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Studying the pathogenic role of catecholamines in the development of retinopathy of prematurity on an experimental model of the disease

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Purpose. *To study the involvement of dopamine and noradrenaline in the pathogenesis of retinopathy of prematurity (ROP) on an original rat model of the disease.* ***Material and methods.*** *The study was conducted on 41 newborn Wistar rats (82 eyes) which were divided into 2 groups: experimental (EROP, rats with experimental ROP; n = 21) and control (n = 20). All rat pups were given binocular enucleation on day 7, 14, 23 and 28 post birth. The eyeballs were dissected along the limbus, and the cornea, lens, hyaloid system, and vitreous were removed. The retina was isolated from the eye cup. Using an ultrasonic homogenizer (Labsonic M, Sartorius), the isolated retinal samples were homogenized in 10 volumes of 0.1 n HClO4 containing 50 pmol/ml (or more) of 3,4-dihydroxybenzylamine (DBA) and centrifuged at 2000g for 20 minutes. Norepinephrine, dopamine, and L-3,4 dihydroxyphenylalanine (a precursor of dopamine, also known as L-DOPA) were identified in the resulting supernatant. The contents of substances were measured using reverse phase high performance liquid chromatography with electrochemical detection (Amperometric detector LC-4B, Bioanalytical Systems, USA) set at the potential of 850 mV.* ***Results.*** *On day 7, avascular retinal zones in both groups of animals were observed, and no significant differences were found in the content of monoamines in the retina of rats with EROP and in the control group. On day 14, the content of noradrenaline, dopamine, and L-DOPA in the retina of the experimental group significantly increased compared with the control. On day 23, corresponding to the peak of neovascularization in the EROP model, the level of norepinephrine in the retina of experimental rat group was significantly higher, while the level of L-DOPA was significantly lower compared to the control group. The dopamine level was comparable in both study groups and similar to the level of L-DOPA in the control group. On day 28, corresponding to the beginning of EROP regression accompanied by vascular activity decrease, the content of dopamine and L-DOPA remained lower than in the control group.* ***Conclusion.*** *During the development of pathological neovascularization of rat pup retina with EROP, the level of noradrenaline is growing, revealing a peak corresponding to the period of pronounced pathological growth of retinal vessels within the applied model, which indicates to the fact of noradrenalin proangiogenic properties and its direct participation in the pathogenesis of ROP. The level of dopamine and its predecessor, L-DOPA, increased approaching the 14th day compared to its detected level on the 7th day, which may be due to the maturation of the amacrine cells producing it. On the 23 day, i.e. during the period corresponding to the maximum peak of angiogenesis, its relative decrease of L-DOPA was noted. It can be assumed that the lack of this monoamine, and hence insufficient manifestation of its anti-angiogenic properties contributes to the development of uncontrolled neovascularization of the retina.*

Keywords: retinopathy of prematurity; experiment; rat model; pathogenesis; norepinephrine; dopamine

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Retinopathy of Prematurity (ROP) is a severely disabling disease that develops in prematurely born children. ROP is characterized by disruption of retinal angiogenesis and leads to the progressive growth of extraretinal pathological vessels with the development of exudative or tractional retinal detachment. A large number of studies have been devoted to the study of mechanisms in the regulation of retinal angiogenesis in prematurely born infants. However, there is still no clear understanding of what factors trigger the process of abnormal vascular growth, as well as, why the disease develops in its classic or aggressive form and in some cases cannot be treated by the existing methods of treatment.

In recent years, an actively researched subject has been the angiogenic properties of monoamines. A significant part of research conducted in this subject is devoted to tumor neoangiogenesis. It has been shown that endogenous dopamine is an important inhibitor of tumor angiogenesis, and, consequently, its growth. It has been established that dopamine, acting via D2-receptors, blocks the effects of vascular endothelial growth factor (VEGF) and normalizes the structure of abnormal tumor blood vessels by affecting two cellular components of the vascular wall: pericytes and endothelial cells [1]. At the same time, it has been revealed that noradrenaline and adrenaline interacting with β-adrenergic (β-AR) receptors, on the contrary, increase the expression of VEGF in a number of human tumors [2], which leads to an increase in tumor angiogenesis, and its growth [3].

