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CORRECTION OF ISCHEMIC DAMAGE TO THE RETINA ON APPLICATION OF PHARMACOLOGICAL PRECONDITIONING OF RECOMBINANT ERYTHROPOIETIN

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Abstract. To study the protective properties of pharmacological preconditioning with recombinant erythropoietin suberitro stimulating dose of 50 IU / kg in the retina of the eye, was the model of retinal ischemia, ischemia with the assessment of the temporary simulation mode of the retina of rats with the instrumental methods of analysis and morphometric studies. The most suitable model was a model 30 minute ischemia with subsequent reperfusion periods of 1 hour to 72 hours. We studied the protective effect preconditioning suberitro stimulating recombinant erythropoietin in a dose of 50 IU/kg, preconditioning distant ischemic and emoxipine 2 mg/kg per rat model of retinal ischemia. In the experiment, it was revealed that the recombinant erythropoietin (50 IU/kg) prevents the development of degenerative retinal layers due to ischemic damage more than distant ischemic preconditioning and emoxipine. The observed protective effect of erythropoietin us with the development of ischemia was confirmed by laser Doppler flowmetry, electroretinography and morphometry. Using suberitro stimulating recombinant erythropoietin in a dose of 50 IU/kg, 30 minutes prior to the pathology simulation eliminates erythropoietic effect, and the lack of positive dynamics in groups with preliminary administration of glibenclamide 5 mg/kg, the key role of ATP-dependent potassium channels in a preconditioning mechanism implementation. Identification and use of pharmacological agents, which have the effect of preconditioning, may be a new approach in the correction and prevention of retinal ischemia, which is the leading element in the pathogenesis of a number of visual pathologies. The possibility of pharmacological preconditioning with erythropoietin ischemic lesions of the retina is essential for the development of anti-ischemic agents for the treatment and prevention of ocular pathologies of ischemic origin.

Keywords: ischemia-reperfusion of the retina; pharmacological preconditioning; erythropoietin; emoxipine; ATP-dependent potassium channels.

Introduction.

In recent years, been an increase in ischemic diseases of the eye associated with the spread of atherosclerosis, arterial hypertension, coronary artery disease, diabetes mellitus, which lead to diseases of the vascular system of the eye. Ischemia is one of the leading causes of low vision, blindness and disability-free for people of different age groups [1].

In this regard, prevention and correction of the consequences of ischemia, developing in various pathological conditions, it is an actual problem of modern medicine. That is why the study of ways to improve tissue tolerance to ischemia is an actual problem of modern experimental and clinical pharmacology.

Treatment available to date, medications may not always be successful enough, that ensures the relevance of the problem to expand the arsenal of drugs for the treatment of ocular diseases associated with ischemic conditions [2].

Due to the fact that the leading element in the pathogenesis of a number of vascular diseases of the fundus is the retinal ischemia [3], prevention and correction of ischemic diseases of the retina is an actual problem of modern ophthalmology and pharmacology, which can be solved with the help of pharmacological preconditioning, the essence of which consists in the activation of endogenous protective mechanisms that reduce the extent of damage in the subsequent prolonged ischemic episode [4].

It is proved that the use of recombinant erythropoietin (EPO) in small doses has no effect on peripheral blood, whereas the positive effects on brain tissue [5] and myocardium [6], skin flap [7], kidney [8], endotelioprotection persists [9].

In connection with the above, it should be noted study the relevance of protective effects of EPO on a model of retinal ischemia-reperfusion.

Objective: to increase the effectiveness of pharmacological correction of retinal ischemia with use of distant ischemic preconditioning and pharmacological preconditioning with recombinant erythropoietin.

Materials and methods.

Experiments were carried out on the Wistar rats weighing 225- 275 g. For the study the rats were taken with no external signs of disease, passed quarantine regime.

Operations and other manipulations were performed on rats under general anesthesia performing intraperitoneal (i/p) adding an aqueous solution of chloral hydrate 300 mg/kg.

DIP was performed 10 min by the clamping the femoral artery to the proximal tourniquet third hip for 40 min before modeling retinal ischemia followed by a 30 minute episode-reperfusion injury [10].

Recombinant erythropoietin was administered i/p (Epokrin, GosNII OCHB (State Research Institute of especially pure biological products) in a dose of 50 IU/kg 30 minutes before the pathology simulation.

Glibenclamide ("Maninil" (Berlin-Chemie AG/ Menarini Group) was administered at a dose of 5 mg/kg once per 60 min before ischemia simulations.

Emoxipine, solution for injection, 10 mg/ml (vials) 1 ml (1% Solution), (Federal State Unitary Enterprise "Moscow Endocrine Plant") was administered at a dose of 2 peribulber mg/kg. Introduction of the preparation was carried out once daily for 4 days including the first administration 30 min before modeling pathology.

Measuring the level of microcirculation in the retina of rats was performed by LDF. Registration is carried out by means of hardware and software Biopac-systems MP-150 and the needle-type sensor TSD-144 (USA) with AcqKnowledge 4.2 program. After anesthesia the animal, carried out an assessment of the microcirculation level in ten points on the circumference of the eyeball, the recording duration of the microcirculation level readings at one point was twenty seconds. From the microcirculation level results at every point of the average volumes, which was taken as the indicator of the level of the microcirculation in the retina of the experimental animal. Indicator microcirculation in the group of animals was calculated as the average of the values obtained from each experimental animal group [11].

For registration of the ERG animals were kept in the dark for 30 minutes [12], then anesthetized (chloral hydrate, 300 mg/kg, i/p) and fixed on the table is isolated from the electromagnetic radiation. Corneal silver electrode was placed on the cornea, pre-wetted with saline to more fully contact the reference EL needle electrode 452 placed subcutaneously in the skull, EL450 ground needle electrode was placed subcutaneously in the base of the tail. Strobe light with white flash connected to the stimulator STM200 company Biopac System, Inc. (USA) placed behind the back of the animal, ERG registration was carried out in response to a single stimulation. Evoked bio potentials were run at a frequency of 1-1000 Hz, amplified, averaged and presented graphically on the screen using the Biopac-systems MP-150 with a computer program AcqKnowledge 4.2 (USA). ERG recording was carried out for 0.5 seconds of each rat in groups to assess the degree of retinal ischemia evaluated by the ratio of the amplitudes of the a- and b- wave ERG - coefficient b/a (Neroev VV et al., 2004) [13]. Report the average, which was added to the protocol from the obtained ten values in each group.

After the LDF and ERG eyes with surrounding tissues subject to enucleation. The obtained sample was fixed in 10% formalin solution for the iμmersion method phosphate buffer for 24 hours.

Then, subject to the eyeball enucleation. For histological examination with eyes iμmediately, adjacent tissues were fixed in 10% formalin solution. After fixation, the material is completely embedded in paraffin wax in the standard mode. Slices oriented in the blocks so as to receive the slices in the manufacture of preparations in the meridian direction substantially through the middle of the eyeball. Sections for standard histological examination and stained with hematoxylin-eosin. A descriptive study of histological preparations were performed under a microscope Axio Scope A1 (Carl Zeiss Microimaging GMbH, Germany) [14]. The morphometric studies were performed on the microscope Mikmed-6, JSC "LOMO" with the use of the program Micro-Analysis View.

For all these descriptive statistics were used: data are checked for normal distribution. Type defines the criteria for the distribution of the Shapiro-Wilk. In case of normal distribution were calculated the average value (M) and standard error of the mean (m). In cases of abnormal distribution were calculated median (Me) and quartile range (QR).

Between-group differences were analyzed by parametric (t-Student criterion) or non-parametric (Mann-Whitney test) methods, depending on the type of distribution. Differences were determined at 0.05

significance level. Statistical analyzes were performed using Statistica 10.0 software.

The main part:

The first step in the study of pharmacological preconditioning protective properties of the retina is the development of models of retinal ischemia.

Based on literature data, modeling laboratory rats retinal ischemia can be carried out by raising the intraocular pressure (IOP). This model was performed by Pathology of the needle connected to a saline solution container, in the anterior chamber of the eye. Further, the capacity is raised to the desired height, creating the necessary IOP [15].

The present method model of retinal ischemia-reperfusion injury, has drawbacks and limitations associated with damage to the anterior chamber, the additional administration of antibacterial drugs to prevent infection with the eye [16].

In connection with the above, our own modification of the model of retinal ischemia-reperfusion was used, in which increased intraocular pressure is carried out by mechanical pressure (110 mm Hg) In the anterior chamber of the eye.

5 series of experiments carried out to search for the optimum time of retinal ischemia. The first group (n = 20) - the group of intact animals, second (n = 20) - a group of animals to the duration of retinal ischemia 10 min, and the third (n = 20) - to ischemia lasting for 20 minutes, and the fourth (n = 20) - with a length 30 minutes ischemia, fifth (n = 20) - with a 40 min ischemia. After anesthesia i/p introducing an aqueous solution of chloral hydrate 300 mg/kg, the animal was fixed in a lateral position, and then carried ischemia. After the simulation after 1 h and 72 h of reperfusion microcirculation was measured in the retina by LDF, determined electrophysiological condition of the retina by the ERG and the excretion of animals was carried out, followed by enucleation of eyes for morphological studies.

After working models and choosing the optimal time simulation retinal ischemia surveyed retinoprotective activity distant ischemic preconditioning and pharmacological preconditioning with recombinant erythropoietin compared to the reference drug emoxipine.

Results.

In accordance with the study design, modeling the duration of retinal ischemia was 10, 20, 30 and 40 min, followed by reperfusion period duration 1 h and 72 h.

In accordance with the protocol simulation pathology after 1 hour and 72 hours of reperfusion the animals were performed anesthesia (chloral hydrate solution 300 mg/kg rat body weight).Then evaluated the level of the microcirculation by LDF, we evaluated electrophysiological condition of the retina by ERG, and then taken out of animal experiments followed by enucleation eye for morphological and morphometric study of the retina.

