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Research Article

Correction of retinal ischemic injuries by using non-selective imidazoline receptor agonists in the experiment

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Abstract

Introduction: Retinoprotective effects of non-selective imidazoline receptor agonists: potassium salt of C7070; sodium salt of C7070; C7070 processed with CO_2 – were investigated in comparison with C7070 on the retinal ischemia-reperfusion model in rats.

Materials and methods: The protective effects of the substances were evaluated by using ophthalmoscopy, laser Doppler flowmetry, electroretinography, histological and morphometric studies of retinal layers.

Results and discussion: The most pronounced retinoprotective effect was observed in potassium salt of C7070 at a dose of 10 mg/kg, which expresses in approaching the normal eye fundus image, achieving the target values of the retinal blood flow, b/a coefficient, and reaching the norm values of morphometric indicators. A less pronounced protective effect was found in sodium salt of C7070 at a dose of 10 mg/kg, which expresses in a 71% decrease ($p < 0.05$) in semi-quantitative assessment of the eye fundus changes, an increase in the retinal blood flow level by 70.4% ($p < 0.05$), in b/a by 94% ($p < 0.05$) in comparison with the group without correction, and reaching the norm of the morphometric indicators. A retinoprotective effect of the substance C7070 processed with CO_2 at a dose of 10 mg/kg is inferior to that of the sodium salt of C7070.

Conclusion: The retinoprotective activity of the substances is expressed in descending order: potassium salt of C7070 (10 mg/kg) ≈ C7070 (50 mg/kg) > sodium salt of C7070 (10 mg/kg) > C7070 processed with CO_2 (10 mg/kg) ≈ C7070 (10 mg/kg). Injections of glibenclamide leveled the neuroretinoprotective effects of the substances to varying degrees, which confirmed the participation of ATP-dependent potassium channels in the implementation of these effects.

Keywords

retinal ischemia-reperfusion, imidazoline receptor agonists, C7070, ATP-dependent potassium channels, Wistar rats.

Introduction

Retinal ischemia may have various etiologies: central retinal artery (CRA) occlusion and occlusion of its branches, atherosclerosis of retinal vessels, of carotid arteries, glaucoma with normal intraocular pressure (IOP), endocrine ophthalmopathy, surgical operations, etc. CRA occlusion is observed in 57% of cases, occlusion of its branches – in 38%. Acute occlusions of retinal arteries in 91.2% of cases occur against the background of cardiovascular system diseases. Nonarteritic anterior ischemic optic neuropathy (NAION), acute ischemia of the anterior segment of the optic nerve, occurs 6 times more often compared with neuropathy developed against the background of systemic vasculitis. The prevalence of the disease is 2.3–10.2 per 100,000 population over 50 years of age (Avetisov et al. 2018).

Retinal ischemia leads to inhibition of metabolic processes in its layers with retinal cell apoptosis (Hayreh 2013, Janáky et al. 2007, Ju et al. 2018). At the heart of ischemic neuropathy of the optic nerve is an acute disorder of arterial blood circulation in the system of vessels feeding the optic nerve. In the development of this pathology, a leading role is played by biochemical, haemodynamic and haemostatic disorders that develop in atherosclerosis, hypertension, diabetes mellitus, blood diseases, and arterial (brachiocephalic, etc.) occlusions (Hayreh 2013).

Currently, there is no unique and effective treatment for NAION, despite the fact that it is a major pathology that affects the optic nerve in the elderly and often leads to a significant loss of visual acuity (Nuzzi and Monteu 2017, Wilhelm et al. 2015).

Type II imidazoline receptors are a new biological target for the treatment of neurological disorders (Li 2017). Type II imidazoline receptors are widely distributed in the brain, and their ligands may have therapeutic potential as neuroprotectors (Abás et al. 2017, Head and Mayorov 2006). Activation of central type I imidazoline receptors leads to a decrease in blood pressure, due to the suppressive impact on the peripheral sympathetic nervous system, which is reasonable when correcting hypertensive changes in the retina and optic nerve, hypertensive neuroretinopathy, where retinal ischemia plays an important role in the pathogenesis (Peresypkina et al. 2018). Type III imidazoline receptors perform their function by regulating the concentration of K^+ and Ca^{2+} in cells and is associated with the activation of ATP-dependent potassium channels (Morgan and Chan 2001), which is expected to have a positive effect in the correction of ischemic conditions.

As previous studies showed, when evaluating the timing of the simulated retinal ischemia in rats based on the measurement of microcirculation, electroretinography (ERG) and morphometric studies, 30-minute ischemia with subsequent reperfusion turned out to be the most optimal simulated ischemia (Shabelnikova et al. 2016). This model was applied in the present study to examine the possibilities of correcting retinal ischemic injuries by using imidazoline receptor agonists.

The substance C7070 is lipophilic (Buzov et al. 2016). At a dose of 50 mg/kg, the neuroprotective activity of C7070 is more pronounced in retinal pathology models than at a dose of 10 mg/kg. The dose of 50 mg/kg of C7070 in rats equals 628 mg for a man weighing 70 kg, which exceeds the minimum therapeutic dose for an adult by 6.28 times (Dovgan 2017). In this regard, the study of hydrophilic derivatives of C7070 with presumed higher bioavailability, namely potassium salt of C7070 with laboratory code K^+C7070, sodium salt of C7070 with laboratory code Na^+C7070, C7070 processed with carbon dioxide, with laboratory code C7070CO_2 looks promising.

Due to the above, it is important to study the possibilities of pharmacological correction of retinal ischemic injuries by using non-selective imidazoline receptor agonists in the experiment.

Objective: to improve the efficacy of pharmacological correction of retinal ischemic injuries in the experiment by using non-selective imidazoline receptor agonists.

