ClinicSearch

Clinical Trials and Clinical Research

Elizaveta I Bon *

Open Access

Research Article

Effect Of Prenatal Administration of Non-Selective No-Synthase Blocker L-Name on Reflex Formation and Motor Activity of Rats in The Postnatal Period

Maksimovich N, Rusak T, Elizaveta I Bon*, Martsun P, Pauliuchenkava D

Grodno State Medical University, Gorkogo St, Grodno, Republic of Belarus.

***Corresponding Author:** Elizaveta I Bon, Candidate of biological science, Assistant professor of pathophysiology department named D. A. Maslakov, Grodno State Medical University; Grodno State Medical University, 80 Gorky St,230009, Grodno, Belarus.

Received Date: July 18, 2023; **Accepted Date: June 14, 2023; Published Date: June 21, 2023**

Citation: Maksimovich N, Rusak T, Elizaveta I Bon, Martsun P, Pauliuchenkava D. (2023), Effect of Prenatal Administration of Non-Selective No-Synthase Blocker L-Name on Reflex Formation and Motor Activity of Rats in The Postnatal Period, *Clinical Trials and Clinical Research*; 2(5): **DOI:** 10.31579/2834-5126/034.

Abstract

According to world statistics, hypertensive conditions during pregnancy occur with a frequency of 10%, pre-eclampsia accounts for 2-8%. Preeclampsia occurs in the second half of pregnancy (after 20 weeks) and is characterized by arterial hypertension in combination with proteinuria and often with edema, manifestations of multiorgan or multisystem dysfunction and failure. The purpose of the article is studying the effect of prenatal administration of non-selective NO synthase blocker L NAME on the formation of reflexes and motor activity of rats in the postnatal period. Administration of L-NAME promotes the development of functional changes revealed in the tests "open field" and "negative geotaxis".

Keywords: reflex formation; motor activity; rats; postnatal period

Introduction:

More than 75 thousand women die annually from complications of pregnancy. The main complications that lead to 75% of all cases of maternal mortality are:

1.hemorrhage (mainly postpartum hemorrhage);
2.infections (usually after delivery);
3.high BP during pregnancy (pre-eclampsia, eclampsia);
4.postpartum complications;
5.unsafe abortion.

According to world statistics, hypertensive conditions during pregnancy occur with a frequency of 10%, pre-eclampsia accounts for 2-8% [1]. Preeclampsia occurs in the second half of pregnancy (after 20 weeks) and is characterized by arterial hypertension (AH) in combination with proteinuria and often with edema, manifestations of multiorgan or multisystem dysfunction and failure. Preeclampsia is not an independent disease. It is a syndrome that causes the inability of the adaptive systems of the mother to meet the needs of the growing fetus. At the heart of preeclampsia are disorders of the general circulation with the development of multi-organ failure. Pathophysiologic mechanisms of preeclampsia development include endothelial dysfunction, placentation disorders, inflammation, oxidative stress, thrombosis and activation of the renin-angiotensin-aldosterone system [2].

Annually, 8.5 million cases of preeclampsia are registered in the world [3]. According to statistics in the Republic of Belarus, the frequency of PE is 7.3-10.5 per 1000 births, while in developing countries it reaches 30-35%. The frequency of preeclampsia in pregnant women on average increased from 7 to 35% in the world from 2014 to 2018. In the structure of causes of maternal mortality in Russia, preeclampsia consistently ranks third and accounts for 11.8-14.8%. Maternal mortality is not registered in our country. Women who have undergone pre-eclampsia or eclampsia suffer from AH (70-72%), kidney disease (9.4%), central nervous system pathology (20%), etc. [4].

The relevance of the pathology of the second half of pregnancy is determined by the development of complications, so in patients with PE/E the risk of cerebral edema and hemorrhage, DIC, pulmonary edema, respiratory distress syndrome, acute renal and hepatic failure, HELLP syndrome, maternal and fetal death, venous

thromboembolism, which leads to disability of women of fertile age increases many times [3].

