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CORRECTION OF RETINAL ANGIOPATHY OF HYPERTENSIVE TYPE BY MINOXIDIL, SILDENAFIL IN EXPERIMENT

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Abstract. For the study of retinoprotective properties of pharmacological agents a model of retinal angiopathy of hypertensive type was created against the background of N-nitro-L-arginine methyl ester (L-NAME) administration in a dose 12.5 mg/kg rat body weight for 28 days. We studied the retinoprotective effects of minoxidil in a dose 0.5 mg/kg, sildenafil in a dose 0.5 mg/kg and distant ischemic preconditioning (DIP) on a model of retinal angiopathy of hypertensive type in Wistar rats. The experiment showed that minoxidil in a dose 0.5 mg/kg prevented the development of ischemic damage and retinal vascular changes of hypertensive type caused by the introduction of L-NAME for 28 days, to a greater extent than sildenafil and distant ischemic preconditioning. The observed retinoprotective effects are confirmed by ophthalmoscopy, laser Doppler flowmetry (LDF) and electroretinography (ERG). The lack of positive dynamics in experimental groups with the introduction of glibenclamide in a dose 5 mg/kg confirms the key role of ATP-dependent potassium channels in the mechanism of realization of preconditioning. Detection of preconditioning properties of pharmacological agents may be a new approach in the correction and prevention of retinal angiopathy, which is the initial part of hypertensive retinopathy.

Keywords: retinal angiopathy of hypertensive type, pharmacological preconditioning, minoxidil, sildenafil, hypertensive retinopathy.

Introduction.

Hypertension, existing for a long time, leads to serious complications in the so-called target organs (kidney, heart, brain, retina). Changes in the eye of high blood pressure among other manifestations of this pathology have a special place. The picture of the fundus and indicators of local hemodynamics significantly complement the representation of researchers about the features of the disease, can detect early signs of organic changes in the retinal vessels and judge by their state with a certain degree of confidence about the changes of regional vascular bed and on the vascular system of the body as a whole [1].

63% of hypertensive patients have evidence of hypertensive angiopathy [2]. To assess the changes in the fundus caused by hypertension, used classification of Krasnov ML, according to which there are three steps: stage I – hypertonic angiopathy; stage II – a hypertonic angiosclerosis; stage III – hypertensive angioretinopathy and neuroretinopathy. Hypertensive angiopathy is characterized by the first phase of hypertension, in which there are only functional vascular disorders and the pressure is unstable.

Speaking about the pathogenesis of hypertensive retinopathy, it should be noted three main factors: the restriction and increased vascular permeability, and arteriosclerosis. Expressed arteriolar narrowing due to spasm of the walls takes place in response to increased blood pressure. The presence of bleeding on the surface of the retina caused by ruptures of the capillaries in the retinal nerve fiber layer in the posterior pole of the eye area [3].

The above changes in the retina, of course, lead to disruption of blood supply and the development of ischemic conditions, which can be corrected by pharmacological preconditioning. The phenomenon of ischemic preconditioning eventually realized by activation of the ATP-dependent potassium channels. As an end-effector of preconditioning, the ATP-dependent potassium channels cause hyperpolarization of the cell membrane, as well as launching a system of nitrous oxide and a number of anti-apoptotic mechanisms [4, 5, 6, 7].

From our point of view, one of the most promising drugs with the effect of pharmacological

preconditioning and unstudied as retinoprotectors are minoxidil and sildenafil.

The cardioprotective effect of minoxidil during heart preconditioning proved in experimental researches in vivo and in vitro [8]. The use of minoxidil (0,5 mg/kg/day) during simulated ischemia of the lower leg muscle helps to increase the level of the microcirculation to the 28th day in 2,3 times in comparison with the control group, levels ischemic damage muscle tissue [9].

Recent studies have shown that inhibitors of phosphodiesterase - 5, in particular, sildenafil, have a stimulating effect on the nitric oxide pathway, exerting pronounced endothelioprotective effect, capable of activating protein kinase G and the ATP-dependent potassium channels [10, 11].

In connection with the above, it should be noted the relevance of study of retinoprotective effects of minoxidil, sildenafil on model of retinal angiopathy of hypertensive type.

Objective: to increase the effectiveness of pharmacological correction of retinal angiopathy of hypertensive type using pharmacological preconditioning by minoxidil, sildenafil.

Materials and methods.

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

Operations and other manipulations were performed on rats under general anesthesia by intraperitoneal (i/p) introducing an aqueous solution of chloral hydrate in a dose 300 mg/kg.

L-NAME was injected in a dose 12.5 mg/kg/day for 28 days i/p daily.

DIP was performed 10 min by the clamping the femoral artery to the proximal tourniquet third thigh for 40 min before the administration of L-NAME in odd days of the experiment.

Minoxidil was administered intragastrically (i/g) in a dose 0.5 mg/kg 60 min before the administration of L-NAME in odd days of the experiment (every 48 hours). This dose of minoxidil is chosen by us, based on the literature data on pharmacological preconditioning by minoxidil [8, 9]. Drug administration explained by possible reduce of expressed hypotensive action due to the short half-life (4 hours) and longer period (48 hours) of preconditioning action [12].