After modeling retinal ischemia (IR) rats by providing mechanical pressure on the anterior chamber after 1 h post-ischemic reperfusion formed reactive hyperemia.

The results of measuring the level of microcirculation rat retina after 1 h reperfusion depending on the duration of ischemia are shown in fig. 1. The level of retinal microcirculation intact animals was 743.6 ± 20.9 p.u.. After 1 hour after the pathology simulation group of animals from 30 min ischemia, the level of microcirculation was 1135.2 ± 43.4 p.u. that exceeds the level of microcirculation under the group of intact animals by 52%, in the group of animals with 40 min ischemia indicator of microcirculation was 1164.9 ± 34.5 p.u., exceeding the figure in the group of intact animals by 57%. In groups of 10, and 20 min ischemia of the microcirculation level exceeded the figure relative to the group of intact animals by 23% and 33%, respectively. The data obtained in all the experimental groups was significantly different from that of the group of intact animals.

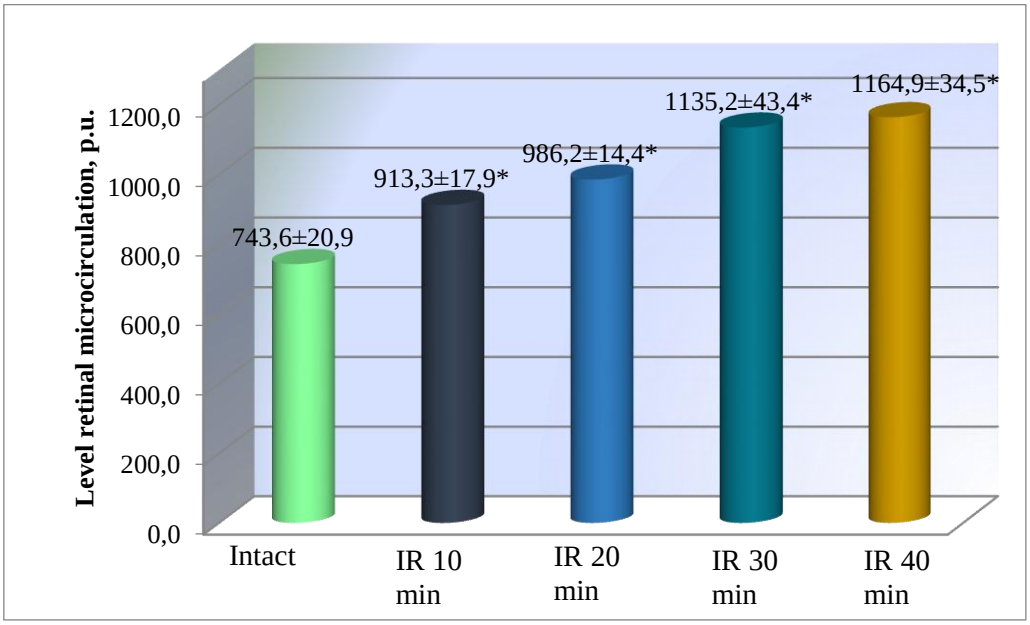

Figure 1. Level retinal microcirculation 1 hour after retinal ischemia simulations
Note. * - p <0.05 compared with the group of intact animals.

After modeling IR rats by providing mechanical pressure on the anterior chamber after 72 hours post-ischemic reperfusion observed deterioration in regional blood flow, which is associated with the development of the pathological cascade of morphological and functional changes in the eye.

The data obtained during the measurements in the microcirculation retina of rats after 72 h of reperfusion, depending on the duration of ischemia are shown in Fig. 2. The level of the microcirculation in the retina intact animals was 740.0 ± 8.1 p.u.. After 72 hours, the animals in groups with a duration of ischemia, 30 and 40 min levels microcirculation reached the least results. In the group of animals with 30 min ischemia of the microcirculation level was 346.1 ± 11.5 p.u., which is 53% lower than this indicator in the group of intact animals. In the group of 40 min ischemia microcirculatory level was 294.4 ± 17.9 p.u., below the level of microcirculation in the intact group by 60%. In the case of 10 and 20 minutes of retinal ischemia model of microcirculation level was lower than in the group of animals intact 15% and 34%, respectively. During simulation pathology in all experimental groups, a significant difference is observed between the level of microcirculation indices in the group of intact animals.

Based on these results, it should be noted that the duration of ischemic episodes caused by increased intraocular pressure greatly affects the level of retinal microcirculation after 1 h and 72 h after reperfusion pathology simulation.

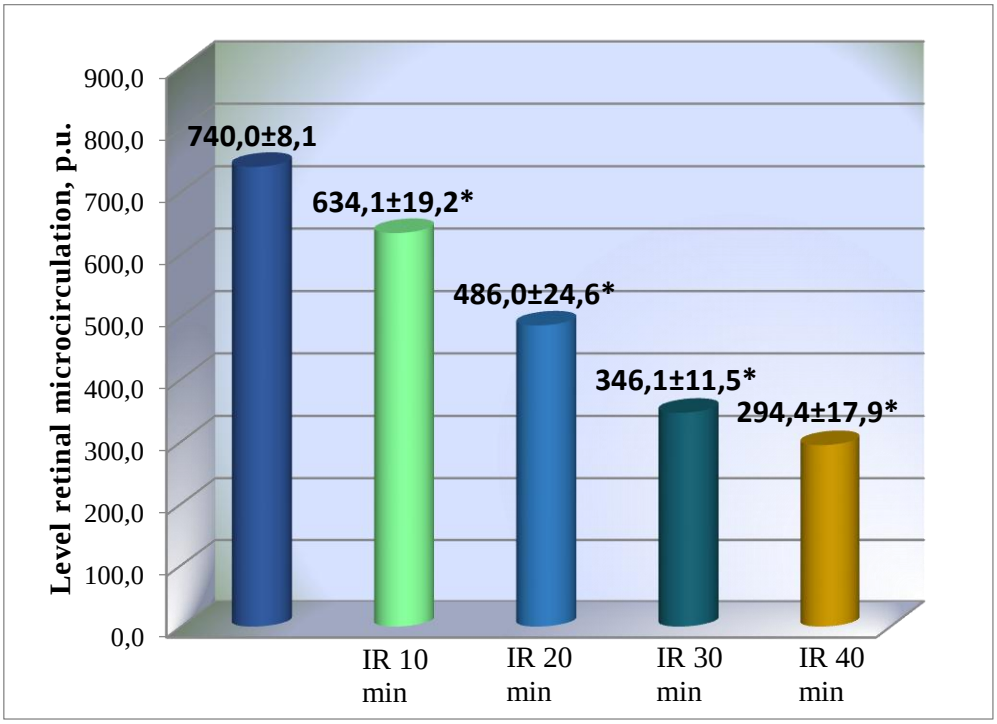

Figure 2. The level of the microcirculation in the retina after 72 h after modeling retinal ischemia.
Note. * - p <0.05 compared with the group of intact animals.

Violations result in hemodynamic changes characteristic of ischemia in the study of retinal electrophysiological state. To assess the severity of retinal ischemia is used with respect to the amplitude of the wave b wave amplitude a ERG, which allows to evaluate the severity of retinal ischemia - coefficient b/a [13].

Fig. 3 shows the results of changing the ratio of the amplitudes of a- and b- waves electroretinogram - ratio b/a 1 h after reperfusion, retinal ischemia simulations of 10, 20, 30 and 40 min.

In the experimental evaluations of electrophysiological condition of the retina of rats, it was found that the ratio b/a in the group of intact animals was 2.5 ± 0.08 r.u., in groups IR lasting 30 and 40 minutes, modeling the figure was significantly different from the values in the group of intact animals and amounted to 2.1 ± 0.08 and 2.0 ± 0.06 r.u. which is lower than under the group of intact animals by 16% and 20%, respectively. In the groups of IR 10 and duration 20 min significant differences from the values in the group of intact rats were revealed.

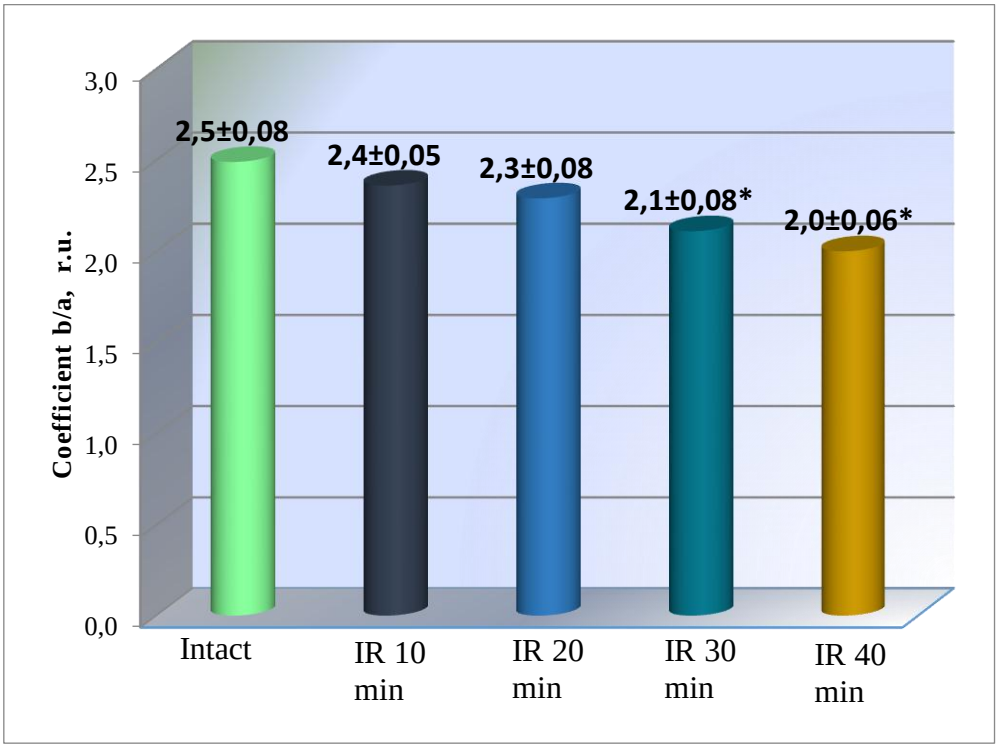

Figure 3. Evaluation of retinal electrophysiological state 1 hour after modeling retinal ischemia.
Note. * - p <0.05 compared with the group of intact animals.