Scientific interest in such obstetric pathologies as preeclampsia (PE), placental insufficiency (PI), fetal growth retardation (FGR) is due not only to the high frequency of these complications of pregnancy, but also to their impact on the course of the perinatal period. It is not completely clear what (or what) causes lead to the development of pre-eclampsia/eclampsia, but in most cases, it is due to a disruption of placentation, resulting in incomplete invasion of the trophoblast into the spiral arteries of the mother. In this regard, pre-eclampsia is often complicated by fetal growth retardation and, conversely, marked fetal growth retardation (FGR) will be accompanied by the development of PE.

The pathogenesis of preeclampsia is polyetiologic. Currently, the greatest attention is paid to endothelial dysfunction. Establishing an animal model is an effective way to reproduce the pathology and evaluate its effect on the fetus. N-nitro-L-arginine methyl ester (L-NAME) is a nitric oxide synthase (NOS) inhibitor and is a widely used drug for modeling pre-eclampsia in rats [5]. Numerous studies have confirmed that after L-NAME administration, rats develop a syndrome similar to preeclampsia, which includes hypertension, proteinuria, and intrauterine fetal growth restriction. To date, most studies have focused on microcirculation and structural changes in the placenta, but brain damage and neurological deficits in offspring born to mothers with PE remain poorly understood.

Objective. To study the effect of prenatal administration of non-selective NO synthase blocker L NAME on the formation of reflexes and motor activity of rats in the postnatal period.

Materials and Methods

The studies were carried out on 12 mongrel white female rats weighing 300±20 g. The rats for the study were obtained from the vivarium of Grodno State Medical University. All animals were kept according to the requirements of the Directive of the European Parliament and Council No. 2010/63/EU of 22.09.2010 on the protection of animals used for scientific purposes. The ethical committee of Grodno State Medical University granted permission to perform these studies. The females of the experimental and control groups were taken of approximately the same age, from the same population. The animals were divided into 2 groups. The control group consisted of pregnant animals (n=6) receiving 0.9% NaCl solution once intramuscularly and their born rats (n=12); the experimental group consisted of rats with L-NAME administration (at a dose of 25 mg/kg) once intramuscularly on the 11th day of pregnancy (n=6) and their born rats (n=12), Table 1.

№ of group	Group characterization	Number of animals (females / offspring)
1	L-NAME	6 / 12
2	Control	6 / 12
TOTAL	12 / 24	

Table 1: Characterization of groups of experimental animals.

Note: *L-NAME – N□-nitro-L-Arginine Methyl Ester*

For fertilization, males were placed with females after 18.00 at the rate of 1 male per 3 females, and pregnancy detection was performed the next day before 9.00 am [6]. The first day of pregnancy was considered to be the day when spermatozoa were detected in vaginal swabs under a microscope. All pregnant females, as well as their offspring, were under the same conditions.

The open-field test was performed by recording the number of crossed squares when the rats were placed on a 36×36 cm square with lines forming 36 squares (Figure 1) [18-21].

In the "open field" test, the time for the animal to leave the center of the square where it is placed at the beginning, activity in the horizontal and vertical planes of space, grooming (washing), and defecation were evaluated. In the "open field" it is possible to observe disturbances in motor activity by recording discoordination and reduction of arbitrary movements [7, 10].

Figure 1: Determination of motor activity of rats in the "open field" test.

The motor activity of rats in the vertical plane is represented by two types of stances: climbing - in which the hind legs of the animal remain on the floor of the surface, while the front legs rest against the wall of the "open field", and rearing (from "rear" - "to stand on the fence") - stances in which the forelimbs remain on the weight [11-13].

To determine muscle strength, the animal was placed on a horizontally placed grid, after which it was slowly moved to a vertical position.

The bar above the grate prevented the animal from climbing to the top edge of the grate and from climbing to the opposite side of the grate (Figure 2) [14-16].

Figure 2: Determination of Muscle Strength

To determine muscle strength, the animal was placed on a horizontally placed grate and then slowly moved to a vertical position.

A bar above the grate prevented the animal from climbing to the top edge of the grate and from climbing to the opposite side of the grate.

Muscular strength was determined by recording the time during which the animal was able to hold on the grate of the device.

Taking into account the stage-by-stage formation of morphofunctional properties of the cerebral cortex in rats, the offspring of different age groups were used for the study: 1-day-old (early postnatal period),

20-day-old (pubertal period) [17].