Sildenafil was administered i/p in a dose 0.5 mg/kg 30 min before the administration of L-NAME in odd days of the experiment. This dose is chosen by us, based on the literature review of study of a possible correction of ischemic-reperfusion injury of various organs and tissues by sildenafil [13, 14, 15].

Glibenclamide, a blocker of ATP-sensitive potassium channels, was administered i/g in a dose 5 mg/kg [16] 90 min before the administration of L-NAME in odd days of the experiment.

To measure blood pressure in rats (tail) a system of non-invasive measurement of blood pressure for small animals NIBP200 was used in the complex Biopac-systems MP-150.

To investigate the fundus of experimental animals a direct ophthalmoscopy was used on 29 day of the experiment (ophthalmoscope Bx a Neitz, Japan). To expand the pupil the eye drops Irifrin 2.5% were used. Ophthalmoscope has been approached to the rat eye and we sent in it a beam of light from a distance of 0.5-2 cm to obtain a clear picture of the fundus image. In the dim image of the fundus we picked up the lens by turning the disc of ophthalmoscope, which gives crisp image details of the fundus. To zoom a lens Osher MaxField 78D model OI-78M has been used.

For subsequent statistical processing the degree of change, detected during ophthalmoscopy, were ranking (Tab. 1).

Table 1.

Methods of integrated semiquantitative evaluation of the fundus changes, detected during ophthalmoscopy (in grades).

A set of attributes of fundus changes	Grades
Optic disc is circular or oval shape and stands out from the fundus in pale - pink. The boundaries of the optic nerve disc are clear. It lies in the plane of the retina. From the middle of the optic nerve exit the central vessels of the retina. Blood vessels of the retina don't have anastomoses. The veins and arteries are straightforward, caliber is uniform, not crimped. The general background is pink.	0
Angiopathy. Symptom Salus-Hun I. It is characterized by the presence of sclerosis of retinal vessels in fundus and "phenomenon of chiasm", which occurs due to indentation of artery at the site of chiasm with extended vein. Expansion of vein on both sides of the chiasm. Symptom Guist - expansion and corkscrew curl of venules located around the macular; observed in hypertensive disease.	1
Angiosclerosis. Symptom of copper wire - yellow glow of the retinal arteries; sign of hypertensive retinal angiopathy. Symptom Salus-Hun II - the formation of bulges in arteries and veins chiasm. Symptom Salus-Hun III - the disappearance of the vein at the site of crossing due to the formation of the arcuate bend, sinking deep into the retinal tissue. Symptom of silver wire. Increased vascular permeability.	2 3
Retinopathy. "Cotton" exudates. Hemorrhages. In the macular area may be deposits of hard exudates in a star shape.	4
Hypertensive neuroretinopathy. Severe discoloration of the optic nerve. Swelling of the optic disc and peripapillary retina. Multiple foci of hemorrhage and "cotton" exudate, indicates the growing ischemia.	5

Measuring of the microcirculation level in the rats retina was performed by LDF after examining the fundus. 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 animal anesthesia assessment of microcirculation level was carried out in ten points on the circumference of the eyeball, the recording duration of the microcirculation level readings at one point was 20 seconds. From the microcirculation level results at every point the average value has been calculated, which was taken as the indicator of the microcirculation level in the retina of the experimental animal. Value of microcirculation in the animal group was calculated as the average of the values obtained from each experimental animal in group [17].

ERG was performed immediately after the registration of the microcirculation level. For this the animals were kept in the dark for 30 minutes [18], further the animals were anesthetized (chloral hydrate, 300 mg/kg, i/p) and fixed on the table, isolated from the electromagnetic radiation. Corneal silver electrode was placed on the cornea that has been soaked by saline solution for better contact, the reference needle electrode EL452 has been placed subcutaneously in the region of the skull, ground needle electrode EL450 has been placed subcutaneously in the base of the tail. Strobe flash of white light that is connected to the stimulator STM200 by company Biopac System, Inc. (USA) has been placed behind the back of the animal, ERG registration was carried out in response to a single stimulation. Evoked biopotentials 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 in each rat in groups. To assess the degree of functional damage to the retina we evaluated the ratio of amplitudes of a- and b-wave of ERG - the coefficient b/a [19]. From ten values in each group were taken the average, which was added to the protocol.

For all data the descriptive statistics were used: data are checked for normal distribution. Distribution type was determined by using the criterion of Shapiro-Wilk. In case of normal distribution the average value (M) and standard error of the mean (m) were calculated. In cases of abnormal distribution the median (Me) and the quartile range (QR) were calculated.

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 by using Statistica 10.0 software.

The main part:

The first step in study of the retinoprotective properties of pharmacological agents is the development of model of retinal angiopathy of hypertensive type.