There are only a few studies on the participation of monoamines in the development of vasoproliferative retinal diseases, and they mainly concern the role of β-adrenergic receptors and noradrenaline. In particular, it has been shown that an increase of noradrenaline synthesis occurs in a simulated oxygen-induced retinopathy in mice. Also, the effectiveness of the β-blocker propranolol in inhibiting neovascularization processes has been investigated [4]. In a pilot randomized controlled trial in stage II zone ROP 2, positive results were obtained in children with respect to the inhibition of ROP progression during oral administration of propranolol, but the development of systemic side effects in the form of bradycardia and hypotension were observed [5]. At the same time, considering the fact that dopamine synthesis in the retina takes place in the amacrine cells of the inner nuclear layer [6, 7], it is of great interest to study its role in the regulation of retinal angiogenesis in vasoproliferative retinal diseases [8].

The **PURPOSE** of our study was to research the role of dopamine and noradrenaline in the pathogenesis of ROP on the original model of the disease.

MATERIAL AND METHODS

The study was performed on 41 rats of the Wistar breed (82 eyes) in accordance with GOST 53434-2009 from 02.12.2009 "Principles of Good Laboratory Practice GLP", the Resolution of the Chief State Doctor of the Russian Federation № 51 from 29.08.2014 "On Approval of SP 2.2.1.3218-14 "Sanitary-and-epidemiological requirements for the device, equipment, and content of experimental and biological clinics (vivariums)", Federal Law No. 61-FZ of 12.04.2010 "On Circulation of Medicines". The study protocol has been approved by the local ethics committee.

The rats were divided into 2 groups: an experimental group (rats with experimental ROP, EROP) (n=21) and a control group (n=20).

In order to reproduce the EROP of newborns, the rats were placed in an incubator together with the female that gave birth to them for 14 days. Every 12 hours, the oxygen concentration in the incubator ranged from 60 to 15%. The rats were then placed in conditions with a normal oxygen content (21%). Throughout the experiment, the room was kept at a constant temperature (+26°C) and light (12 hours a day, 12 hours a night).

The control group consisted of rats which were kept in conditions with normal oxygen content from the moment of birth.

The rats were taken out of the experiment on the 7^{th}, 14^{th}, 23^{rd}, and 28^{th} day. Binocular enucleation was carried out on all rats within the specified period of time. The content of noradrenaline and dopamine precursor L-3,4 dihydroxyphenylalanine (L-DOPA) was determined in the samples of the retina. Using an ultrasonic homogenizer (Labsonic M, Sartorius), the isolated retina was homogenized in 10 volumes of 0.1 n $HClO_4$ containing a minimum of 50 pmol/ml 3,4-dihydroxybenzylamine (DHBA) and centrifuged at 2000g for 20 minutes. From the obtained supernatant, the content of catecholamines was determined by reverse-phase high-performance liquid chromatography with electrochemical detection (Amperometric detector LC-4B, Bioanalytical Systems, USA) at the potential of 850 mV.

The *results were statistically* processed using the Microsoft Excel statistical package. Reliability of the differences measured between groups with a level of significance of at least 95% was estimated using the Student's parametric t-test.

RESULTS

On the 7^{th} day, when avascular zones of the retina were observed in both groups of animals [9], no signifi-

cant differences in the content of catecholamines in the retina of rats with EROP and in the control group were observed (Fig. 1). However, it should be noted that the level of noradrenaline in both groups was significantly and reliably reduced in comparison with the level of L-DOPA, the immediate precursor of dopamine.

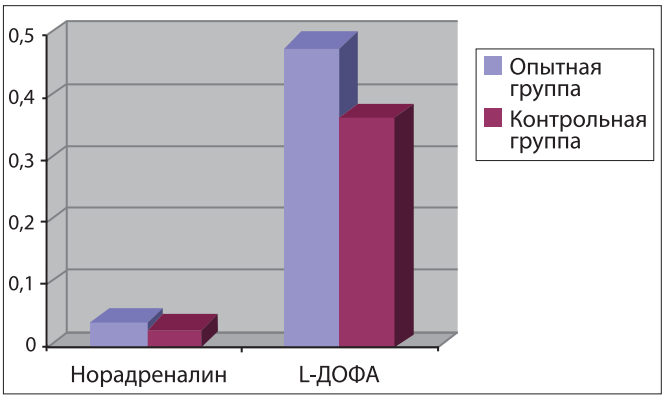

Fig. 1. The level of catecholamines in the retina of rats of the experimental and control groups on the 7th day of the experiment, pmol/g