Fig. 4 shows the change in the coefficient b/a within 72 h after reperfusion, retinal ischemia simulations of 10, 20, 30 and 40 min.

After 72 h of reperfusion in all experimental groups showed a significant contrast ratio b/a of the electroretinogram values in the group of intact animals. This indicator reached the level of 2.1 ± 0.07 r.u. in the group with the 10-min ischemia, which is lower than with respect to the group of intact animals by 16%. In the group with the 20-min ischemia ratio b/a was 1.8 ± 0.08 r.u., which is below this indicator relative to the group of intact animals by 28%. In the group with the 30-min ischemia ratio b/a was equal to 1.2± 0.06 r. u., which is below this indicator relative to the group of intact animals by 52%. In the group with the 40-min ischemia ratio b/a was 1.1 ± 0.03 r.u. in the group, which is lower than this indicator relative to the group of intact animals by 56%.

Based on the conclusion that the modeling of pathology can be made of data is a violation of the electrophysiological state of the inner layers of the retina, which is characterized by a decrease in the electrophysiological activity of the bipolar, Muller cells, amacrine and horizontal cells, due to violations of the retinal blood flow and the occurrence of ischemia.

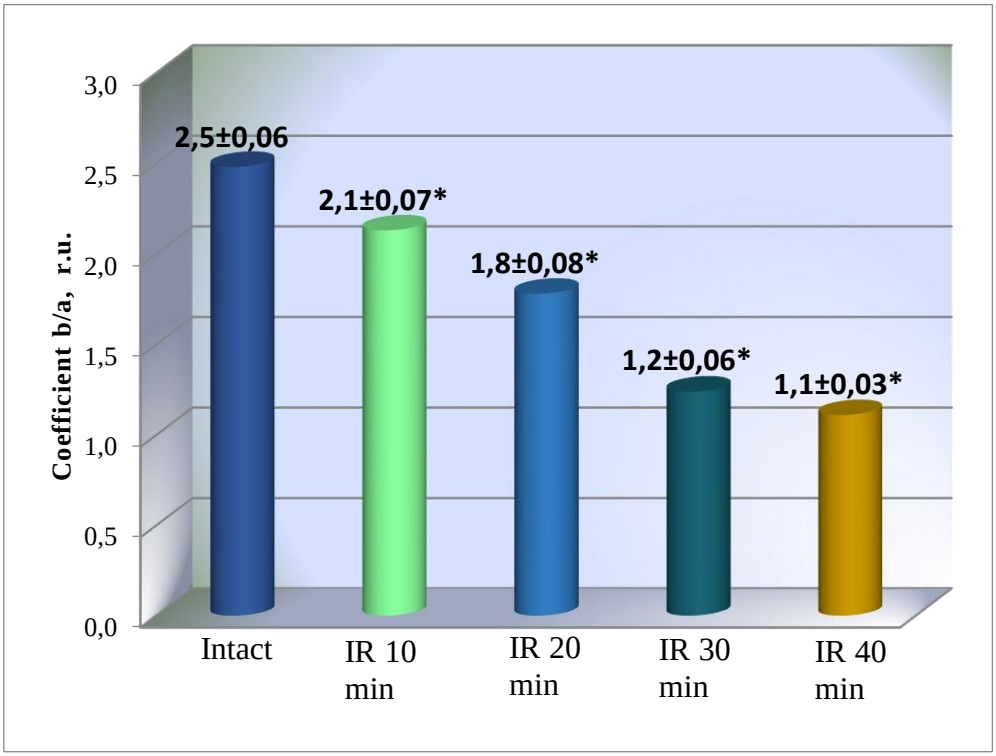

Figure 4. Assessment of retinal electrophysiological state 72 hours after modeling retinal ischemia.
Note. * - p <0.05 compared with the group of intact animals.

Changing the ratio b/a of the electroretinogram in the experimental groups occurred after 72-hours eperfusion, more pronounced, than in the experimental groups at 1 hour of reperfusion, which is associated with the development of degenerative changes in the retina that occurred during this time.

After electroretinography analyzed morphometric parameters of retina. Morphometric assessment was subject to the inner nuclear layer and photoreceptor layer of the retina of experimental animals.

Example of measuring the inner nuclear layer thickness and the photoreceptor layer is given in Fig. 5 and Fig. 6, respectively.

In the morphometric analysis of the inner nuclear layer thickness and a layer of photoreceptors was determined after 1 h reperfusion in duration with IP groups 10 and 20 minutes, increasing the thickness of the inner nuclear layer was not significantly different from the group of intact animals. In the experimental groups with 30 min and 40 min ischemia thickness of the inner nuclear layer was significantly different from the group of intact animals. As a result of the morphometric analysis photoreceptor layer thickness it was found that significant differences in all the experimental groups with respect to no group intact.

An example of a morphometric analysis through 1 hour after pathology simulation presented in Fig. 7 and 8. Results of morphometric 1 h after reperfusion are shown in Tab. 1.

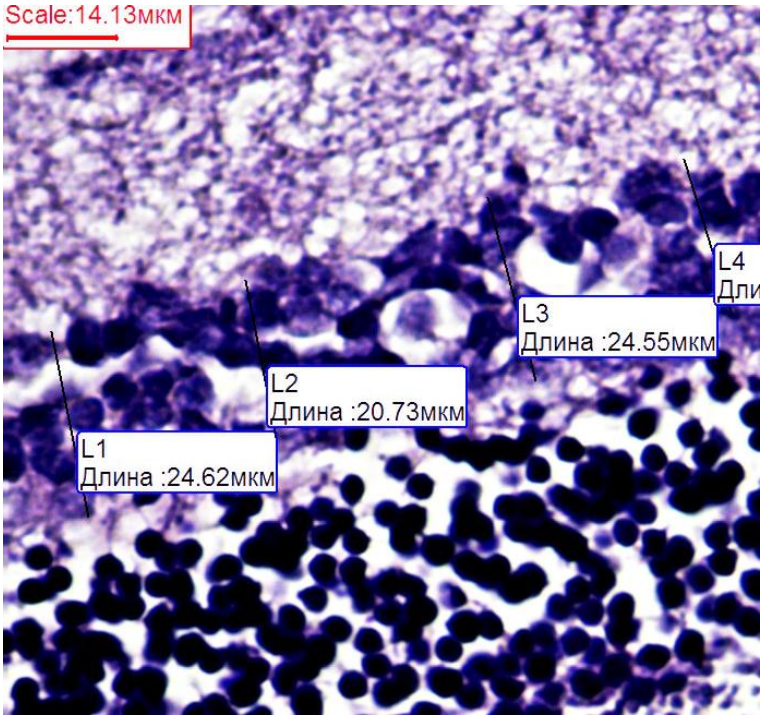

Figure 5. An example of measuring the thickness of the inner nuclear layer of the retina intact animal. H & E stain. Increasing the X400

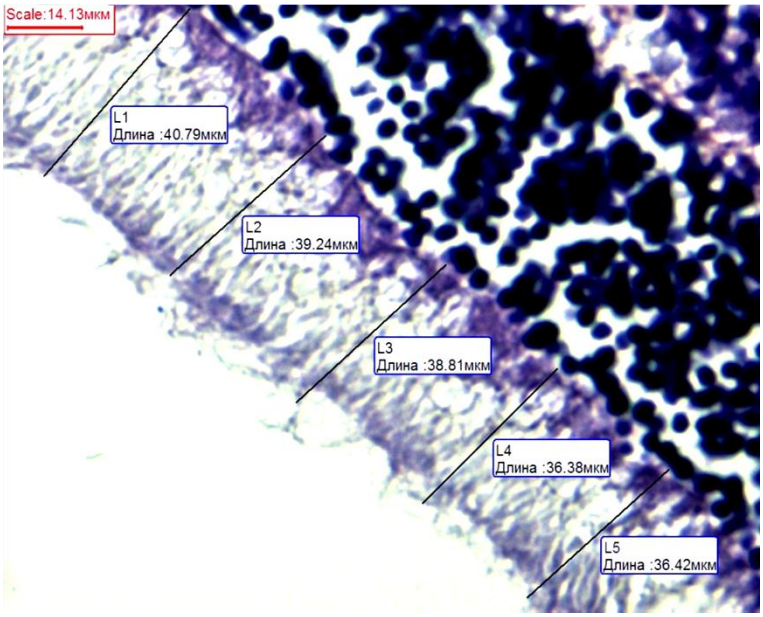

Figure 6. Example of measurement of the thickness of the retinal photoreceptor layer is intact animal eyes. H & E stain. Increasing the X400

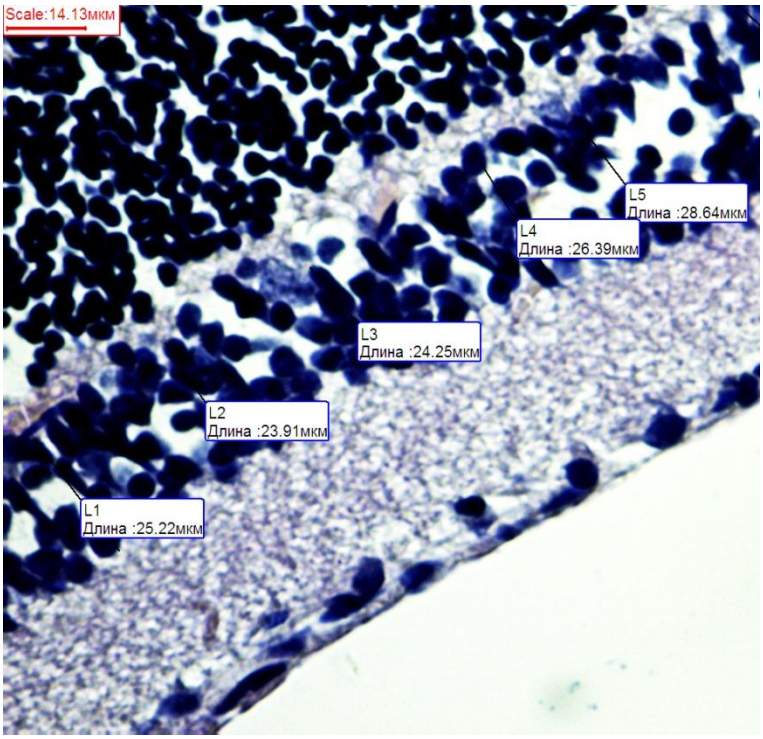

Figure 7. An example of morphometric measurements of the inner nuclear layer of the retina thickness rats 1 hour after reperfusion disease modeling (30 minute model). Ochre. hematoxylin and eosin. Increase 0 x40.