Materials and methods

The experiments were performed on white Wistar rats, males and females weighing 250 ± 25 g, with no external signs of the disease, which had passed quarantine. During the experiment, the rats were kept in a standard experimental biologically clean room, the air temperature was 22–24 °C, with a 12 h/12 h light/dark cycle; all the animals received granular feed and filtered water. Operations and other manipulations on the rats were carried out under general anesthesia by intraperitoneal (i/p) injection of chloral hydrate solution at a dose of 300 mg/kg. The withdrawal of the animals from the experiment was carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes adopted by the Council of Europe (Strasbourg, France, 1986) and Council Directive 86/609/EEC of 24.11.1986 on the protection of animals used for experimental and other scientific purposes, with an overdose of chloral hydrate administered i/p. The animals were divided into groups by stratified randomization with stratification by body weight, conditions of maintenance and nutrition, age, as well as by operations and manipulations.

Retinal Ischemia-reperfusion simulation

Retinal ischemia was simulated by increasing IOP to 110 mm Hg by mechanical pressure on the anterior eye chamber for 30 minutes. Prio to that, IOP had been registered in the animals by introducing into the anterior chamber a 30G needle of TSD104A sensor (MP150, BIOPAC SYSTEMS, Inc., Goleta, CA, USA). Rendering mechanical pressure on the anterior chamber was carried out basing on a step of the cylinder calibration system, with the registration of an increase in IOP, which made it possible to estimate the level of IOP increase at a certain position. In this paper, this model of pathology was used, followed

by a 72-hour reperfusion episode in order to assess the retinoprotective effects of non-selective imidazoline receptor agonists.

Semi-quantitative assessment of changes in the eye fundus

To study the fundus in the laboratory rats, ophthalmoscopy was used after 72 hours of reperfusion by using a Bx a Neitz Ophthalmoscope (Japan). For drug-induced mydriasis, eye drops with phenylephrine (Irifrin 2.5%, PROMED EXPORTS, India) were used by instillation into the conjunctival sac.

Doses were calculated using the dose conversion factor from an adult to rats weighing 250 g, according to the guidelines contained in the Guideline for Preclinical Studies of Drugs. For adults, a single instillation of a 2.5% solution is used during ophthalmoscopy. To create mydriasis, it is enough to inject 1 drop of 2.5% Irifrin into the conjunctival sac. One eye drop of 2.5% Irifrin contains 0.05 ml of the active substance. The conversion factor for an adult weighing 70 kg is 39. For rats weighing 250 g, the conversion factor is 7.0. Thus, the calculated dose (CD) of Irifrin is (Formula 1):

$$CD = \frac{0.05 \times 39}{70 \times 7.0} = 0.004 \text{ml} / \text{kg} \quad (1)$$

A solution of eye drops was injected into the conjunctival sac by using a micropipette in conversion to the body weight of rats. After the pupil was fully dilated, the ophthalmoscope was brought closer to the rat's eye, with a beam of light directed at it from a distance of 0.5–2 cm to obtain a clear image of the retina. In case an image of the fundus was unclear, a lens to render clear images of the details of the fundus was selected by turning the disk of the ophthalmoscope. An OI-78M lens (VOLK Optical Inc, Mentor, OH, USA) was used to magnify and obtain images of the eye fundus.

For further statistical processing, the degree of changes in the eye fundus during ophthalmoscopy against the background of simulating and correcting retinal ischemia-reperfusion was assessed in points (Table 1).

Table 1. Method of Integral Semi-quantitative Assessment of Changes in the Eye Fundus Caused by Simulating and Correcting Retinal Ischemia-Reperfusion by Pharmacological Agents, points.

Features of eye fundus changes observed during ophthalmoscopy	Score
Norm. The optic disc (OD) is round or oval, of pale pink colour against the fundus. The boundaries of OD are clear, located in the plane of the retina. The central retinal vessels emerge at the center of OD. Veins and arteries are rectilinear, their caliber is uniform, with no tortuosity. The overall background is pink.	0
Correction of neuronal injuries. The OD is round or oval, of pale pink colour against the background of the fundus. The OD has clear boundaries and is located in the plane of the retina. There is spasm of arteries (filamentous arteries), dilation and tortuosity of veins, and microaneurysms.	1
Correction of vascular injuries. Veins and arteries are rectilinear, of the uniform caliber, without tortuosity, microaneurysm or haemorrages. The OD is edematous, enlarged, and pale. The boundaries of OD are not clear.	2
Model of retinal ischemia-reperfusion. The OD is edematous, enlarged, and pale. The boundaries of OD are not clear. There is spasm of arteries (filamentous arteries), dilation and tortuosity of veins, microaneurysms., and hemorrhages. Retina is pale.	3

Evaluation of blood circulation in the retina using laser Doppler flowmetry

Retinal blood flow in rats was measured by laser Doppler flowmetry (LDF) 72 hours after simulating ischemia. The registration was carried out using MP-150 (BIOPAC Systems), a computer-based data acquisition system with software AcqKnowledge 4.2, and a TSD-144 needle probe (USA). After the rats were anesthetized, a microcirculation level was measured at 10 points on the circumference of the eyeball; the recording of the microcirculation level readings at one point lasted for 20 seconds. From the microcirculation level results at every point, the mean value was calculated, which was taken as an indicator of the microcirculation level in the retina of the experimental animal. The value of microcirculation in the animal group was calculated as the average of the values obtained from each experimental animal in the group.

Assessment of the electrophysiological state of the retina using ERG

A decrease in the amplitude of wave b is an indicator of ischemic retinal injury. ERG was performed immediately after recording the microcirculation level. To perform ERG, the rats were kept in the dark for 30 min (Zahng et al. 2013), then anesthetized (chloral hydrate, 300 mg/kg of rat body weight, i/p). A corneal silver electrode was placed on the cornea pretreated with saline solution for better contact; a reference needle electrode EL452 (BIOPAC SYSTEMS, Inc., Goleta, CA, USA) was placed subcutaneously in the region of the skull, a ground needle electrode EL450 (BIOPAC SYSTEMS, Inc., Goleta, CA, USA) was placed subcutaneously at the base of the tail. A TSD122B stroboscope with a flash of white light connected to a STM200 stimulator (BIOPAC SYSTEMS, Inc., Goleta, CA, USA) was placed behind the animal's back. The evoked biopotentials were transmitted at a frequency of 1–1000 Hz, amplified, averaged, and presented graphically on the screen using the MP150 data acquisition and analysis system (BIOPAC SYSTEMS, Inc., Goleta, CA, USA) with ACQKNOWLEDGE 4.2. software. The ERG was recorded for 0.5 sec in each rat in the groups. To assess a degree of retinal ischemia, the ratio of the amplitudes of ERG b- and a-waves, a b/a coefficient, was evaluated (Sachidanandam et al. 2015). The mean was derived for each group from ten values received and was introduced into the protocol.