The methods for assessing the development of the nervous system during the newborn period included the study of the rate of emergence of motor reflexes during the nursing period ("flipping on the plane", "negative geotaxis", "cliff avoidance", "open field") [8].

The study of the reflex "negative geotaxis" was carried out by placing rats on an inclined plane (25°) head downward and estimating the rate of turning by 180° with determination of the retention time. The reflex was considered to be formed if all rats turned 180° (average - day 7).

Registration of the reflex "turning over on the plane" was carried out when the rats were laid on their backs on a flat surface, after which the time required to return the rat to the normal position on all 4 legs was measured.

The reflex "cliff avoidance" was studied when the rats were placed on the edge of the table by recording the time of crawling away from the edge of the platform. The formation of the reflex was completed (average - day 9) if the rats crawled away from the edge of the platform within 10 sec.

As a result of histological studies, quantitative continuous data were obtained and processed using the licensed computer program Statistica 10.0 for Windows (StatSoft, Inc., USA). Since small samples were used in the experiment, which had non-normal distribution, the analysis was performed by methods of nonparametric statistics. Data are presented as Me (LQ; UQ), where Me is the median, LQ is the value of the lower quartile; UQ is the value of the upper quartile [9].

Differences between indices were considered reliable at $p < 0.05$ (Mann Whitney U-test).

Results and discussion

When evaluating motor activity and reflex formation in the offspring of rats of the control group, the early reflexes under study were formed in time: "cliff avoidance" by the 9th day, "flipping on a plane" by the 2nd day, and "negative geotaxis" by the 5th day. The 180◦ turning time of control group rats on the 5th day of postnatal development was 16 sec (14.0;18.0).

When the offspring were tested in the "open field" test on the 16th day (all individuals of the group had their eyes open), the number of crossed squares, the number of "climbing" and "rearing" stands, the number of washings, and the number of defecations were investigated (Table 2).

Number of crossed squares	Number of racks type «climbing»	Number of racks type «rearing»	Washing	Defecation
20,0 (16;30)	7,0 (6;7)	0 (0;1)	6 (3;7)	0 (0;0)

Table 2: Results of the open field test in control group rats, Me (LQ; UQ).

Thus, motor activity and formation of reflexes in rats of the control group corresponded to the age norms for this species of animals.

In rats of the experimental group the early reflexes under study were also formed in time, however, there was revealed a tendency to delay their formation when tested in the test "negative geotaxis". When rats were placed on an inclined plane (25°) head downwards, there was noted a lengthening of the time required to turn around by 180° in rats of the experimental group compared to the control by 40% (Table 3).

Group	Turnaround time, sec
Control	16,0 (14,0;18,0)
L-NAME	27,0 (12,5;40,0)

Table 3: Results of the "negative geotaxis" test in rat offspring during the newborn period, Me (LQ; UQ).

***Note:** * - $p < 0.05$ compared to control.*

In the "open field" test, there was a tendency to decrease the motor activity of rats born to females receiving L NAME on day 11 of gestation compared to the control group, manifested in a decrease in the number of "climbing" stands and the number of squares crossed (Table 4).

Group	Number of crossed squares	climbing	rearing	Washing	Defecation
Контроль	20,0 (16;30)	7,0 (6;7)	0 (0;1)	6 (3;7)	0 (0;0)
L-NAME	14,5 (8;33)	4,0 (1.5;7.0)	0 (0;0)	6 (3;13)	0 (0;0)

Table 4: Results of the "open field" test in rat offspring during the newborn period, Me (LQ; UQ).

***Note:** * - $p < 0.05$ in comparison with the control.*

In the tests "rollover on the plane" and "cliff avoidance" no differences in the timing of reflexes formation in rats of the experimental group in comparison with the control group were revealed.

Thus, direct administration of L-NAME promotes the development of functional changes revealed in the tests "open field" and "negative geotaxis".