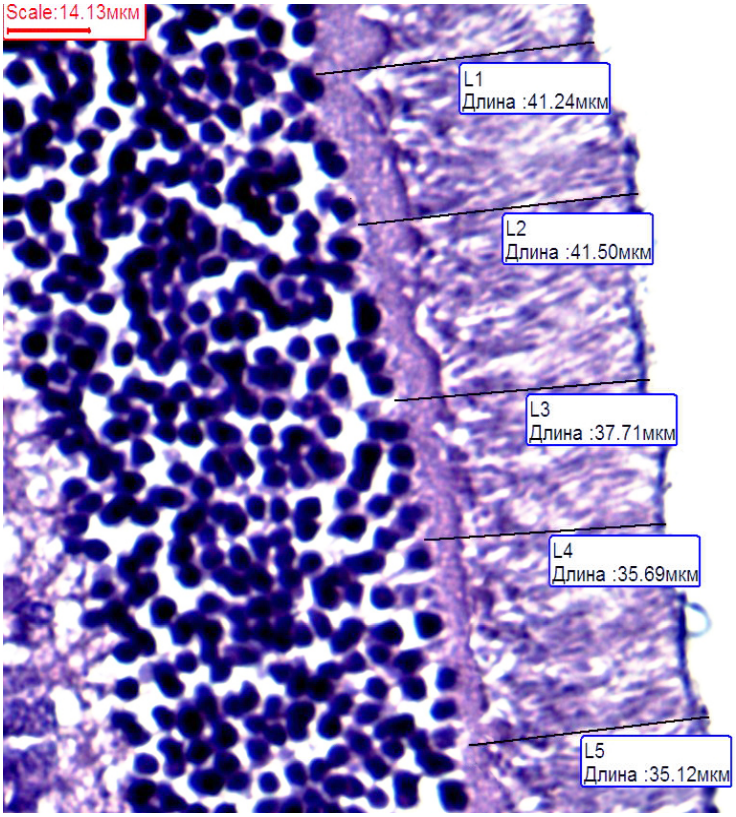

Figure 8. Example morphometric measurement of the thickness of the retinal photoreceptor layer of the rat at 1 hour after reperfusion disease modeling (30 minute model). H & E stain. Increasing the X400.

Table 1.

The thickness of the inner nuclear layer and retinal photoreceptors in rat experimental groups after 1 h reperfusion (M ± m; n = 10)

No	Experimental groups	Inner nuclear layerthickness, µm	The thickness of the photoreceptor, µm
1.	Intact	23.6 ± 0.69	38.2 ± 0.95
2.	IR 10 min	24.0 ± 0.74	38.5 ± 0.73
3.	IR 20 min	24.4 ± 0.86	38.6 ± 0.63
4.	IR 30 min	25.8 ± 0.65 *	38.8 ± 0.64
5.	IR 40 min	26.1 ± 0.70 *	39.1 ± 0.74

Note. * - P <0.05 compared with the group of intact animals

Thickness of the inner nuclear layer of the retina intact rats was 23.6 ± 0.69 µm. In the group of animals with a model of ischemia and 40 minutes, 30 of the inner nuclear layer thickness was 25.8 ± 0.65 µm and 26.1 ± 0.70 µm, respectively, which was significantly different from the index of the group of intact animals.

Morphometrically picture retinal layers after 72 h of reperfusion is shown in Fig. 9. and Fig. 10.

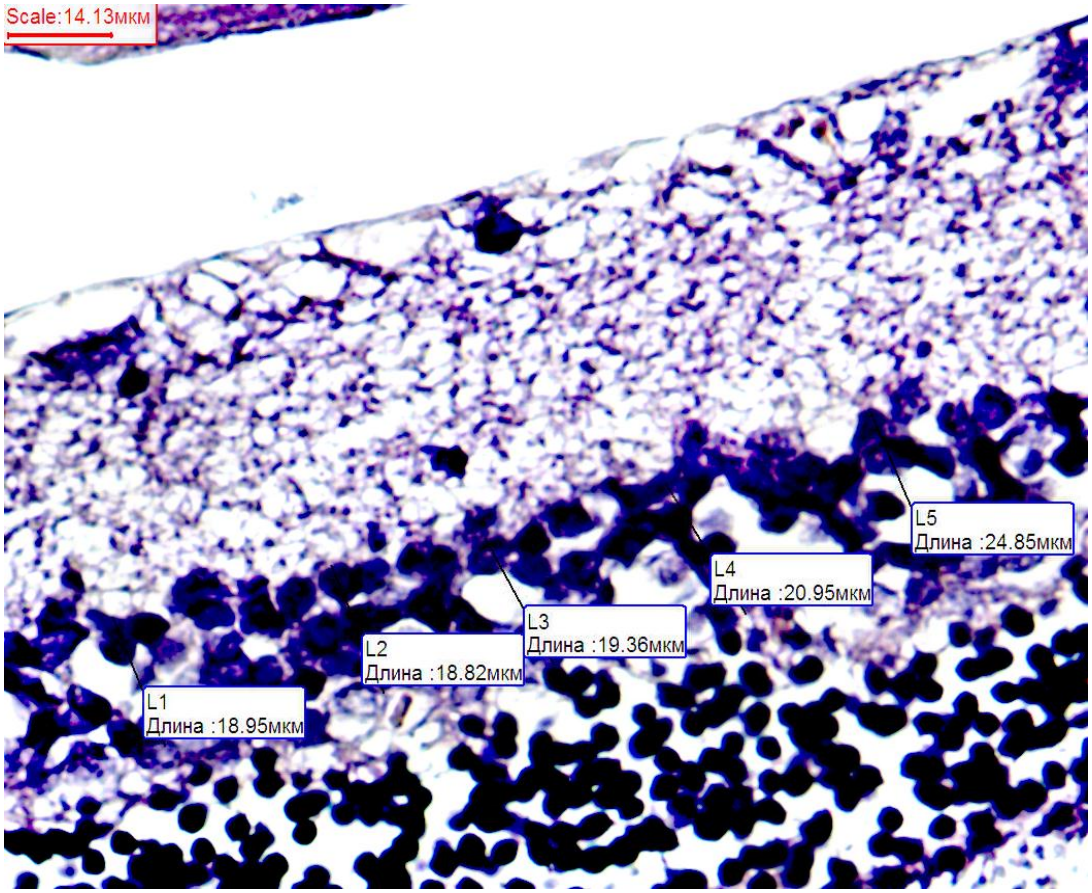

Figure 9. Example morphometric measurement of the thickness of the inner nuclear layer of the retina of rat after 72 hours of reperfusion with a moment disease modeling (30 minute model). H & E stain. Increasing the X400.

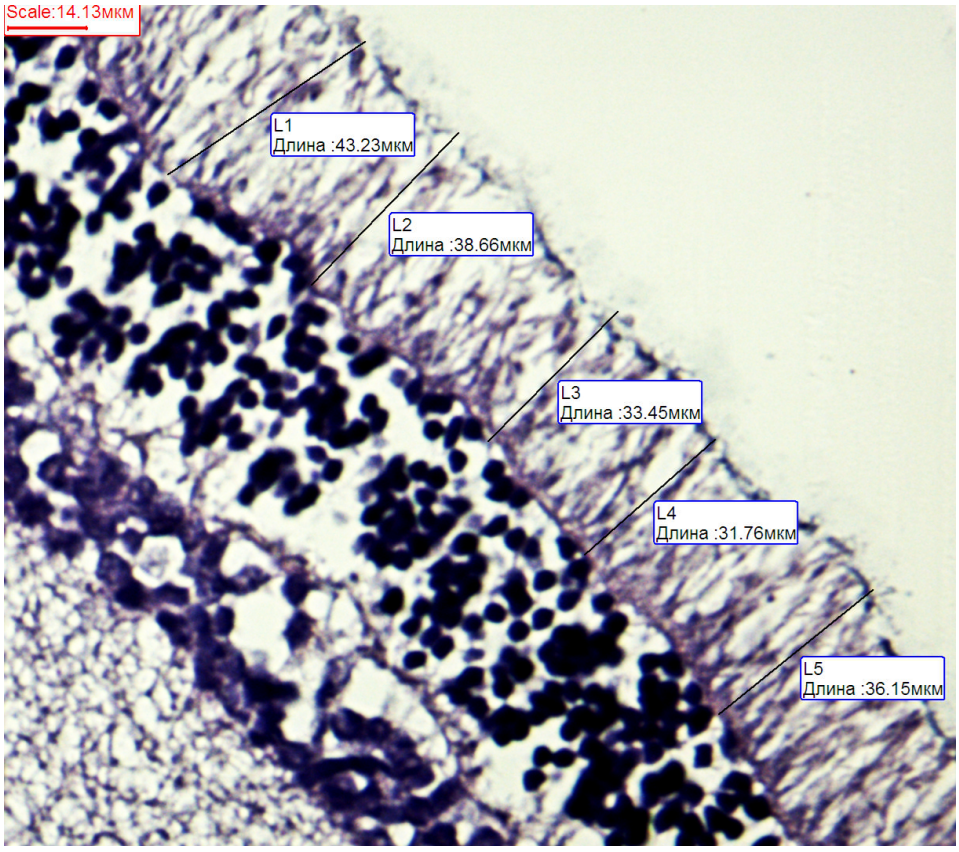

Figure 10. Example morphometric measurements of the retinal photoreceptor layer thickness rats 72 hours after reperfusion disease modeling (30 minute model). H & E stain. Increasing the X400.