Assessment of retinal changes using histological, morphometric studies

For a histological study, the eyes were removed with the adjacent tissues and fixed by immersion in a 10% formalin solution. After fixing, the eyes were sectioned into two parts longitudinally through the centre, and both halves were processed into paraffin by routine methods. Serial sections were also made longitudinally, with a thickness of 5–7 μm, stained with haematoxylin and eosin. The stages of histological processing were performed using LEICA equipment (Germany). For microscopy, morphometry and archiving, the prepared microslides were scanned using MIRAX DESK, a computerised archiving and image analysis system. Image analysis and morphometry were carried out by using a PANNORAMIC VIEWER 1.15.4. The thickness of the layers of the retina was measured in a strictly perpendicular direction to within onehundredth of a micrometre. In each animal, five representative areas, free from artifacts of treated material, were measured. The quantitative data were recorded in MS Excel spreadsheets.

Calculation of the specific number of neuronal nuclei in the inner nuclear layer was carried out by G. Avtandilov point test on standard computer images obtained via a MIRAX DESK system for scanning and archiving images and PANNORAMIC VIEWER 1.15.4 software. A computer version of the 0.3 mm^2 grid projected on the image was used.

In order to objectify the assessment of the detected qualitative changes in the retina, a degree of changes was recorded by conditional points: 0 points – no changes, 1 point – minimal changes, 2 points – moderately expressed changes, 3 points – pronounced changes in neuronal structures. In each group, 30 retinal regions were evaluated, and the results were expressed as a "coefficient of change", which was calculated by the formula 2:

$$CC = \frac{(1 \times n + 2 \times n + 3 \times n)}{N} \quad (2)$$

where: n is the number of regions with the corresponding (0; 1; 2; 3) degree of change; N is the total number of regions studied.

Study design. Mode of administration and dose of pharmacological agents

Potassium salt of C7070 (potassium salt of 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentancarbonic acid, $C_{16}H_{19}KN_2O_2$) with laboratory code K^+C7070 (JSC Experimental Plant VladMiVa, Belgorod, Russia) was injected intragastrically (i/g) at a dose of 10 mg/kg as a single dose, 60 min before the retinal ischemia simulation in a 1% starch solution.

Sodium salt of C7070 (sodium salt of 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentancarbonic acid, $C_{16}H_{19}NaN_2O_2$) with laboratory code Na^+C7070 (JSC Experimental Plant VladMiVa, Belgorod, Russia) was injected i/g at a dose of 10 mg/kg as a single dose, 60 min before the retinal ischemia simulation in a 1% starch solution.

C7070 processed with CO_2, with laboratory code C7070CO_2 (JSC Experimental Plant VladMiVa, Belgorod, Russia) was injected i/g at a dose of 10 mg/kg as a single dose, 60 min before the retinal ischemia simulation in a 1% starch solution.

Substance with laboratory code C7070 (3-(1H-benzimidazole-2-il)-1,2,2-trimethyl cyclopentancarbonic acid) (JSC Experimental Plant VladMiVa, Belgorod, Russia) was injected i/g at doses of 10 mg/kg, 50 mg/kg as a single dose, 60 min before the ischemia simulation in a 1% starch solution when correcting experimental retinal ischemia-reperfusion as a reference substance (Peresypkina 2018).

Glibenclamide, Maninil (Berlin-Chemie AG, Germany), a blocker of ATP-dependent potassium channels, was injected i/g at a dose of 5 mg/kg (Dowlatshahi et al. 2015, Mohamed et al. 2018, Peresypkina et al. 2017), once 60 minutes before ischemia simulation to confirm the implementation of a retinoprotective action of the pharmacological agents through the activation of ATP-dependent potassium channels.

Evaluation of the effectiveness of the studied substances on the retinal ischemia-reperfusion model was performed after 72 hours of reperfusion, with the use of ophthalmoscopy, integral semi-quantitative assessment of changes in the eye fundus, LDF, ERG and morphological studies.

The design of the experiment is presented in Table 2.

Table 2. The research design of the protective effects of the imidazoline receptor agonists K^+C7070, Na^+C7070, C7070CO_2 on the retinal Ischemia-reperfusion model.

Wistar rats (n=120). Weight 250 ± 25 g. Housing conditions: standard experimental biologically clean room. Illumination 12 h/12 h light/dark cycle, t (22–24) °C
1. Intact (n = 10)
2. Simulated retinal ischemia-reperfusion (n = 10)
3. Correction by K^+C7070, 10 mg/kg (n = 10)
4. Correction by Na^+C7070, 10 mg/kg (n = 10)
5. Correction by C7070CO_2, 10 mg/kg (n = 10)
6. Correction by C7070, 10 mg/kg (n = 10)
7. Correction by C7070, 50 mg/kg (n = 10)
8. Correction by K^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg (n = 10)
9. Correction by Na^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg (n = 10)
10. Correction by C7070CO_2, 10 mg/kg + glibenclamide, 5 mg/kg (n = 10)
11. Correction by C7070, 10 mg/kg + glibenclamide, 5 mg/kg (n = 10)
12. Correction by C7070, 50 mg/kg + glibenclamide, 5 mg/kg (n = 10)

Statistical data processing

For all the data, descriptive statistics were used, and the data were checked for normal distribution. A distribution type was determined by using the Shapiro-Wilk criterion. In case of normal distribution, the mean value (M) and standard error of the mean (m) were calculated. Outliers at each time point were identified using a Grubbs statis-

tical test. If for any value, the value of Z exceeded the critical number of measurements N for this value, this experiment was excluded from further calculations. 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 a distribution type. Differences were determined at a 0.05 significance level. Statistical analyses were performed using Statistica 10.0 software.