We propose a model of retinal angiopathy of hypertensive type, which pathogenesis is associated with the development of hypertension in rats on the background of daily i/p administration of L-NAME in a dose 12.5 mg/kg/day for 28 days (SBP 204.8 mmHg, DBP 164.2 mmHg in a group with pathology; SBP 139.2 mmHg, DBP 104.2 mmHg in the intact group, $p < 0.05$). The confirmation of the formation of vascular changes in hypertensive type in the retina were results of ophthalmoscopy, LDF and ERG.

The study of retinoprotective action of minoxidil, sildenafil compared to DIP on model of retinal angiopathy of hypertensive type in experiment includes the following groups:

- The first (n = 10) - the group of intact animals,
- the second (n = 10) - the group with the modeling of retinal angiopathy (control),
- the third (n = 10) - with the correction of pathology by minoxidil,
- the fourth (n = 10) - with the correction of pathology by sildenafil,
- the fifth (n = 10) - with the correction of pathology by DIP,
- the sixth (n = 10) - with the introduction of glibenclamide and modeling of retinal angiopathy,
- the seventh (n = 10) - with the introduction of glibenclamide and correction of pathology by minoxidil,
- the eighth (n = 10) - with the introduction of glibenclamide and correction of pathology by sildenafil,
- the ninth (n = 10) - with the introduction of glibenclamide and correction of pathology by DIP.

Results.

We have developed a model of retinal angiopathy of hypertensive type in Wistar rats on the background of daily i/p administration of L-NAME in a dose 12.5 mg/kg/day for 28 days.

In accordance with the protocol the anesthesia of animals was carried out (chloral hydrate solution i/p 300 mg/kg) on 29 day of the experiment. Then were performed: ophthalmoscopy, assess the level of microcirculation in the retina by LDF, retinal electrophysiological status by ERG.

Example of ophthalmoscopy on intact animal is shown in fig. 1.

Example of ophthalmoscopy on animal with retinal angiopathy of hypertensive type shown in fig. 2.

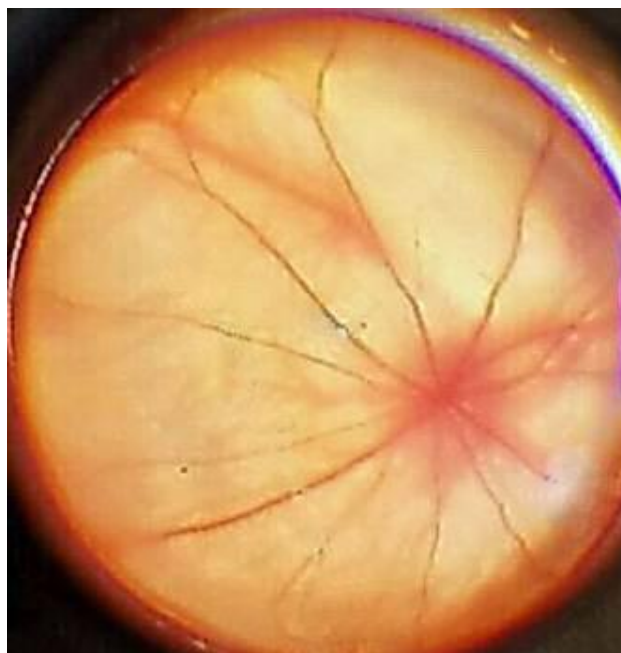


Figure 1. Example of ophthalmoscopy on intact Wistar rat. Optic disc is circular or oval shape and stands out from the fundus in pale - pink. The boundaries of the optic nerve disc are clear. It lies in the plane of the retina. From the middle of the optic nerve exit the central vessels of the retina. Blood vessels of the retina don't have anastomoses. The veins and arteries are straightforward, caliber is uniform, not crimped. The general background is pink.

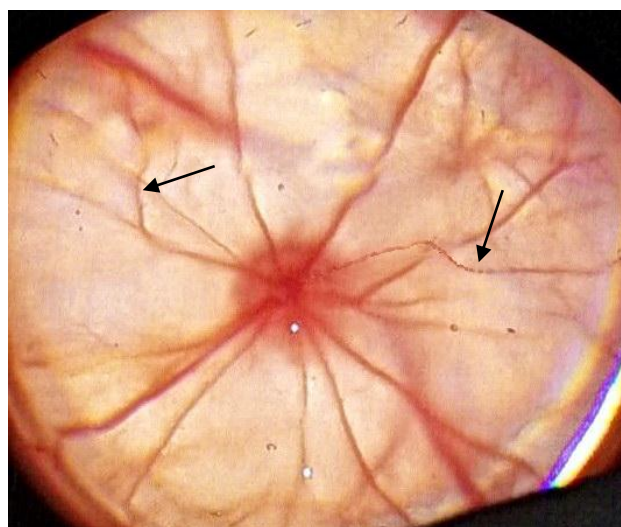


Figure 2. Example of ophthalmoscopy on Wistar rat with retinal angiopathy of hypertensive type. Optic disc is circular or oval shape and stands out from the fundus in pink. The boundaries of the optic nerve disc are clear. Veins are congested, full-blooded, crimped at the periphery. Arteries are narrowed, slightly crimped. Retina is palely (ischemic). No hemorrhage. Symptom Salus-Hun I-III (arrows show).