These morphometric study of the retina eyes of animals 72 hours after pathology simulation are presented in Table. 2.

Table 2.

The thickness of the inner nuclear layer and retinal photoreceptors in rat experimental groups, after 72 h of reperfusion (M ± m; n = 10)

No	Experimental groups	Inner nuclear layerthickness, µm	The thickness of the photoreceptor, µm
1.	Intact	23.4 ± 0.61	38.0 ± 0.89
2.	IR 10 min	22.2 ± 0.84	38.1 ± 0.67
3.	IR 20 min	21.7 ±0.61	37.3 ± 0.66
4.	IR 30 min	20.4 ± 0.80 *	36.6 ± 0.65
5.	IR 40 min	19.5 ± 0.51 *	36.1 ± 0.69

Note. * - P <0.05 compared with the group of intact animals

After 72 hours of reperfusion, animals in groups ah model of ischemia 10 and 20 m thickness of the inner nuclear layer was not significantly different from this index in the group of intact animals. The groups ah animal models of ischemia 30 and 40 min thickness of the inner nuclear layer was 20.4 ± 0.83 µm and 19.5 ± 0.51 µm respectively that differs significantly from the index of the group of intact animals, after 72 h of reperfusion. Results morphometry photoreceptor layer in all experimental groups showed no statistically significant differences.

Study: microcirculation level in the retina by LDF, electrophysiological activity by ERG, morphometric parameters, allowed us to estimate the degree of damage to the retina at the pathology of this model depending the duration of ischemic episodes.

Most acceptable (from our perspective) model is a model of a 30-minute IC, which is characterized by:

- statistically significant difference in the level of retinal microcirculation in rats after 1 hour and 72 hours after reperfusion by IP indicators group of intact animals;

- significant reduction index b/a simulation pathology of electroretinogram after 1 hour and 72 hours of reperfusion compared with those in the group of intact animals;
- statistically significant difference between morphometric parameters of the layers of the retina relative to the group of intact animals.

After working pathology model evaluated protective effect of ischemic preconditioning distant and recombinant erythropoietin compared to emoxipine.

Estimated at the level of the microcirculation in the retina, after pathology simulation was carried out after 1 h and 72 h of reperfusion by LDF. The results obtained after 1 hour of reperfusion are shown in Tab. 3.

The level of the microcirculation in the retina intact rats was 738,9 ± 37.6 p.u. The level of microcirculation after ischemia simulations in the control group reached after 1 h of reperfusion 1155.0 ± 51.9 p.u. , which was significantly higher than the value in the group intact farm animals ($p < 0.05$). Against the background of the correction of pathology DIP level of microcirculation in the retina after 1 h of reperfusion significantly reduced to 952.0 ± 25.8 p.u. ($p < 0.05$) compared with the control group (IC) (Tab. 3).

Table 3.

The level of the microcirculation in the retina of rats After 1 h of reperfusion (M ± m), PE

Number pp	experimental groups	The level of the microcirculation
1.	Intact (n = 10)	738.9 ± 37.6 ^{y}
2.	Control (n = 10)	1155.0 ± 51.9 *
3.	Correction DIP (n = 10)	952.0 ± 25.8 * ^{y}
4.	Correction EPO, 50 IU/kg (n = 10)	798.5 ± 12.3 ^{y}
5.	Control + glibenclamide 5 mg/kg (n = 10)	1135.8 ± 31.2 *
6.	Correction DIP + glibenclamide 5 mg/kg (n = 10)	1144.7 ± 20.7 *
7.	Correction EPO, 50 IU/kg + glibenclamide 5 mg/kg (n= 10)	1148.5 ± 14.3 *
8.	Correction emoxipin, 2 mg/kg (n = 10)	998.0 ± 19.4 * ^{y}

Note. * - $P < 0.05$ compared with the group of intact animals; ^{y} - $p < 0.05$ compared with the control group

When the correction of the modeled pathology EPO microcirculation level in the group is reduced to 798.5 ± 12.3 p.u. and also significantly different from the values in the control group ($p < 0,05$). Pretreatment of Emoxipine led to a decrease in the level of microcirculation to 998.0 ± 19.4 p.u. Introduction glibenclamide, a blocker of ATP-sensitive potassium channels in the group correction DIP EPO and prevented reduction of microcirculation, which confirms the action precondition recombinant EPO at a dose of 50 IU / kg to retinal ischemia model of rats after 1 hour of reperfusion.

The results of evaluation of microcirculation level in rat retina after 72 hours after reperfusion and its IR simulation DIP correction EPO at a dose of 50 IU/kg and emoxipin 2 mg / kg are presented in Table. 4.

The level of the microcirculation in the retina intact rats was 743.9 ± 5.0 p.u. The level of microcirculation after IC simulation in the control group was 72 h after reperfusion 353.3 ± 11.7 p.u. which was significantly lower than in the group of intact animals ($p < 0.05$), indicating that the formation of ischemia reperfusion for 72 hours . Against the background of the correction of pathology DIP level of microcirculation in the retina after 72 h of reperfusion significantly increased to 638.5 ± 15.8 p.u. ($p < 0.05$) compared with the control group (Tab. 4).

Table 4

The level of the microcirculation in the retina after 72 h of reperfusion (M ± m), p.u.

Number pp	experimental groups	The level of the microcirculation
1.	Intact (n = 10)	743.9 ± 5.0 ^{y}
2.	Control (n = 10)	353.3 ± 11.7 *
3.	Correction DIP (n = 10)	638.5± 15.8 * ^{y}
4.	Correction EPO, 50 IU/kg (n = 10)	724.0 ± 4.1 ^{y}
5.	Control + glibenclamide 5 mg/kg (n = 10)	359.4 ± 10.3 *
6.	Correction DIP + glibenclamide 5 mg/kg (n = 10)	361.7 ± 13.9 *
7.	Correction EPO, 50 IU/kg + glibenclamide 5 mg/kg (n = 10)	372.3 ± 13.4 *
8.	Correction emoxipin, 2 mg/kg (n = 10)	672.3 ± 12.7 * ^{y}

Note. * - $P < 0.05$ compared with the group of intact animals; ^{y} - $p < 0.05$ compared with the control group

When the correction of the modeled pathology EPO microcirculation level in the group increased to 724.0 ± 4.1 p.u. , which was significantly different from the values in the control group ($p < 0.05$), and tends to the value in the group of intact animals. In the group of animals treated with emoxipine microcirculation level was 672.3 ± 12.7 p.u.

Group Introduction glibenclamide correction DIP and EPO prevented improving microcirculation, which confirms the presence precondition recombinant EPO effect in a dose of 50 IU/kg per rat model of retinal ischemia reperfusion after 72 hours.

After simulating ischemia and measuring the level of the microcirculation in the retina was performed on electroretinography evoked potential. The data obtained are presented in Table. 5.

Table 5

The results of evaluation of retinal electrophysiological state after 1 h of reperfusion (M ± m; n = 10)

Number pp	Experimental groups	b/a, r.u.
1.	Intact	2.6 ± 0.09 ^{y}
2.	Control	2.0 ± 0.09 *
3.	Correction DIP	2.3 ± 0.07 * ^{y}
4.	Correction EPO, 50 IU/kg	2.5 ± 0.07 ^{y}
5.	Control + glibenclamide 5 mg/kg	2.2 ± 0.06 *
6.	Correction DIP + glibenclamide 5 mg/kg	2.1 ± 0.08 *
7.	Correction EPO, 50 IU/kg + glibenclamide 5 mg/kg	2.2 ± 0.09 *
8.	Correction emoxipin, 2 mg/kg (n = 10)	2.3 ± 0.09 ^{y}

Note. * - P <0.05 compared with the group of intact animals; ^{y} - p <0.05 compared with the control group

Ratio b/a in the control group was 2.0 ± 0.09 conventional units, which was significantly different from the group of intact animals. Increase of this indicator in the group with the correction of recombinant EPO to 2.5 ± 0.07 r.u. and DIP - up to 2.3 ± 0.07 r.u. says the preservation of retinal function after electrophysiological disease modeling. In the group of animals with the correction emoxipin ratio b/a was 2.3 ± 0.09 r. u., which was significantly different from that of the group with retinal ischemia and approaches the values in the group of intact animals.

Introduction of glibenclamide in the groups of animals with the correction of the EPO and DIP led to a decrease in the index b/a to values significantly different from that of the group of intact animals, indicating that the blockade of the ATP-dependent potassium channel and confirms the presence of precondition properties of EPO in a dose of 50 IU/kg and DIP IR model for 1 h after reperfusion.

Conducting the ERG at 72 h after reperfusion was performed measuring the level of retinal microcirculation in experimental animals. The results of the study are presented in Table. 6.