References:

1. V. V. Kulakov, (2006). Obstetrics and gynecology. *Clinical recommendations*. 136-171.
2. Oludare, G. O., O. J. Ilo, B. A. Lamidi (2017). Effect of lipopolysaccharide and high-salt intake on blood pressure, angiogenic factors and liver enzymes of pregnant rats, *Niger. J. Physiol*. 129-136.
3. Sidorova, I.S., N.A. Nikitina (2014).Preeclampsia in the focus of attention of a practicing physician.*Obstetrics and Gynecology*. 4-9.
4. O. S. Filippov, (2016). Results of the confidential audit of maternal mortality in the Russian Federation in 2016 (methodological letter).
5. Herraiz, S. (2012). Sildenafil citrate improves perinatal outcome in fetuses from preeclamptic rats. *BJOG*. 1394-1400.
6. I. P. Zapadnyuk et al. (1983). Laboratory animals (breeding, maintenance, use in experiments) - *Kiev: Vishcha Shkola*.
7. Maksimovich, N. E, E. I. Bon, S. M. Zimatkin. - Grodno: GrSMU, (2020). *Rat brain and its reaction to ischemia*.
8. Smolnikova, N. M. (1982).Methodological approaches to the study of antenatal effects of drugs on the development of offspring .*Pharmacology and Toxicology*. 100-104.
9. Batin, N. V. (2008). Computer statistical data analysis: textbook. Minsk: *Institute for training of scientific personnel of the National Academy of Sciences of Belarus*.
10. Bon, E. I. N. E. Maksimovich. S. M. Zimatkin, (2019). Morphological changes in the parietal cortex of rats after subtotal brain ischemia and against the background of L-NAME administration, *Vestnik VSMU*. 14-20.
11. Maksimovich, N. E. (2003).The concept of the nitroxidergic system of the brain. The role of neuronal. *Journal of GrSMU*.7-11.
12. Maksimovich, N. E. (2004).The concept of the nitroxidergic system of the brain. The role of extraneuronal sources. *Journal of GrSMU*. 3-5.
13. Maksimovich, N. E., (2005). Platelet aggregation during modulation of the L Arginine-NO pathway in rats with cerebral ischemia. *Pathophysiology and Experimental Therapy*.14-15.
14. Maksimovich, N. E., E. I. Bon, S. M. Zimatkin (2020). Grodno Rat brain and its reaction to ischemia. *GrSMU*.

15. Maksimovich, N. Ye. T. S. Rusak, E. I. Bon, (2023).Results of Using the Method of Studying the Degree of Chromatophilia of Brain Neurons in Animals Under Prenatal Administration. *Journal of Transplantation Proceedings and Research*.1-3.
16. Maksimovich, N. Ye. T. S. Rusak, E. I. Bon, (2023).Effect of administration of a non-selective no synthase inhibitor during pregnancy on cortical development in newborn rats. *Journal of Psychiatry and Neurochemistry Research*. 1-3.
17. T. S. Rusak, et al. (2023).Histological Changes in the Neurons of the Frontal Cortex of One and Twenty-Day-Old Rats After Prenatal Administration. *Lupine Online Journal of Sciences*.18-20.
18. Rusak., T. S. N. E. Maksimovich., E. I. Bon., F. G. Gadzhieva., S. A. Sidorovich. - Grodno, (2023). Structural abnormalities of pyramidal neurons of the frontal cortex of rat offspring after prenatal administration. Spring Anatomical Readings: Proceedings of the Republican Scientific and Practical Conference dedicated to the 65th anniversary of the Department of Normal Anatomy GrSMU. *Grodno State Medical University*; 169-173.
19. Rusak, T. S., N. A. Gavrisheva, (2023). Methodological approaches to counting neurons, different in degree of cytoplasm chromatophilia, in the norm and after prenatal administration. Current issues of experimental and clinical medicine: a collection of abstracts LXXXIV scientific and practical conference with international participation, St. Petersburg. *Petersburg State Medical University*. 232-233.
20. Rusak, T. S. A. A. Turevsky, Grodno, (2023). Features of chromatophilia and the size of neurons of the cerebral cortex of newborn rats under the conditions of L-NAME administration during pregnancy.*Grodno State Medical University*. 666-667.
21. T. S. Rusak, A. O. Smolyak, O. A. Yaroshuk (2023). Features of chromatophilia and the size of neurons of the cerebral cortex of 20-day-old rats under the conditions of L-NAME administration during pregnancy Proceedings of the Republican Scientific and Practical Conference of Students and Young Scientists, dedicated to the 100th anniversary of the birth of Professor A. A. Turevsky, Grodno, 27-28, *Grodno State Medical University*; 668-669.