Thus, the results of fundus research during ophthalmoscopy in experimental animals have found that the modeling of retinal angiopathy of hypertensive type with administration of L-NAME for 28 days leads to a pronounced vascular changes in

the retina and signs of ischemic damage. Integral evaluation showed, respectively, 0 and 3 points for intact rats and for rats with retinal angiopathy of hypertensive type (tab. 2).

Table 2.

Integral assessment of fundus changes, detected during ophthalmoscopy (scores 0-5; n = 10).

Experimental groups	Integral assessment in scores
Intact	0
With the modeling of retinal angiopathy of hypertensive type	3*

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

The microcirculation level in the retina of intact animals was 743.6 ± 20.9 p.u. After pathology simulation in animal group the microcirculation level on 29 day of experiment was 431.4 ± 13.8 p.u., which is less than in the group of intact animals by 42%. LDF data obtained in the control group was significantly different from that of the group of intact animals ($p < 0.05$) and confirm the formation of ischemia during the modeling of retinal angiopathy of hypertensive type.

Violations in hemodynamics led to changes inherent to retinal angiopathy of hypertensive type, which is also confirmed by the results of electrophysiological studies of retinal conditions. To assess the severity of the functional changes in the retina we used the ratio b-wave amplitude to the amplitude of a wave of the ERG - the coefficient b/a [19].

In the experimental evaluations of electrophysiological condition of rat retina it has been found that the ratio b/a in the group of intact animals was 2.6 ± 0.07 r.u., in the group with pathology simulation this index was significantly different from the values in the group of intact animals and was 2.2 ± 0.09 r.u. ($p < 0.05$), lower than in the group of intact animals by 15%.

The data obtained allow us to conclude that the modeling of retinal angiopathy of hypertensive type causes a disturbance of electrophysiological state of inner retinal layers, which is characterized by decrease in the electrophysiological activity of the bipolar cells, Muller cells, amacrine and horizontal cells, due to violations of the retinal blood flow and the ischemia formation.

Data obtained during ophthalmoscopy, an integrated evaluation of the fundus changes, LDF

and ERG in intact and control groups confirm the adequacy of the proposed model of retinal angiopathy of hypertensive type on Wistar rats for further research of retinoprotective properties of pharmacological agents.

This model is characterized by:

- severe vascular changes of hypertensive type in the retina and attributes of ischemic injury (3 points, $p < 0.05$ compared with the group of intact animals) during ophthalmoscopy and integral evaluation of the fundus changes;
- statistically significant difference between values of the microcirculation level in the retina of rats in control group from values in the intact group on day 29 of the experiment after pathology modeling;
- significant reduction of coefficient b/a of electroretinogram after the pathology simulation on 29 day of the experiment in comparison with the value in the group of intact animals.

Further the study of retinoprotective action of minoxidil, sildenafil on the model of retinal angiopathy of hypertensive type compared to distant ischemic preconditioning and proof implementation of pharmacological effect through the participation of the ATP-dependent potassium channels were carried out.

On day 29 of the experiment anesthesia of animals was performed (chloral hydrate solution i/p 300 mg/kg). Further ophthalmoscopy were performed in groups of experimental animals. The experiment included 90 Wistar rats.

Example of ophthalmoscopy on Wistar rat with the correction of retinal angiopathy of hypertensive type by minoxidil is shown in fig. 3.

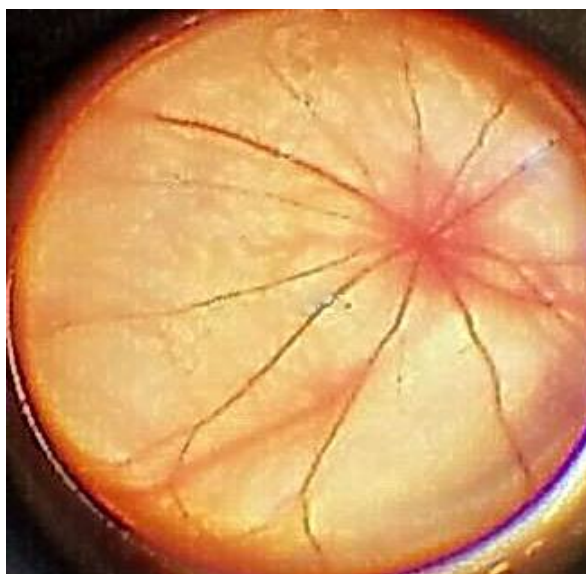


Figure 3. Example of ophthalmoscopy on Wistar rat with the correction of retinal angiopathy of hypertensive type by minoxidil in a dose of 0.5 mg/kg on day 29 of the experiment. Optic disc is circular or oval shape and stands out from the fundus in pale - pink. The boundaries of the optic nerve disc are clear. It lies in the plane of the retina. Blood vessels of the retina don't have anastomoses. The veins and arteries are straightforward, no crimping. Slightly dilated veins at the periphery. The general background is pink, not ischemic.