Results and discussion

After 72 hours of reperfusion after an ischemic episode, in all the experimental groups the rats were anesthetized for the purpose of further studying the eye fundus image.

An example of the eye fundus image of a Wistar rat in the intact group is shown in Figure 1A and can be described as follows: OD is round or oval, of pale pink colour against the background of the fundus. The boundaries of OD are clear. OD is located in the plane of the retina; the retina is tightly attached over the entire area to the choroid. The central retinal vessels emerge at the center of OD. The retinal vessels have no anastomoses. The veins and arteries are rectilinear; their caliber is uniform, with no tortuosity. The overall background of the retina is pink.

The examples of the eye fundus images of the laboratory rat with the simulated retinal ischemia-reperfusion are shown in Figure 1B, C and can be described as follows: OD is obviously decolorated, edemous and increased in size. The boundaries of OD are not clear. There arteries are stenosed; the veins are dilated; there are hemorrhages. The overall background of the retina is pale (ischemic).

The ophthalmoscopic image when correcting retinal ischemia-reperfusion by means of potassium salt of C7070 at a dose of 10 mg/kg is shown in Figure 2A, and its description is close to normal: OD is round or oval, of pale pink colour against the background of the fundus. The boundaries of OD are clear. OD is located in the plane of the retina. The veins and arteries are rectilinear; their caliber is uniform, with no tortuosity. The overall background of the retina is pink.

When sodium salt of C7070 was administered to the rats at a dose of 10 mg/kg to correct the simulated pathology, the adjustment of the retinal vessels calibers was observed, but the veins remained plethoric; no microaneurysms were observed. The OD was slightly increased, pink. The boundaries of OD were clear (Fig. 2B).

When correcting retinal ischemic injury by means of C7070CO2 at a dose of 10 mg/kg, the ophthalmoscopic image in the group is as follows: the OD is slightly increased, partly decolorized. The boundaries of the disk are clear. The veins are dilated, with sporadic microaneurysms. The retina is pale (Fig. 2C).

The fundus image when administering C7070 as a reference drug at a dose of 10 mg/kg to the rats with simulated retinal ischemia-reperfusion is shown in Figure 2D: OD is slightly increased, partly decolorized. The boundaries of the disk are clear. The arteries are filamentous, the veins are plethoric. The retina is ischemic.

When administering C7070 at a dose of 50 mg/kg to the rats with simulated retinal ischemia-reperfusion, the fundus image is close to the norm and shown in Figure 2E.

In the experimental groups with the injection of glibenclamide to correct retinal ischemic injury by K^+C7070 at a dose of 10 mg/kg and Na^+C7070 at a dose of 10 mg/kg, a spasm of the retinal arteries is observed. The state of the OD is close to normal, similar to that in the groups with the injection of these substances. When administering glibenclamide in the groups with correction by C7070CO_2 at a dose of 10 mg/kg, as well as by the reference substance C7070 at doses of 10 mg/kg and 50 mg/kg, the images of the eye fundus were not very different from a pathology model: obviously, edemous and decolorated OD; unclear boundaries of the OD, arterial spasm, plethoric veins, pale retina, microaneurysms and haemorrhages. Presumably, the blockade of ATP-dependent potassium channels by glibenclamide led to the elimination of the positive effects of these substances at the studied doses on the model of retinal ischemia-reperfusion.

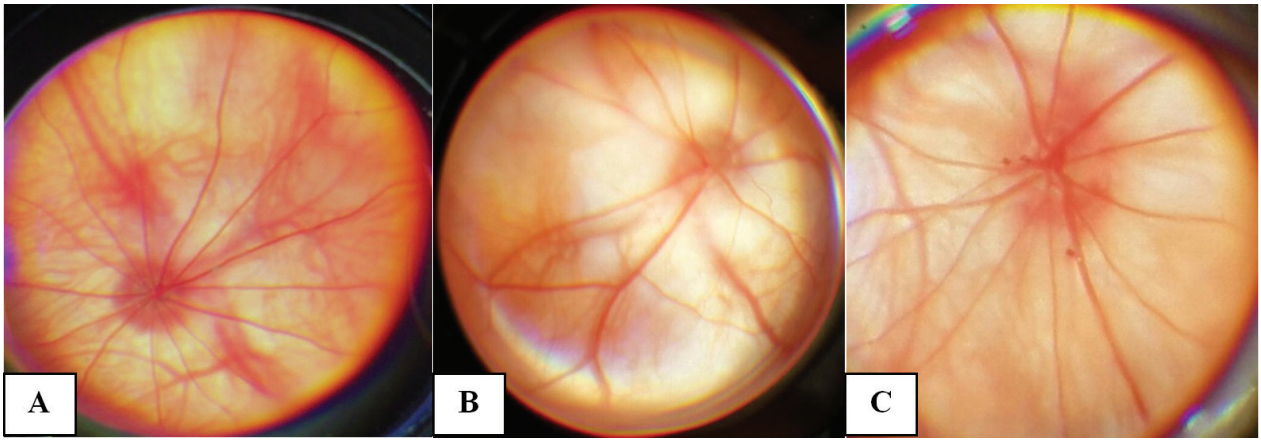

Figure 1. Eye fundus images of Wistar rats: **A** – from intact group; **B, C** – with the simulated retinal ischemia-reperfusion.

