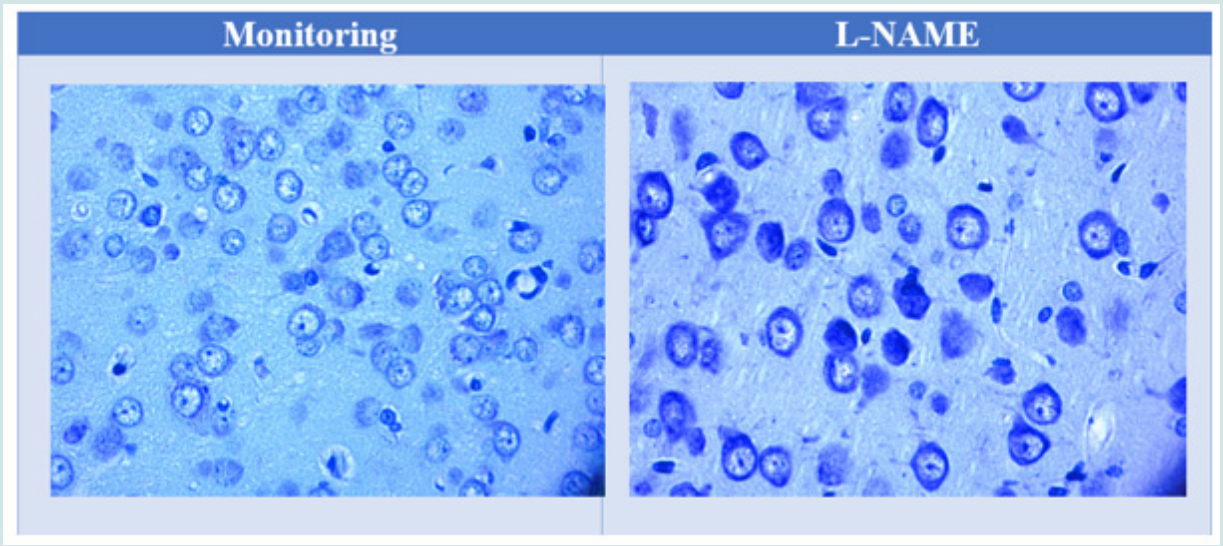

Figure 2: Neurons in the Frontal Cortex of Twenty-day-old rats. Digital micrograph. Nissl staining. Magnification x 40.

Conclusion

Thus, the administration of a non-selective NOS inhibitor L-NAME to female rats during placenta caused morphological changes in the frontal cortex neurons of rat offspring on day 1 of postnatal development, which were manifested by an increase in cell size and a proportion of hypochromic neurons. In 20-day-old rats, the effects of L-NAME administration were manifested by a decrease in neuronal size and an increase in the proportion of hyperchromic neurons as well as by the appearance of hyperchromic wrinkled neurons. This effect may be due to a decrease in NO production in cortical neurons and in the endothelium of cerebral vessels with subsequent impairment of cerebral circulation, development of oxygen starvation and, consequently, posthypoxic cell energy deficiency.

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