On the 14th day, the results of our previous studies confirmed that the expression of the PCNA antigen in endothelial cells would be detected, which further indicated the activation of the cells replicative potential [9]. At this time period in the current study, the content

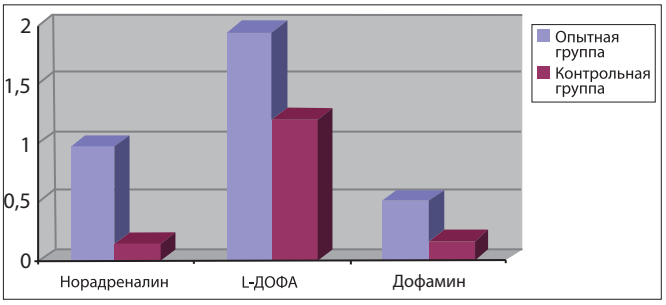

Fig. 2. The level of catecholamines in the retina of rats of the experimental and control groups on the 14th day of the experiment, pmol/g

of L-DOPA, dopamine, and noradrenaline in the retina of the experimental group was significantly increased in comparison with the control (Fig. 2).

When analyzing the stage of the experiment where the group of rats with EROP did not yet have pronounced vascularization, and the control group completed the process of retinal vascularization [9], it is important to note the severity of the increase in the level of noradrenaline in the experimental group in comparison with the control group. This may have had an important pathogenetic value in the induction of pathological angiogenesis in EROP (Fig. 3).

The level of noradrenaline in the retina of the experimental group was significantly higher than in the

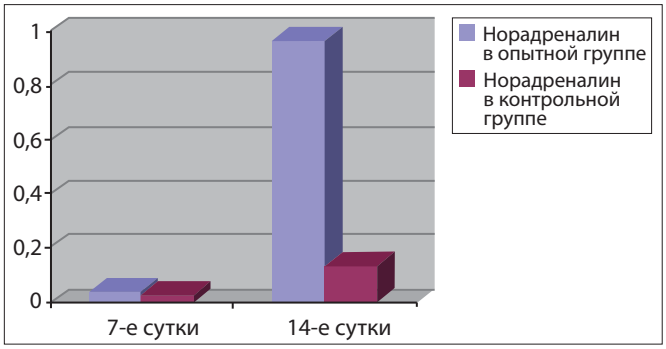

Fig. 3. Dynamics of the level of noradrenaline in the retina of experimental and control rats (7–14 days), pmol/g

control group by 23 days, corresponding to the peak of neovascularization in the EROP model [8]. On the other hand, the level of L-DOPA was significantly lower in the retina of rats with EROP in comparison with the control group (Fig. 4), and the level of dopamine was comparable in both groups and comparable to the level of L-DOPA in the control group.

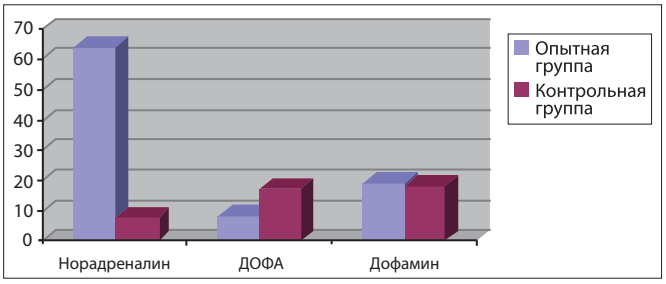

Fig. 4. The level of catecholamines in the retina of rats of the experimental and control groups on the 23rd day of the experiment, pmol/g

The measurements obtained indicated pronounced proangiogenic properties of noradrenaline. Regarding dopamine metabolism, the data may indicate a critical "consumption" of L-DOPA for the synthesis of dopamine during the active formation of pathological vessels in the retina of rats with EROP as an attempt to “inhibit” this process. It is worth mentioning that the pathological process is always reversible in rat models with ROP. This process consists of only 3 stages, and this mechanism can directly participate in the induction of the reverse development of neovascularization.

On the 28th day, at the beginning of EROP regression with a decrease in vascular activity [9], the content of both dopamine and L-DOPA in the retina of EROP rats was reduced in comparison with the control group (Fig. 5).

When we further evaluated the dynamics of L-DOPA content in the retina of both groups of rats, a high degree of increase was noted in the control group (Fig. 6).