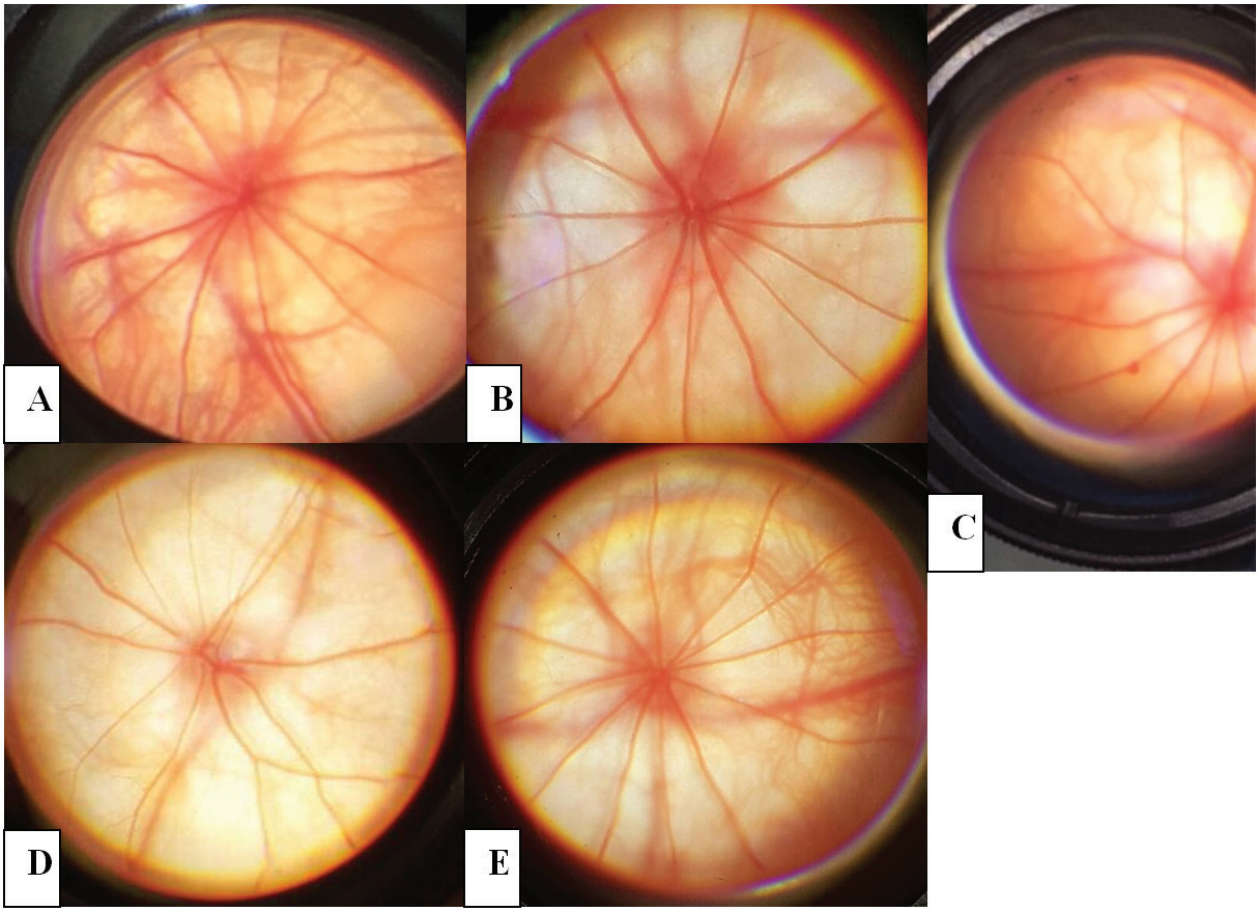

Figure 2. Eye fundus image of rats with correction of retinal ischemia-reperfusion by: **A** – potassium salt of C7070 at a dose of 10 mg/kg, **B** – sodium salt of C7070 at a dose of 10 mg/kg, **C** – C7070 processed with CO_2 at a dose of 10 mg/kg, **D** – C7070 at a dose of 10 mg/kg, **E** – C7070 at a dose of 50 mg/kg.

The results of the integral semi-quantitative assessment of the changes in the eye fundus in the experimental groups are presented in Table 3.

Based on the obtained data of the integral semi-quantitative assessment of the changes in the eye fundus on the model of retinal ischemia-reperfusion in Wistar rats, it follows that the studied substances have the retinoprotective effect (in descending order): potassium salt of C7070 (10 mg/kg) > C7070 (50 mg/kg) > sodium salt of C7070 (10 mg/kg) > C7070 (10 mg/kg) > $C7070CO_2$ (10 mg/kg).

The results of the LDF are presented in Table 4.

TBased on the obtained assessment data of the retinal microcirculation level in the experimental groups, it follows that the studied substances have a positive influence on the condition of retinal blood flow (in descending order): C7070 (50 mg/kg) > potassium salt of C7070 (10 mg/kg) > sodium salt of C7070 (10 mg/kg) > $C7070CO_2$ (10 mg/kg) > C7070 (10 mg/kg).

The influence of the studied pharmacological agents on the amplitudes of a- and b-waves of electroretinograms obtained in the experimental groups is presented in Table 5.

In each group, the b/a coefficient was calculated, the values of which are presented in Table 6.

When simulating retinal ischemia, after 72 hours of reperfusion, the b/a coefficient decreased by 53% ($p < 0.05$) in comparison with the group of the intact animals. On the background of the injection of K^+C7070 at a dose of 10 mg/kg, as well as C7070 at a dose of 50 mg/kg, b/a significantly increased by more than 2 times in comparison with the group without correction ($p < 0.05$). In the group with the injection of Na^+C7070 at a dose of 10 mg/kg, b/a increased by 94% in comparison with the value in the group without correction ($p < 0.05$). When injecting $C7070CO_2$ at a dose of 10 mg/kg to the animals, b/a in the group increased by 84% ($p < 0.05$), which is also significantly different from the mean in the group without correction. When correcting by means of C7070 at a dose of 10 mg/kg, b/a significantly increased by 68% ($p < 0.05$) in comparison with the group with pathology simulation.

Injection of glibenclamide in the groups with correction of pathology by K^+C7070, Na^+C7070, b/a increased by 68% and 52%, respectively, in comparison with the group with the simulated retinal ischemia-reperfusion, which indicated partial preservation of retinoprotective effects against the background of blockade of ATP-dependent potassium channels. In the groups with correcting retinal ischemic injury by means of $C7070CO_2$ at a dose of 10 mg/kg; C7070 at a dose of 10 mg/kg; C7070 at a dose of 50 mg/kg, the injection of glibenclamide prevented the increase of the b/a coefficient.