Example of ophthalmoscopy on Wistar rat with the correction of retinal angiopathy

of hypertensive type by sildenafil is shown in fig. 4.

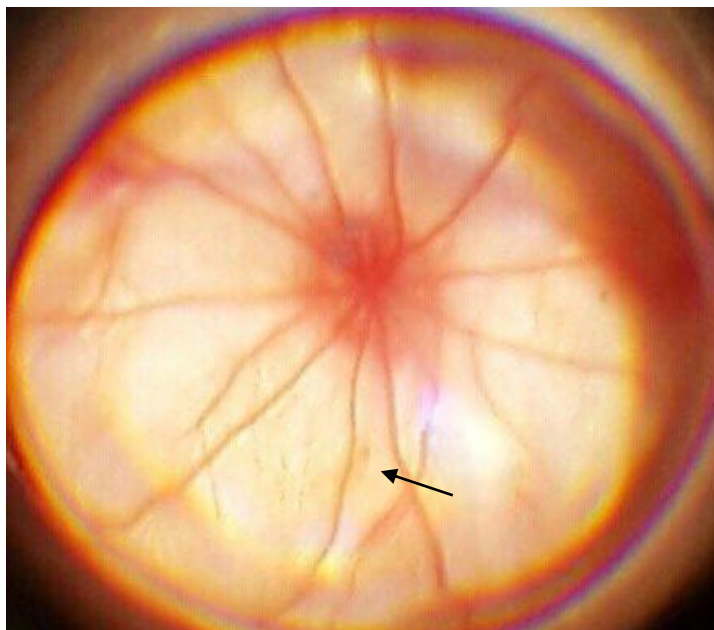


Figure 4. Example of ophthalmoscopy on Wistar rat with the correction of retinal angiopathy of hypertensive type by sildenafil in a dose 0.5 mg/kg on day 29 of the experiment. Optic disc is circular or oval in shape and stands out from the fundus in pale - pink. The boundaries of the optic nerve disc are clear. It lies in the plane of the retina. There is a slight vasoconstriction, "the phenomenon of chiasm", a symptom Salus-Hun I (arrow). Veins are crimped at the periphery. The background is slightly pale.

Example of ophthalmoscopy of retinal angiopathy of hypertensive type by DIP is shown in fig. 5.

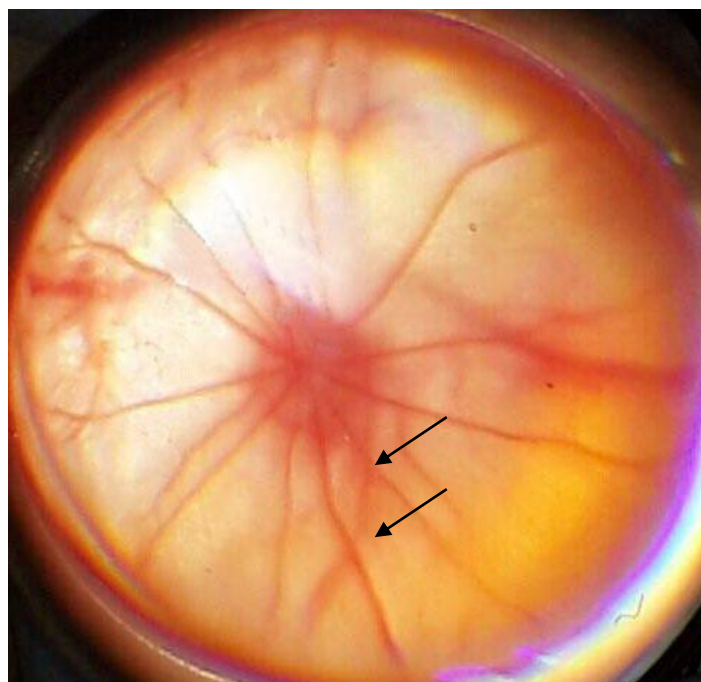


Figure 5. Example of ophthalmoscopy on Wistar rat with the correction of retinal angiopathy of hypertensive type by distant ischemic preconditioning on the 29 day of the experiment. Optic disc is circular or oval shape and stands out from the fundus of the eye in pink. The boundaries of the optic nerve disc are clear. It lies in the plane of the retina. There is a slight vasoconstriction, "the phenomenon of chiasm", a symptom Salus-Hun I, II (arrows show). Expansion of veins on both sides of the chiasm. Veins are crimped at the periphery. The background is slightly pale.

Integral assessment of fundus changes in experimental groups with the correction of pathology,

detected during ophthalmoscopy, is presented in the table. 3.

Table 3.

Influence of minoxidil, sildenafil and DIP on the complex fundus changes, found during ophthalmoscopy, on model of retinal angiopathy of hypertensive type (n = 10).