Table 6

The results of evaluation of retinal electrophysiological state after 72 h of reperfusion (M ± m; n = 10)

No	Experimental groups	b / a, r. u
1.	Intact	2.5 ± 0.10 ^{y}
2.	Control	1.2 ± 0.04 *
3.	Correction DIP	2.0 ± 0.08 *^{y}
4.	Correction EPO, 50 IU/kg	2.3 ± 0.06 ^{y}
5.	Control + glibenclamide 5 mg/kg	1.2 ± 0.05 *
6.	Correction DIP + glibenclamide 5 mg/kg	1.2 ± 0.0 4 *
7.	Correction EPO, 50 IU/kg + glibenclamide 5 mg/kg	1.2 ± 0.06 *
8.	Correction emoxipin, 2 mg/kg (n = 10)	2.1 ± 0.07 *^{y}

Note. * - P <0.05 compared with the group of intact animals; ^{y} - p <0.05 compared with the control group

The coefficient b / a in the control group was 1.2 ± 0.04 relative units, statistically significantly different from that of the group of intact animals. The increase of this indicator in the group with the correction of recombinant EPO to 2.3 ± 0.06 r. u., DIP - to 2.0 ± 0.08 r. u. and emoxipine to 2.1 ± 0.07 r. u. talks about maintaining electrophysiological retinal function after IR simulation.

Introduction of glibenclamide in groups of rats with the correction of the EPO and DIP led to a reduction coefficient b/a to values significantly different from the group of intact rats, indicating that the blockade of the ATP-dependent potassium

channel and confirms the presence of precondition properties of EPO in a dose of 50 IU/kg and DIP on IR model after 72 h of reperfusion.

Reducing the ratio b/a in animals with simulated ischemia (control) due to inhibition of the positive b-wave ERG, which indicates violation of electrophysiological function of bipolar and Muller cells with the possible contribution of the horizontal and amacrine cells. Saving the electrophysiological function of the photoreceptor layer is confirmed by the absence of adverse changes in the wake of a. Example b-wave suppression electroretinogram is shown in Fig. 3.9.

Electroretinogram retinas of rats experimental groups №1-8 (Tab. 6) are shown in Fig. 11, 12, 13, 14.

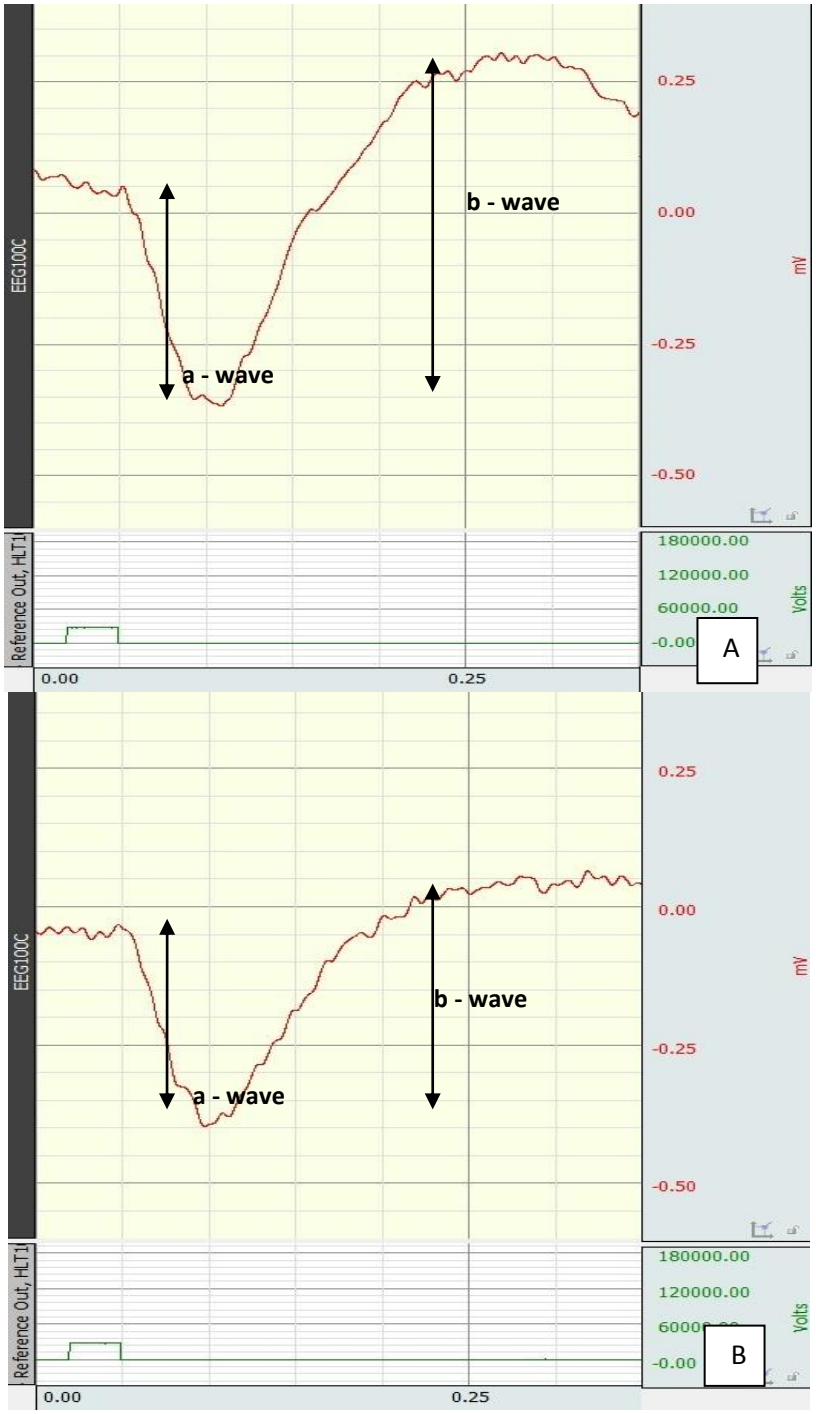

Figure 11. Electroretinogram retina of rats: A - ERG retina intact animal; B - ERG animal retinal ischemia (inhibition observed b-wave of electroretinogram) after 72 h of reperfusion

Figure 12. Retina ERG in rats after 72 h of reperfusion in the experimental groups: A - correction of the EPO; B - correction DIP.

Figure 13. ERG retina of rats after 72 h of reperfusion in the experimental groups: A - control + glibenclamide; B - correction DIP + glibenclamide.

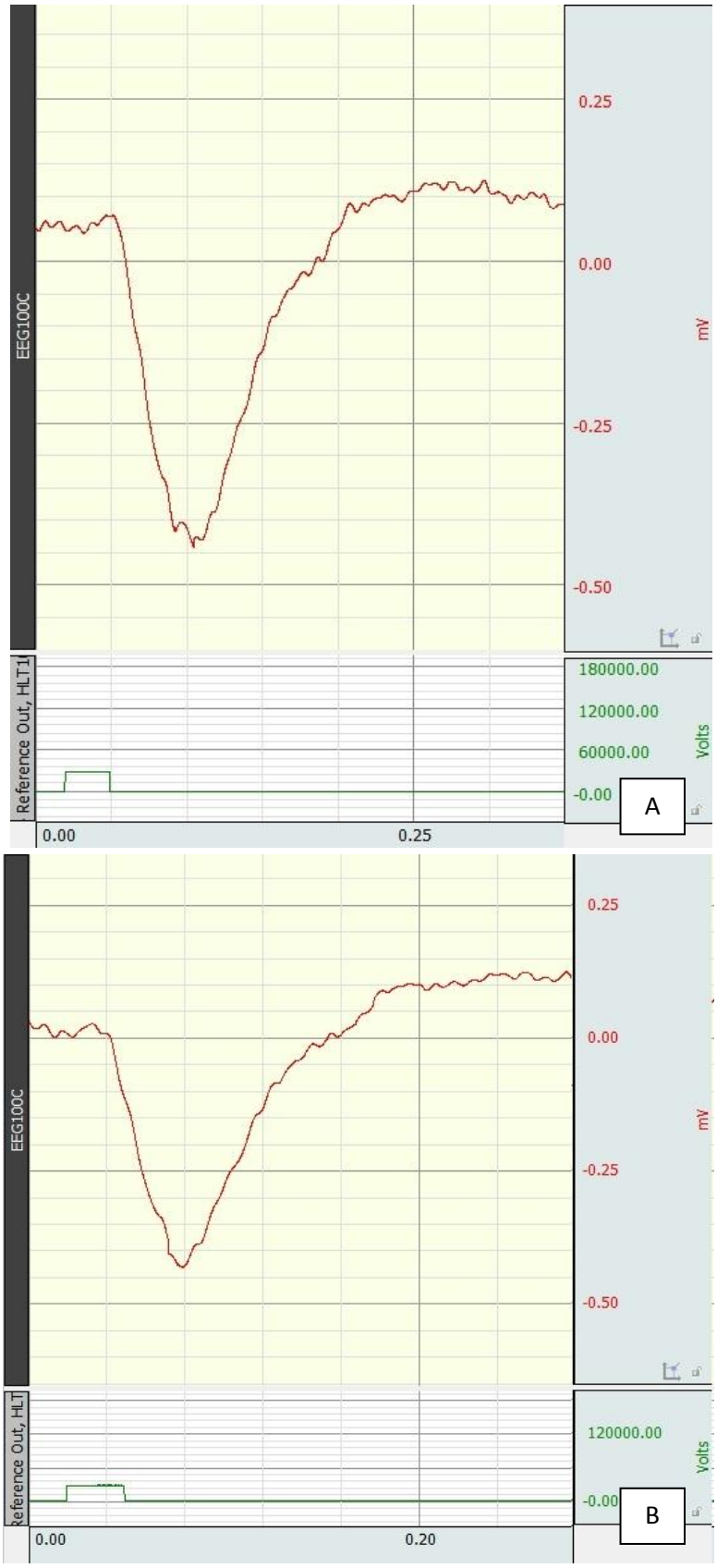

Figure 14. ERG retina of rats after 72 h of reperfusion in the experimental groups: A - EPO correction + glibenclamide; B - correction emoxipin.

After the ERG, eye enucleation was subject to subsequent morphometric analysis. Morphometric assessment subject to the inner nuclear layer and photoreceptor. Morphometry of retinal layers was carried out by Micro-Analysis View software. Results of morphometric analysis after DIP correction EPO emoxipine and combined use with glibenclamide, reperfusion after 1 hour are shown in Table. 7.