Table 3. Influence of Potassium Salt of C7070; Sodium Salt of C7070; C7070 Processed with CO_2 and C7070 on the Results of Semi-quantitative Assessment of Changes in the Eye Fundus When Correcting Retinal Ischemic Injury (M ± m; n = 10), points.

Experimental groups	Score
1. Intact	0.2 ± 0.2
2. Simulated retinal ischemia-reperfusion	2.8 ± 0.3*
3. Correction by K^+C7070, 10 mg/kg	0.2 ± 0.2^y
4. Correction by Na^+C7070, 10 mg/kg	0.8 ± 0.2*^{y}
5. Correction by C7070CO_2, 10 mg/kg	1.4 ± 0.3*^{y}
6. Correction by C7070, 10 mg/kg	1.2 ± 0.1*^{y}
7. Correction by C7070, 50 mg/kg	0.4 ± 0.2^y
8. Correction by K^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	1.1 ± 0.1*^{y}
9. Correction by Na^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	1.2 ± 0.2*^{y}
10. Correction by C7070CO_2, 10 mg/kg + glibenclamide, 5 mg/kg	2.7 ± 0.2*
11. Correction by C7070, 10 mg/kg + glibenclamide, 5 mg/kg	2.8 ± 0.2*
12. Correction by C7070, 50 mg/kg + glibenclamide, 5 mg/kg	2.5 ± 0.3*

Note: * – $p < 0.05$ in comparison to the intact group; ^{y} – $p < 0.05$ in comparison to the group with pathology simulation.

Table 4. Influence of Potassium Salt of C7070; Sodium Salt of C7070; C7070 processed with CO_2; C7070 on the Level of Retinal Microcirculation When Correcting Retinal Ischemic Injury (M ± m), perfusion units.

Experimental groups	Microcirculation level. PU
1. Intact	743.9 ± 5.0
2. Simulated retinal ischemia-reperfusion	353.3 ± 11.7*
3. Correction by K^+C7070, 10 mg/kg	732.7 ± 16.9^y
4. Correction by Na^+C7070, 10 mg/kg	602.1 ± 15.0*^{y}
5. Correction by C7070CO_2, 10 mg/kg	398.2 ± 11.6*^{y}
6. Correction by C7070, 10 mg/kg	394.1 ± 9.2*^{y}
7. Correction by C7070, 50 mg/kg	740.6 ± 12.1^y
8. Correction by K^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	450.4 ± 14.2*^{y}
9. Correction by Na^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	428.5 ± 13.3*^{y}
10. Correction by C7070CO_2, 10 mg/kg + glibenclamide, 5 mg/kg	365.1 ± 10.3*
11. Correction by C7070, 10 mg/kg + glibenclamide, 5 mg/kg	354.8 ± 12.1*
12. Correction by C7070, 50 mg/kg + glibenclamide, 5 mg/kg	365.2 ± 15.3*

Note: * – $p < 0.05$ in comparison to the intact group; ^{y} – $p < 0.05$ in comparison to the group with pathology simulation.

Table 5. Influence of Potassium Salt of C7070; Sodium Salt of C7070; C7070 processed with CO_2; C7070 on the a- and b-waves Amplitudes When Correcting Retinal Ischemia-reperfusion (M ± m; n = 10), mV.

Experimental groups	a-wave amplitudes	b-wave amplitudes
1. Intact	0.35 ± 0.03	0.88 ± 0.07^y
2. Simulated retinal ischemia-reperfusion	0.37 ± 0.03	0.44 ± 0.03*
3. Correction by K^+C7070, 10 mg/kg	0.35 ± 0.02	0.84 ± 0.03^y
4. Correction by Na^+C7070, 10 mg/kg	0.36 ± 0.02	0.83 ± 0.05^y
5. Correction by C7070CO_2, 10 mg/kg	0.37 ± 0.03	0.81 ± 0.06^y
6. Correction by C7070, 10 mg/kg	0.36 ± 0.02	0.72 ± 0.06^y
7. Correction by C7070, 50 mg/kg	0.36 ± 0.03	0.86 ± 0.06^y
8. Correction by K^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	0.35 ± 0.02	0.70 ± 0.06^y
9. Correction by Na^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	0.36 ± 0.04	0.65 ± 0.06*^{y}
10. Correction by C7070CO_2, 10 mg/kg + glibenclamide, 5 mg/kg	0.37 ± 0.02	0.48 ± 0.04*
11. Correction by C7070, 10 mg/kg + glibenclamide, 5 mg/kg	0.37 ± 0.04	0.48 ± 0.06*
12. Correction by C7070, 50 mg/kg + glibenclamide, 5 mg/kg	0.38 ± 0.04	0.49 ± 0.06*

Note: * – $p < 0.05$ in comparison to the intact group; ^{y} – $p < 0.05$ in comparison to the group with pathology simulation.

Table 6. Influence of Potassium Salt of C7070; Sodium Salt of C7070; C7070 Processed with CO_2; C7070 on the Value of the b/a Coefficient When Correcting Retinal Ischemia-reperfusion (M ± m; n = 10), R.U.

Experimental groups	b/a
1. Intact	2.51 ± 0.07
2. Simulated retinal ischemia-reperfusion	1.19 ± 0.05*
3. Correction by K^+C7070, 10 mg/kg	2.40 ± 0.11^y
4. Correction by Na^+C7070, 10 mg/kg	2.31 ± 0.06^y
5. Correction by C7070CO_2, 10 mg/kg	2.19 ± 0.09^y
6. Correction by C7070, 10 mg/kg	2.05 ± 0.20*^{y}
7. Correction by C7070, 50 mg/kg	2.39 ± 0.09^y
8. Correction by K^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	2.00 ± 0.12*^{y}
9. Correction by Na^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	1.81 ± 0.09*
10. Correction by C7070CO_2, 10 mg/kg + glibenclamide, 5 mg/kg	1.30 ± 0.09*
11. Correction by C7070, 10 mg/kg + glibenclamide, 5 mg/kg	1.33 ± 0.11*
12. Correction by C7070, 50 mg/kg + glibenclamide, 5 mg/kg	1.29 ± 0.08*

Note: * – $p < 0.05$ in comparison to the intact group; ^{y} – $p < 0.05$ in comparison to the group with pathology simulation.