Experimental groups	Integral assessment in scores
Intact	0
With the modeling of retinal angiopathy of hypertensive type (control)	3*
Control + minoxidil, 0.5 mg/kg	0-1
Control + sildenafil, 0.5 mg/kg	1
Control + DIP	2*
Control + glibenclamide, 5 mg/kg	3*
Control + minoxidil, 0.5 mg/kg + glibenclamide, 5 mg/kg	2-3*
Control + sildenafil, 0.5 mg/kg + glibenclamide, 5 mg/kg	3*
Control + DIP + glibenclamide, 5 mg/kg	3*

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

Thus, the results of research of fundus during ophthalmoscopy and integral evaluation of the fundus changes in animal groups with the correction by minoxidil, sildenafil, DIP found pronounced retinoprotective effect of minoxidil in a dose 0.5 mg/kg, exceeding sildenafil in a dose 0.5 mg/kg and DIP consisting in reducing of ischemic injury to the retina and vascular changes of hypertensive type, which were observed in the control group. Integral evaluation showed, respectively, 0-1, 1 and 2 points for the groups of animals with the correction of minoxidil, sildenafil and DIP.

Introduction of glibenclamide in a dose 5 mg/kg 90 minutes before the administration of L-NAME in odd days of the experiment resulted in complete elimination of the positive effects of the correction of retinal angiopathy of hypertensive type by minoxidil, sildenafil, DIP, which confirms the implementation of protective effects due to the participation of ATP-sensitive potassium channels.

Assessment of the microcirculation level in the retina in experimental groups was performed by the LDF on 29 day of the experiment after ophthalmoscopy. The results are shown in table. 4.

The microcirculation level in the retina of intact rats was 743.6 ± 20.9 p.u. After the modeling of pathology in group of animals on day 29 of the experiment the level of microcirculation was $431.4 \pm$

13.8 p.u., which was significantly different from the values in the group of intact animals ($p < 0.05$). In group with correction of pathology by minoxidil the microcirculation level in the retina was 730.5 ± 15.9 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. Against the background of the correction of pathology by sildenafil the level of microcirculation in the retina was 605.1 ± 19.8 p.u., which was significantly different from the value in the control group ($p < 0.05$) and the value in the group of intact animals ($p < 0.05$). Against the background of the correction of retinal angiopathy of hypertensive type by DIP the level of microcirculation in the retina was 510.8 ± 16.5 p.u., which was significantly different from the value in the group with pathology ($p < 0.05$) and the value in the group of intact rats ($p < 0.05$).

With the administration of glibenclamide i/g in a dose 5 mg/kg the improving of blood flow in the retina were not observed in any of the experimental groups with the correction of pathology - microcirculatory level values were not significantly different from the value in the control group. This fact confirms the implementation of protective effects of minoxidil, sildenafil and DIP through the participation of ATP-dependent potassium channels.

Table 4.

Level of microcirculation in rat retina on day 29 of the experiment ($M \pm m$; n = 10), p.u.

Experimental groups	Level of microcirculation
Intact	$743,6 \pm 20,9^y$
With the modeling of retinal angiopathy of hypertensive type (control)	$431,4 \pm 13,8^*$
Control + minoxidil, 0.5 mg/kg	$730,5 \pm 15,9^y$
Control + sildenafil, 0.5 mg/kg	$605,1 \pm 19,8^{*y}$
Control + DIP	$510,8 \pm 16,5^{*y}$
Control + glibenclamide, 5 mg/kg	$439,4 \pm 14,5^*$
Control + minoxidil, 0.5 mg/kg + glibenclamide, 5 mg/kg	$436,2 \pm 12,9^*$
Control + sildenafil, 0.5 mg/kg + glibenclamide, 5 mg/kg	$441,4 \pm 15,8^*$
Control + DIP + glibenclamide, 5 mg/kg	$430,6 \pm 13,2^*$

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

ERG on evoked potential was performed after the measuring of the microcirculation level in the

retina. The data obtained are presented in table 5.

Table 5.

The results of evaluation of retinal electrophysiological state on day 29 of the experiment (M ± m; n = 10) r.u.

Experimental groups	b/a
Intact	2,6±0,07 ^y
With the modeling of retinal angiopathy of hypertensive type (control)	2,2±0,09*
Control + minoxidil, 0.5 mg/kg	2,5±0,09 ^y
Control + sildenafil, 0.5 mg/kg	2,3±0,08*
Control + DIP	2,3±0,10*
Control + glibenclamide, 5 mg/kg	2,2±0,08*
Control + minoxidil, 0.5 mg/kg + glibenclamide, 5 mg/kg	2,3±0,09*
Control + sildenafil, 0.5 mg/kg + glibenclamide, 5 mg/kg	2,2±0,05*
Control + DIP + glibenclamide, 5 mg/kg	2,2±0,09*

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 control group was 2.2 ± 0.09 r.u., which was significantly different from the value in the group of intact animals. Increase of this indicator in group with the correction by minoxidil up to 2.5 ± 0.09 r.u. says about the preservation of retinal electrophysiological function after disease modeling. In groups of animals with the correction by sildenafil and DIP ratio b/a was 2.3 ± 0.08 r.u. and 2.3 ± 0.10 r.u. accordingly, that significantly differs from the group of intact animals and confirms the conservation of functional retinal activity in both cases.