An example of measuring the thickness of the inner nuclear layer and a layer of photoreceptors in the group of animals with EPO is shown in Figure 15 and Figure 16, respectively.

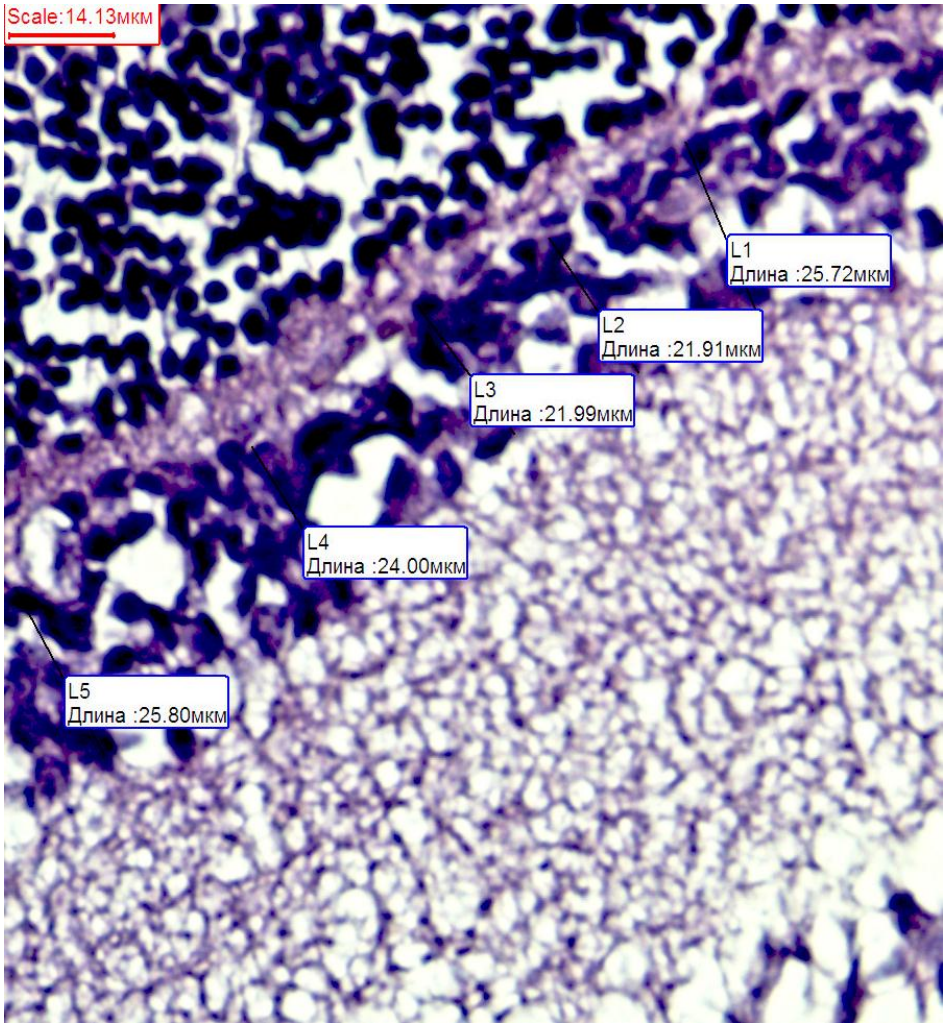

Figure 15. Example morphometric measurement of thickness of the inner nuclear layer of the retina rats 1 hour after reperfusion of EPO on a background correction. H & E stain. Increased X400.

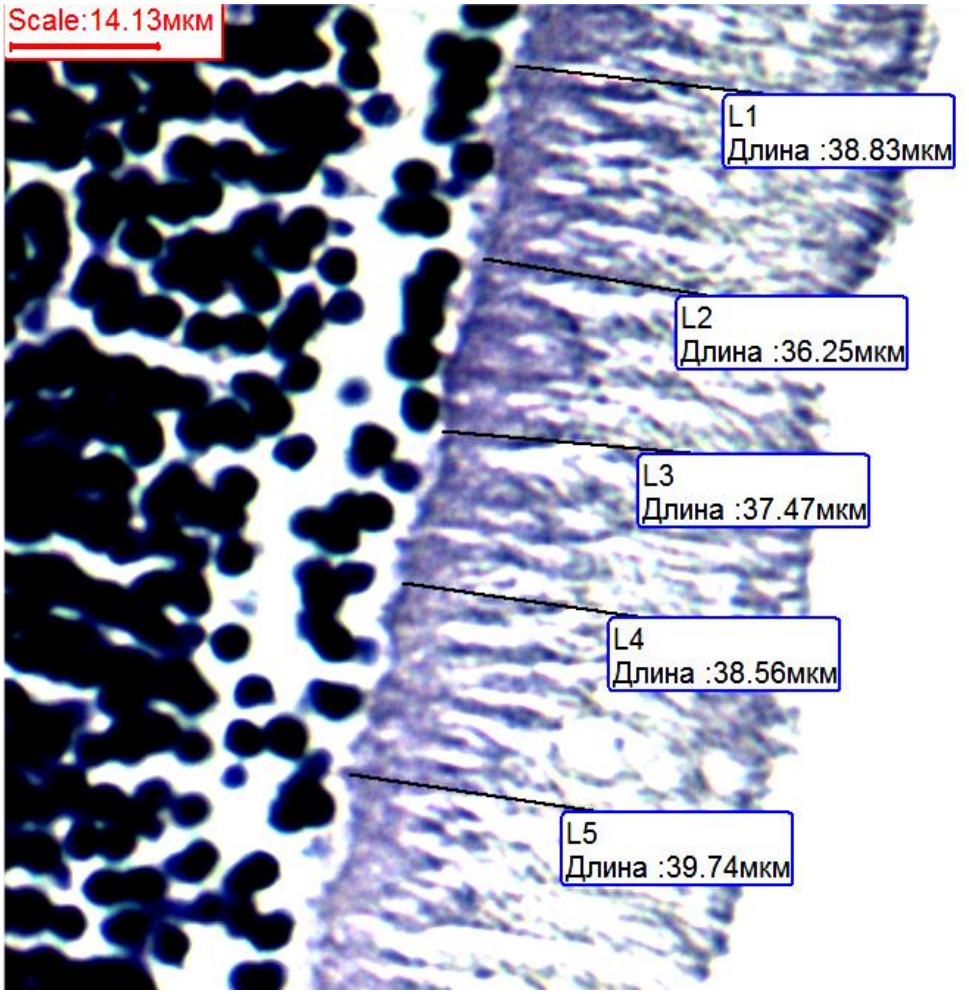

Figure 16. Example of morphometric measurements of the retinal photoreceptor layer thickness rat through 1:00 reperfusion on the background correction of EPO. H & E stain. Increasing the X400.

During the morphometric analysis of the inner nuclear layer and a photoreceptor layer thickness it was found that after 1 hour of reperfusion in the control group increased thickness of the inner nuclear and of 25.9 ± 0.6 μm, which is significantly different group of intact animals.

Pretreatment of EPO reduces the thickness of the inner nuclear layer to 23,8±0.6 μm, which was significantly different from the control group, and is close to the values in the group of intact animals. In the group with DIP and emoxipine thickness of the inner nuclear layer was 24.0 ±0.5 μm and 24.2±0.4 μm, respectively, figures significantly differ from the control group. Pretreatment with glibenclamide in a group with EPO DIP and led to an increase the thickness of the inner nuclear layer, the indicators in groups made up 25.8 ±0.6 μm and 25.7±0.6 μm, respectively, which was significantly different from the group of intact animals and confirms the protective effect of preconditioning on the retina, where the key role played by K^+-ATP channels. Measurements of the photoreceptor layer showed no statistically significant differences in the groups.

Table 7

Morphometric indices retinal layers experimental animals 1 hour after reperfusion (M ± m; n = 10)

No	Experimental groups	The inner nuclear layer, µm	A layer of photoreceptors, µm
1.	Intact	23.5± 0.8 ^{y}	38.4 ± 0,8
2.	Control	25.9 ± 0.6 *	39.1 ± 0,7
3.	DIP	24.0 ± 0.5 ^{y}	38.4 ± 0,9
4.	EPO	23.8± 0.6 ^{y}	38.3 ± 0,9
5.	Control + Glibenclamide	26.0 ± 0.7 *	39.1 ± 0,6
6.	DIP + Glibenclamide	25.8 ± 0.6 *	39.2 ± 0,6
7.	EPO + Glibenclamide	25.7 ± 0.6 *	39.0 ± 0,5
8.	Emoxipine	24.2 ± 0.4 ^{y}	38.6 ± 0,6

Note. * - P <0.05 compared with the group of intact animals; ^{y} - p <0.05 compared with the control group

Morphometry results confirmed the data obtained by ERG changes b - wave of ERG, accompanied by changes in the inner nuclear layer, preserving a - wave of ERG, accompanied by a lack of photoreceptor layer changes.

The results of morphometric analysis after a DIP correction EPO emoxipine and combined use with glibenclamide, after 72 hours of reperfusion are shown in Table. 8.

Table 8

Morphometric indices retinal layers experimental animals at 72 hours of reperfusion (M ± m; n = 10)

No	Experimental groups	The inner nuclear layer, µm	A layer of photoreceptors, µm
1.	Intact	23.8 ± 1.0 ^{y}	38.1 ± 1.2
2.	Control	20.3 ± 0.8 *	36.9 ± 0.9
3.	DIP	21.7 ± 0.4 * ^{y}	37.8 ± 0.8
4.	EPO	23.3 ± 0.7 ^{y}	38.0 ± 1.0
5.	Control + Glibenclamide	20.5 ± 0.4 *	37.1 ± 0.8
6.	DIP + Glibenclamide	20.6 ± 0.6 *	36.9 ± 0.8
7.	EPO + Glibenclamide	20.3 ± 0.5 *	37.0 ± 0.9
8.	Emoxipine	22.5 ± 0.5 ^{y}	37.8 ± 0.9

Note. * - p <0.05 compared with the group of intact animals; ^{y} - p <0.05 compared with the control group

In the morphometric analysis revealed that significant changes in photoreceptor layer thickness after 72 hours of reperfusion in animals of experimental groups is not observed in any of the series, that confirms the absence of a negative change - ERG wave.