Thus, based on the obtained values of the amplitudes of b-wave and the b/a coefficient in the experimental groups, it follows that the studied substances have positive effects on the electrophysiological state of the retina when correcting its ischemic injuries (in descending order): potassium salt of C7070 (10 mg/kg), C7070 (50 mg/kg) > sodium salt of C7070 (10 mg/kg) > C7070CO_2 (10 mg/kg) > C7070 (10 mg/kg).

The results of the counting the specific number of neuronal nuclei in the inner nuclear layer in the experimental groups are presented in Table 7.

From the data obtained, it follows that the neuroprotective activity in relation to neurons of the inner nuclear layer of the retina is most pronounced in K^+C7070 at a dose of 10 mg/kg; in C7070 at a dose of 50 mg/kg; and in Na^+C7070 at a dose of 10 mg/kg.

The results of calculating the "coefficient of change" of retinal layers in the experimental groups are presented in Table 8.

Thus, based on the complex of obtained histological qualitative and quantitative parameters in the experimental groups, it follows that the studied substances have the protective effects when correcting retinal ischemic injuries (in descending order): potassium salt of C7070 (10 mg/kg), C7070 (50 mg/kg) > sodium salt of C7070 (10 mg/kg) > C7070CO_2 (10 mg/kg), C7070 (10 mg/kg).

The results of a complex analysis, including the integral semi-quantitative assessment of changes in the eye fundus during ophthalmoscopy, measurement of the microcirculation level in the retina, electroretinography and quantitative assessment of the functional state of the retina, the results of the histological and morphometric studies after 72 hours of reperfusion after a long ischemic episode in the retina, make it possible to conclude that potassium salt of C7070 at a dose of 10 mg/kg has the most pronounced retinoprotective action comparable to that of the reference substance C7070 at a dose of 50 mg/kg, when correcting retinal ischemia-reperfusion in Wis-

Table 7. Influence of Potassium Salt of C7070; Sodium Salt of C7070; C7070 Processed with CO_2; C7070 on the Specific Number of Neuronal Nuclei in the Inner Nuclear Layer When Correcting Retinal Ischemia-reperfusion (M ± m; n = 10), absolute units.

Experimental groups	Specific number of neuronal nuclei
1. Intact	12.2 ± 0.8^{y}
2. Simulated retinal ischemia-reperfusion	6.3 ± 0.6*
3. Correction by K^+C7070, 10 mg/kg	11.9 ± 0.8^{y}
4. Correction by Na^+C7070, 10 mg/kg	11.0 ± 0.7^{y}
5. Correction by C7070CO_2, 10 mg/kg	8.6 ± 0.5*^{y}
6. Correction by C7070, 10 mg/kg	8.1 ± 0.4*^{y}
7. Correction by C7070, 50 mg/kg	11.4 ± 0.8^{y}
8. Correction by K^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	10.5 ± 0.6^{y}
9. Correction by Na^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	9.4 ± 0.5*^{y}
10. Correction by C7070CO_2, 10 mg/kg + glibenclamide, 5 mg/kg	7.0 ± 0.5*
11. Correction by C7070, 10 mg/kg + glibenclamide, 5 mg/kg	6.5 ± 0.6*
12. Correction by C7070, 50 mg/kg + glibenclamide, 5 mg/kg	7.3 ± 0.5*

Note: * – $p < 0.05$ in comparison to the intact group; ^{y} – $p < 0.05$ in comparison to the group with pathology simulation.

Table 8. Influence of Potassium Salt of C7070; Sodium Salt of C7070; C7070 Processed with CO_2; C7070 on the "Coefficient of Change" of Retinal Layers When Correcting Retinal Ischemia-reperfusion (M ± m; n = 10), points.

Experimental groups	Coefficient of change		
	Photoreceptor	Inner nuclear	Ganglionic and nerve fibres
1. Intact	0.1 ± 0.1^{y}	0.1 ± 0.1^{y}	0.2 ± 0.1^{y}
2. Simulated retinal ischemia-reperfusion	1.9 ± 0.3*	2.8 ± 0.2*	2.9 ± 0.2*
3. Correction by K^+C7070, 10 mg/kg	0.4 ± 0.1^{y}	0.2 ± 0.1^{y}	1.7 ± 0.1*^{y}
4. Correction by Na^+C7070, 10 mg/kg	0.8 ± 0.1^{y}	0.5 ± 0.1^{y}	1.9 ± 0.2*^{y}
5. Correction by C7070CO_2, 10 mg/kg	1.4 ± 0.2*	2.2 ± 0.2*	2.6 ± 0.2*
6. Correction by C7070, 10 mg/kg	1.2 ± 0.1*	2.0 ± 0.2*	2.5 ± 0.2*
7. Correction by C7070, 50 mg/kg	0.4 ± 0.1^{y}	0.3 ± 0.1^{y}	1.5 ± 0.1*^{y}
8. Correction by K^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	0.6 ± 0.2^{y}	0.5 ± 0.1^{y}	2.1 ± 0.2*
9. Correction by Na^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	0.6 ± 0.1^{y}	0.7 ± 0.1^{y}	2.3 ± 0.1*
10. Correction by C7070CO_2, 10 mg/kg + glibenclamide, 5 mg/kg	1.7 ± 0.2*	2.6 ± 0.2*	2.8 ± 0.1*
11. Correction by C7070, 10 mg/kg + glibenclamide, 5 mg/kg	1.9 ± 0.2*	2.8 ± 0.2*	2.9 ± 0.1*
12. Correction by C7070, 50 mg/kg + glibenclamide, 5 mg/kg	1.4 ± 0.2*	2.4 ± 0.3*	2.4 ± 0.1*

Note: * – $p < 0.05$ in comparison to the intact group; ^{y} – $p < 0.05$ in comparison to the group with pathology simulation.

tar rats, which is expressed in the adjusting the retinal vessels calibers, eliminating microaneurysm formation and haemorrages in the retina, reducing OD edema, reaching the target levels of the retinal blood flow, the b-wave amplitude and the b/a coefficient, preserving the microstructure of the retinal layers, neurons of the inner nuclear layer and photoreceptors. Sodium salt of C7070 at a dose of 10 mg/kg is inferior to potassium salt of C7070 at a dose of 10 mg/kg and C7070 at a dose of 50 mg/kg in terms of renoprotective effects in the simulated retinal ischemia-reperfusion. The renoprotective action of C7070 at a dose of 10 mg/kg is comparable with the action of C7070 processed with carbon dioxide at a dose of 10 mg/kg, and according to the results of a complex assessment of the degree of ischemic injury correction in the retina in the experiment, is inferior to that of sodium salt of C7070.