Thus, it can be assumed that the lack of this catecholamine, and therefore the insufficient manifestation of its antiangiogenic properties, contributes to the development of uncontrolled neovascularization of the retina in the process of EROP development.

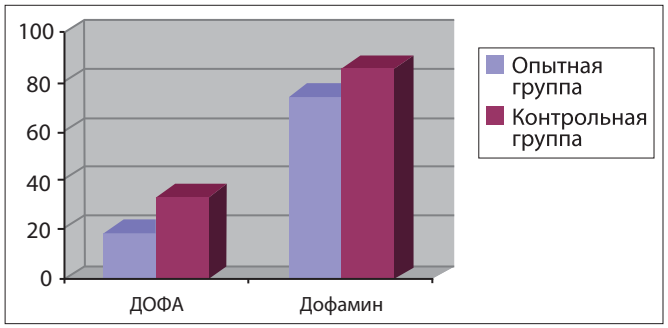

Fig. 5. The level of catecholamines in the retina of the experimental and control rats on the 28th day of the experiment, pmol/g

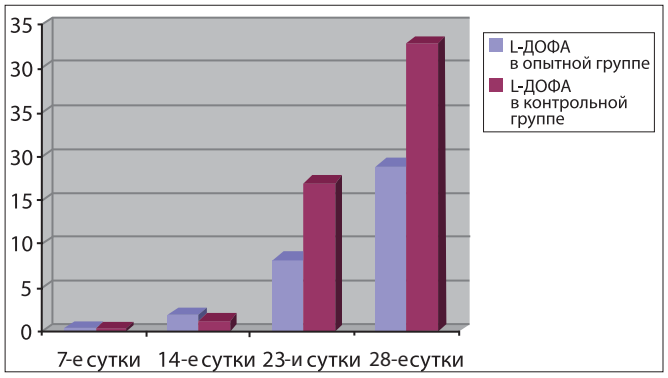

Fig. 6. Dynamics of L-DOPA in the retina of experimental and control rats (7–28 days), pmol/g

DISCUSSION

The main source of dopamine synthesis in the retina is considered to be a subclass of specialized dopaminergic amacrine cells (DAC). The precursor of dopamine is L-tyrosine, which is hydroxylated by the enzyme tyrosine hydroxylase with the formation of L-DOPA, which is further decarboxylated by the enzyme L-DOPA-decarboxylase and converted into dopamine. Afterwards, dopamine is metabolized to noradrenaline (insignificantly in the retina [10]), 3,4-dihydroxyphenylacetic acid (DOPAC), or 3-methoxytyramine.

The world literature contains rare publications devoted to the content of the products of dopamine metabolism occurring in the retina with EROP [10, 11]. N. Zhang, et al. [11] in the rat model and N. Spix, et al. [10] in the mouse ROP model detected a decrease in the dopamine and DOPAC content in the retina of EROP animals. The authors managed to prove that the dopamine deficiency was caused by the death of DAC. The amount of DAC was estimated by detecting the activity of tyrosine and hydroxylase 14 days after the animal's birth. As observed in our work, no reliable differences between the experimental and control groups were revealed at the earliest stages of the study. The presence of single DACs and their dendrites for 14 days in the experimental and control groups and the gradual formation of the DAC network with an increase in the number of their dendrites by day 57 was noted. At the same time, the number of DACs expressing tyrosine kinase in the EROP group at the peak of neovascularization was significantly lower than the control level, which coincided with our data. We also obtained a significant decrease in the L-DOPA level and an increase in the content of noradrenaline in the retina of the experimental group of rats. This change may serve as evidence of a decrease in the number of DACs in EROP. The increased content of L-DOPA in the experimental group on day 14 in our work can be explained by the DAC's maintained functionality and by the attempt to suppress the beginning of neovascularization by increasing dopamine synthesis. N. Spix, et al. also managed to prove on the mouse model that in the case of EROP, the decrease in tyrosine hydroxylase activity and the decrease in the level of retinal dopamine are associated with the death of DAC, and the amount of DAC is not restored after regression of neovascularization [10]. This is fully consistent with our data; on the 28th day, i.e. during the regression period of EROP, the L-DOPA content in our study remained lower compared to the control group.

CONCLUSION

The data obtained in this study gives evidence to the participation of dopamine and noradrenaline in the regulation of angiogenesis in ROP. Such information further broadens the scope for finding new approaches to the treatment of this serious disease. Clarification of mechanisms of their participation in pathological neovascularization requires further research.

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