Most sensitive to the action of ischemia/reperfusion turned inner nuclear layer. After 72 hours, the thinning observed in connection with ischemic processes in the retina, in the group of animals with the simulation pathology inner nuclear layer thickness was 20.3 ± 0.8 µm, which is significantly different from the group of intact animals.

In the group of animals treated with EPO, inner nuclear layer thickness after 72 hours of reperfusion was 23.3 ± 0.7 µm, which is significantly different from the group with the IR values.

In the group with DIP and in the group with emoxipine inner nuclear layer thickness was 21.7 ± 0.4 µm and 22.5 ± 0.5 µm respectively.

Pretreatment with glibenclamide contributed to the elimination of protective actions as DIP and EPO, confirming the presence of anti-ischemic effect on the model of retinal ischemia in rats DIP and EPO in a dose of 50 IU/kg by precondition action.

In all experimental groups, the thickness of the layer of photoreceptors had no statistically significant differences.

Morphometry results in 72 hours of reperfusion confirmed the data obtained by the ERG, oppression b - wave of ERG, due to the thinning of the inner nuclear layer, and the preservation of negative a - wave of ERG, accompanied by a lack of photoreceptor layer changes.

The study found that EPO has a stronger retinoprotective action compared with DIP and emoxipine that after pathology simulation confirmed changes in microcirculation line retina through 1 hour, 72 hours of reperfusion resulted in normalization of ERG and morphometric parameters of the experimental groups.

Use of EPO suberitrostimulating dose of 50 IU / kg 30 minutes prior to simulation, the action precludes eritrostimulating EPO and increased resistance to ischemic retinal tissue.

Pretreatment with glibenclamide abolished the beneficial effects of EPO and DIP, which indicates the implementation retinoprotection by preconditioning, the mechanism of which is realized at the expense of ATP-sensitive potassium channels.

Emoxipine at a dose of 2 mg/kg has a less pronounced effect than retinoprotection DIP 1 hour of reperfusion, but was superior to the performance indicators DIP 72 hours.

Discussion

The main factors of development of IR are: breach of the general hemodynamics, local changes in the walls of blood vessels and coagulation and lipoprotein changes in the blood. The first factor is usually caused by GB, hypotension, atherosclerosis, diabetes, occlusive diseases of the large blood vessels, blood diseases. From local changes are the most important vessels atheromatosis, violations of the vascular endothelium [16, 17, 18, 19].

In this connection there is a need to find new methods for retinoprotection possible reduction in the damaging effect of ischemia and reperfusion of the retina. Segment of drugs to treat diseases of the choroid as the complications of systemic diseases is expedient to expand due to the increasing incidence of and lack of funds for targeted ischemic lesions of the choroid correction [20].

As drugs for correcting retinal ischemic damage is nonspecific therapy and do not have the desired result, that the problem can be solved by AF, which is able to protect the retina from ischemic injury.

The role of the DIP and the PT proved by many authors in various organs and tissues of the body. Versatility preconditioning mechanism gives the background to the study of this phenomenon on the retina. One of the most promising is the AF EPO agents precondition his action has been studied in the brain [21], the spinal cord [22], kidney [23], the small intestine [24] heart[25, 26].

ATP-dependent potassium channel opening during ischemia, plays a central role in the mechanism of cytoprotective effect of SP. Initially, their activity was detected at sarcolemma membrane, and later at the mitochondrial level. Those and other isoforms are inhibited by physiological concentrations of intracellular ATP and open when the concentration of ATP is significantly reduced, that is, act as sensors (sensors) the availability of sufficient amounts of oxygen and glucose (ATP source) [27].

Because most researchers tend to believe that all their isoforms are able to participate in the implementation of the protective effect of preconditioning in the study, we used a non-selective blocker of ATP-sensitive potassium channels, glibenclamide.

Given the fact that these electrophysiological studies often have a decisive importance in the early and differential diagnosis of retinal disorders [28], to study the correction of degenerative changes in the retina, you must conduct a comprehensive analysis, including electroretinography, microcirculatory and morphological studies. Analysis of the dynamics of retinal electrogenesis allows you to evaluate the nature and topography of retinal disorders, as well as to identify the most labile hypoxic retinal structure, their response to recombinant EPO DIP correction and emoxipin.

The foregoing predetermined the need to develop and systematize methodological approaches to the assessment of the functional state of the retina and the subsequent optimization of the correction of ischemic damage of the retina.

So, in the course of our research in the experiment on Wistar rats had developed a set of methodological approaches to assess the functional status of the retina, which includes tools (LDF, ERG) and morphological methods.

The first step in exploring the possibility of correction of ischemic damage to the retina using distant and pharmacological preconditioning with recombinant EPO was to develop a model of retinal ischemia Wistar laboratory rats. A prerequisite was to study the effect of different time periods of ischemia on functional condition of the retina. To do this, we evaluated the microcirculation level in the retina, electrophysiological and morphological picture of the state of the layers of the retina followed by morphometry of the most important layers.

Simulation IR laboratory rats was performed by raising IOP to 110 mm Hg while providing mechanical pressure to the anterior chamber. After simulating ischemia lasting 10, 20, 30 and 40 minutes of retinal condition evaluation was carried out after 1 h and 72 h of reperfusion. This time period is selected based on the literature [29]. After the simulation IR through 1 hour post-ischemic reperfusion reactive hyperemia is formed. After the simulation IR rats at 72 hours post-ischemic reperfusion observed deterioration in regional blood flow.

After examining the functional status of the retina instrumental methods of analysis and morphometric studies was the most appropriate model is a 30-minute IR. Such a period of 1 hour after ischemia reperfusion, contributed to an increase of the inner nuclear layer and resulted in pronounced degenerative changes at 72 hours of reperfusion,

which were not observed at 10 and 20 minutes of ischemia.

Summarizing the above, it should be noted that the proposed method complex functional and morphological changes associated with the development of IR, be sufficient to objectively assess retinoprotective effects of pharmacological agents.

The results of investigating the possibility of IR correction using distant and pharmacological preconditioning recombinant EPO have determined that in the groups of the experimental animals treated with EPO, there was a significant difference between all the measured parameters (level of microcirculation in the retina, the values of the coefficient b/a of electroretinogram, the thickness of the retinal layers) on the values of in the group of animals with IR, which makes it possible to talk about the ability of EPO at a dose of 50 IU / kg retinoprotection exert effects on IR model experiment.

Coefficient value b/a ERG and the thickness of the inner nuclear layer of the retina in the group correction DIP statistically significantly different from the values in the control group and the group of intact animals, which makes it impossible to speak about full retinoprotection, which is observed in the group of animals treated with the EPO.

Proof of protection layers of the retina due to the effect of preconditioning served in the additional administration of glibenclamide 5 mg / kg dose, blocking ATP-dependent potassium channels. In groups of animals treated with correction DIP and EPO administration of glibenclamide resulted in the elimination of the observed cytoprotective effect, which confirms precondition effect of DIP and recombinant EPO in a dose of 50 IU / kg on an experimental IR model, the mechanism of realization of which is the basis of the activation of ATP-dependent potassium channels .

The results obtained in the group of animals with emoxipin correction comparable to the group correction DIP, but inferior performance in the group correction EPO.

Thus, the prospects become apparent to optimize pharmacotherapy conditions accompanied by retinal ischemia, which are closely linked with the task of forming the methodology of the study antiischemic activity of pharmacologic agents based on an adequate assessment of the functional condition of the retina instrumental and morphological studies.

Optimization pharmacological IP correction may be performed by specific recombinant EPO therapy at a dose of 50 IU / kg and DIP, since in both cases there is a positive pharmacological effect.

Conclusion:

The study developed a model of retinal ischemia, with justification temporary ischemia simulation mode of the retina of rats, assessment of the level of microcirculation, retinal electrophysiological state and morphometric parameters of retina, allow to fully appreciate the functional condition of the retina after a period of ischemia followed by reperfusion. The optimum pathology model was a model 30-minute ischemia with subsequent retinal reperfusion periods lasting 1 hour to 72 hours.

This model allowed us to estimate the possibility of retinal preconditioning with recombinant erythropoietin at a dose of 50 IU/kg and distant ischemic preconditioning. A single injection of recombinant erythropoietin in a dose of 50 IU/kg 30 minutes before the pathology simulation resulted in reduced microcirculatory level 1 hour after reperfusion, retinal preservation electrophysiological activity, which is also confirmed by morphometric. Distant ischemic preconditioning conducted 40 minutes before the disease modeling, after 1 hour of reperfusion helped prevent damage to the retinal tissue is less pronounced compared to the group with pre-administration of recombinant erythropoietin. Pre peribulber emoxipine administration at a dose of 2 mg / kg resulted in a less pronounced effect retinoprotecion compared with recombinant erythropoietin and distant ischemic preconditioning.

After 72 hours of reperfusion, the most pronounced retinoprotective action was observed in the group of animals with pharmacological preconditioning recombinant erythropoietin in a dose of 50 IU / kg distant ischemic preconditioning rendered less effective protective effect on the retinal tissue in comparison with the group of animals with daily peribulber emoxipine 2 mg / kg.

Pretreatment with glibenclamide 5 mg / kg, offsetting the positive effects provided by erythropoietin at a dose of 50 IU / kg and distant ischemic preconditioning, which confirms the implementation retinoprotection by preconditioning, carried out with the participation of ATP-dependent potassium channels.

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