Injection of glibenclamide in all the groups with correction of the pathology by the studied substances resulted in partial elimination of their positive effects, presumably due to the blockade of ATP-dependent potassium channels, which confirms the involvement of ATP-dependent potassium channels in the mechanism of implementing the renoprotective action by the imidazoline receptor agonists, most probably of type III, in the simulated retinal ischemia-reperfusion.

Ischemic injuries in the retina and optic nerve are linked with haemocirculation disorders: systemic (hypertension, atherosclerosis, etc.), organ-histological, and microcirculatory. Ischemia leads to the accumulation of impaired metabolism products in the retina, which subsequently leads to dystrophy, up to atrophy, of the optic nerve, in the first place (Huang et al. 2018, Palmhof et al. 2018).

One of the drugs for specific correction of retinal injuries on the background of ischemia may be of imidazoline receptor ligands of types II and III, the proposed mechanism of neuroretinoprotective action of which may be associated with inhibition of NMDA receptors in the retina (Han et al. 2013), inhibition of Na^+/H^+ ion-exchangers in retinal neurons (type II) (Ernsberger 1999), and activation of ATP-dependent potassium channels (type III) (Morgan and Chan 2001). The fragments of a hypothetical mechanism for the implementation of retinoprotective effects of imidazoline receptor agonists: potassium salt of C7070; sodium salt of C7070; C7070 processed with CO_2; C7070 on the model of retinal ischemia-reperfusion is presented in Figure 3.

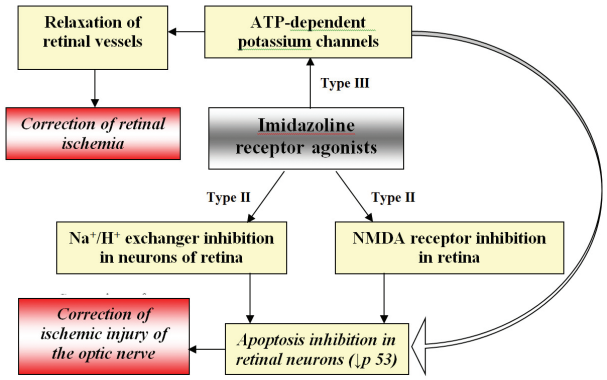

Figure 3. Hypothetical mechanism for the implementation of neuroretinoprotective effects of imidazoline receptor agonists: potassium salt of C7070; sodium salt of C7070; C7070 processed with CO_2; C7070 on the model of retinal ischemia-reperfusion.

Conclusion

The main findings of the study are:

1. Potassium salt of C7070 at a dose of 10 mg/kg has a pronounced protective effect comparable to that of C7070 at a dose of 50 mg/kg when correcting retinal ischemia-reperfusion, which is expressed in improving at most to reach the normal eye fundus image; reaching the target values of blood flow in the retina and the b/a coefficient; preserving the microstructure of retinal neurons and reaching the norm of the specific number of neuronal nuclei in the inner nuclear layer and the "coefficient of change" of the photoreceptor layer and the inner nuclear layer.
2. Sodium salt of C7070 at a dose of 10 mg/kg has a protective effect when correcting retinal ischemia-reperfusion which is inferior to that of potassium salt of C7070 at a dose of 10 mg/kg, which is expressed in a significant 71% decrease ($p < 0.05$) of the integral semi-quantitative assessment of the changes in the eye fundus during ophthalmoscopy in comparison with the mean value of the group without correction; a 70.4% increase in the retinal blood flow level ($p < 0.05$), a 94% increase in the b/a coefficient ($p < 0.05$) in comparison with group without correction; approaching the norm of the specific number of neuronal nuclei in the inner nuclear layer and the "coefficient of change" of the photoreceptor layer and the inner nuclear layer.
3. According to the results of a complex assessment of the degree of correction of retinal ischemic injuries in the experiment, the retinoprotective action of C7070 processed with carbon dioxide at a dose of 10 mg/kg is inferior to that of sodium and potassium salts of C7070, which is expressed in the presence of microaneurysms and vascular changes in the eye fundus; no improvement in the microcirculation level in the retina. The b/a coefficient in the group increased by 84% ($p < 0.05$) in comparison with the group without correction. The specific number of neuronal nuclei in the inner nuclear layer increased by 37% ($p < 0.05$) in comparison with the group without correction, and was also significantly different from the mean of the group of the intact animals. The "coefficient of change" of the photoreceptor, inner nuclear and ganglion layers did not differ significantly from the corresponding values in the group without correction.
4. According to the results of a complex assessment of the degree of correction of retinal ischemic injury in the experiment, from the comparative perspective, the neuroretinoprotective activity of the studied imidazoline receptor agonists is expressed in following descending order: potassium salt of C7070 (10 mg/kg) ≈ C7070 (50 mg/kg) > sodium salt of C7070 (10 mg/kg) > C7070 processed with CO_2 ≈ C7070 (10 mg/kg).
5. Injection of glibenclamide at a dose of 5 mg/kg in the groups with correction of retinal ischemia-reperfusion by the studied non-selective imidazoline receptor agonists at the studied doses leveled their neuroretinoprotective effects to different extents, which confirms the involvement of ATP-dependent potassium channels in the implementation of these effects. Presumably, type III imidazoline receptors are ATP-dependent potassium channels in the vessels and neurons of the retina.

Conflict of interest

The authors have no conflict of interest to declare.

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