Introduction of glibenclamide in animal groups with the correction of pathology led to decrease of the index b/a on 29 day of the experiment to a value, significantly different from the value of the group of intact animals, indicating on the blockade of the ATP-dependent potassium channels and confirms the preconditioning properties, in particular, minoxidil in a dose 0.5 mg/kg on the model of retinal angiopathy of hypertensive type.

Reducing of the ratio b/a in animals with simulated pathology caused by inhibition of the positive b-wave, which indicates violation of electrophysiological function of bipolar and Muller cells, as well as the possible contribution of the horizontal and amacrine cells. Saving of the electrophysiological function of the photoreceptor layer is confirmed by the absence of adverse changes of a-wave (fig. 6).

The observed changes in the functional activity of the retina during the ERG in modeling of retinal angiopathy of hypertensive type confirm the adequacy of the proposed model of pathology.

Discussion.

The main factors in the development of retinal angiopathy of hypertensive type are disorders of common hemodynamics, local changes in the vessel walls. From local changes are the most important violations of the vascular endothelium [20].

In this regard, there is a need to find new methods of retinoprotection for possible reduction of the damaging effect of ischemia, formed in the retinal angiopathy of hypertensive type. Segment of drugs for the treatment of vascular diseases of the eye as a complication of systemic diseases is expedient to expand due to the increasing of incidence and lack of funds for targeted correction of ischemic lesions of the eye vessels [21].

Drugs used for correction of retinal ischemic damages are nonspecific therapy and do not have the desired result, that problem can be solved by pharmacological preconditioning, which is able to protect the retina from ischemic injury [22]. Versatility of preconditioning mechanism gives the background to the study of this phenomenon on the retina.

ATP-dependent potassium channel opening during ischemia plays a central role in the mechanism of cytoprotective effects of ischemic preconditioning. Initially, their activity was detected at sarcolemmal membrane, and later at the mitochondrial level.

Based on the fact that electrophysiological studies often have a decisive importance in the early and differential diagnosis of retinal disorders [23], to study the correction of functional changes in the retina, researcher must conduct a comprehensive analysis, including ophthalmoscopic, electroretinography, microcirculation research. Analysis of the dynamics of retinal electrogenesis allows to evaluate the nature and topography of retinal disorders, as well as to identify the most labile hypoxic retinal structure, their reaction to the correction by the medications.

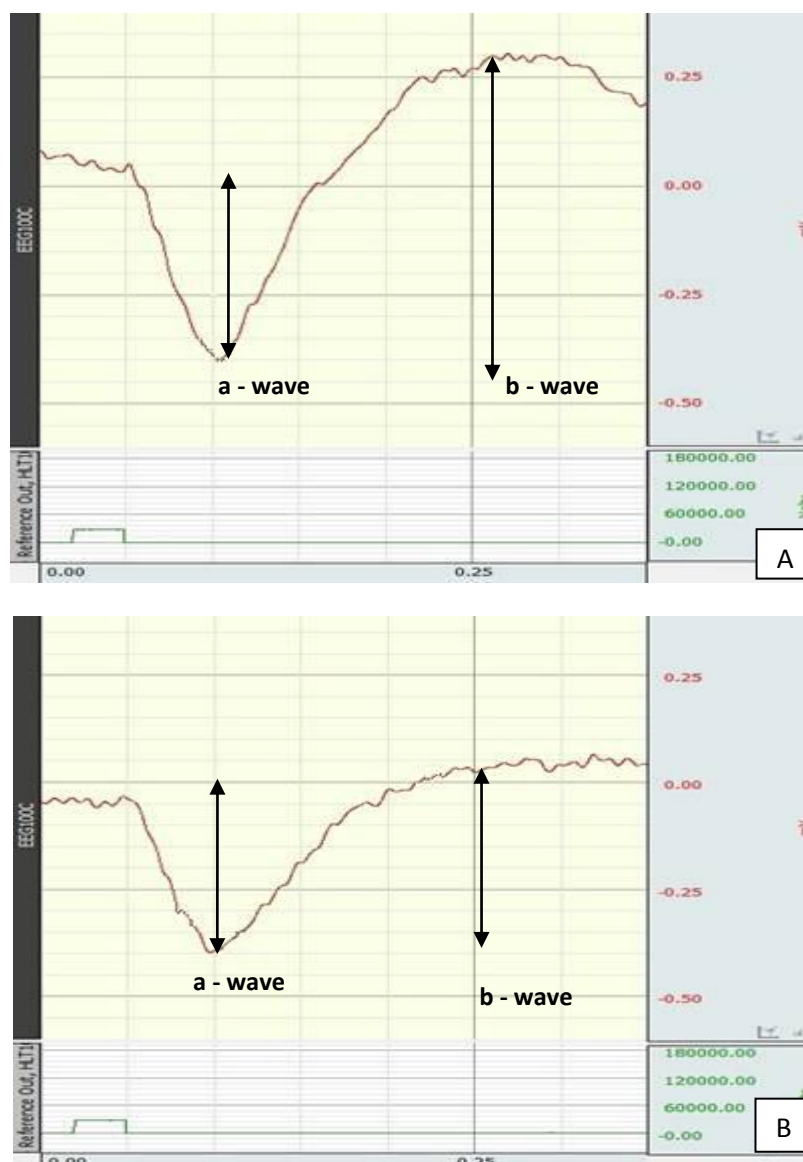


Figure 6. Electroretinogram of rats: A – ERG of intact animal; B – ERG of animal with retinal angiopathy of hypertensive type (inhibition of b-wave is observed) on day 29 of the experiment

The foregoing predetermined the need to develop and systematize the methodological approaches to the assessment of the functional state of the retina and the subsequent optimization of correction of retinal angiopathy of hypertensive type. The studies conducted in our experiments on Wistar rats had developed a set of methodological approaches to assess the functional status of the retina, including instrumental methods of analysis (ophthalmoscopy, LDF, ERG).

The first step in exploring of the possibility of correction of retinal angiopathy of hypertensive type using distant and pharmacological preconditioning by minoxidil, sildenafil has been the development of model of retinal angiopathy of hypertensive type on Wistar rats. We evaluated the fundus changes, the

microcirculation level in the retina and its electrophysiological condition.

Simulation of retinal angiopathy of hypertensive type performed by administration of nonselective NO-synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME) in a dose 12.5 mg/kg body weight of rat for 28 days.

28 days to Wistar rats corresponds to about 3.5 years for humans. During this time, the person on a background of hypertension has generated retinal vascular changes of hypertensive type. Hypertensive neuroretinopathy formed over decades, often developing in the late period of hypertensive disease and usually is a poor prognostic sign. It is characterized not only by changes in the blood vessels and retinal tissue, and involvement in the process of the optic nerve, which becomes swollen,

increased in size, swelling extends to the retina. Around the disk and on it hemorrhages are marked. Ophthalmoscopic picture is similar to the symptoms of stagnant disc, but unlike it marked a dramatic violation of color vision, decreased visual function: the decline of central vision and the narrowing of the field of view. At the end of neuroretinopathy the atrophy of the optic nerve may develop.

Summarizing the above, it should be noted that the proposed methodical complex of evaluation of functional changes associated with the development of retinal angiopathy of hypertensive type, makes possible to sufficiently evaluate objectively the retinoprotective effects of pharmacological agents.

The experiment showed that minoxidil in a dose 0.5 mg/kg prevented the development of ischemic damage and retinal vascular changes of hypertensive type caused by the introduction of L-NAME for 28 days, to a greater extent than sildenafil and distant ischemic preconditioning. It was revealed that in experimental group of animals treated with minoxidil in a dose 0.5 mg/kg was a significant difference of all measured parameters (the integral evaluation of the fundus changes, detected during ophthalmoscopy, the level of the microcirculation in the retina, the values of the coefficient b/a of electroretinogram) from value in the control group, which makes possible to talk about the ability of minoxidil in a dose 0.5 mg/kg to exert retinoprotective action on model of retinal angiopathy of hypertensive type in experiment.

The coefficient b/a of ERG in groups with correction by sildenafil and DIP statistically significantly different from the value in the group of intact animals, which makes impossible to speak about full retinoprotection, which is observed in the group of animals treated with minoxidil.

In proof of protection of retinal layers due to the effect of preconditioning of study drugs served the additional administration of glibenclamide in a dose 5 mg/kg, blocking ATP-dependent potassium channels, resulting in the elimination of the observed retinoprotective effects and confirms the preconditioning effects of minoxidil in a dose 0.5 mg/kg and sildenafil in a dose 0.5 mg/kg on the model of retinal angiopathy of hypertensive type.

Thus, the prospects become apparent to optimize pharmacotherapy of 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 by instrumental methods of analysis.

Conclusion.

As a result, we developed a model of retinal angiopathy of hypertensive type. An integrated assessment of the fundus, the level of microcirculation, retinal electrophysiological state have been carried out, that allows us to appreciate fully the functional condition of the retina in the modeling of pathology and its correction.

This model of pathology allowed to estimate the possibility of preconditioning of retina by minoxidil in a dose 0.5 mg/kg, by sildenafil in a dose 0.5 mg/kg compared to the distant ischemic preconditioning. Intra-gastric administration of minoxidil in a dose 0.5 mg/kg 60 minutes before the administration of L-NAME in odd days of experiment (every 48 hours) resulted in prevention of ischemic injury and retinal vascular changes of hypertensive type, significant increase in the level of microcirculation, preservation of electrophysiological retinal activity more than sildenafil in a dose 0.5 mg/kg and distant ischemic preconditioning.

Prior administration of glibenclamide in a dose 5 mg/kg eliminated the positive effects of the study drugs and DIP, which confirms the implementation of retinoprotection by preconditioning, carried out with the participation of ATP-dependent potassium channels.

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