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Analysis of the Protective Properties of Erythropoetin and Nicorandil on the Basis of the Model of the Retina Ischemia/Reperfusion.

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ABSTRACT

It was shown at the model of the ischemia/reperfusion of the rat retina that erythropoietin (50 IU/kg), Nicorandil (0,6 mg/kg) prevent the development of the degenerative changes of retinal layers. The protective effect of the specified pharmacological agents was confirmed by methods of electroretinography, laser Doppler flowmetry and histomorphometry. The conclusion is drawn that the use of the pharmacological products featuring the pre-conditioning effect may become the new approach to correction and prevention of the retinal ischemia.

Keywords: Retinal ischemia/reperfusion, pharmacological pre-conditioning, Nicorandil, recombinant Erythropoietin, electroretinography, microcirculation.

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INTRODUCTION

Pharmacological pre-conditioning may be considered as a universal method of prevention of the ischemic and reperfusion tissue damage. The protective effect of Erythropoietin has been proved by the model of ischemia/reperfusion of brain [1], muscle tissues of vessels [2, 3], heart [4], limbs [5], liver [6], pedicle flap [7].

Nicorandil shows an expressed cardio-protective effect [8, 9]. The objective of the present paper is the study of the protective effect of Erythropoietin and Nicorandil by the model of the eye ischemia/reperfusion.

PROCEDURE

The tests were performed on the 90 white laboratory bisexual rodents with the weight of 225-275 g. The simulation of the retinal ischemia/reperfusion was performed under anesthesia (chloral hydrate, 300 mg/kg of the animal weight, ip) by applying mechanical pressure (110 mm Hg) to the ocular anterior chamber for 30 minutes.

The distant ischemic preconditioning (DIP) was performed by means of 10-minute long compression of femoral artery through application of tourniquet to the proximal third of the hip 40 minutes prior to the simulation of the retinal ischemia followed by the 30-minutes long reperfusion episode (series). In order to study the preconditioning effect the recombinant Erythropoietin ("Epocrin" GosNII OCHB (State Research Institute of especially pure biological products) was administered intraperitoneally in the single dose of 50 IU/kg within 30 minutes prior to the ischemia simulation. Nicorandil in the dose of 0,6 mg/kg ("Coronel" (PIK-PHARMA (Prospects Innovations Quality - PHARMA) was administered intragastrically within 30 minutes prior to the ischemia simulation. The blocker of the K^+ATP channels Glibenclamidum ("Maniil" (Berlin-Chemie AG/Menarini Group)) was administered in the single dose of 5 mg/kg within 60 minutes prior to the ischemia simulation.

The intensity of the protective effect was estimated by the electro-physiological changes in the rats' retina after 72 hours since reperfusion. The microcirculation rate in the rats' retina was determined immediately after electroretinography performed with the use of the laser Doppler flow meter Biopac-systems MP-150 and needle transducer TSD-144 (USA). The descriptive analysis of the tissue specimen was performed with the microscope Axio Scope A1 (Carl Zeiss Microimaging GMbH, Germany). For morphometry of the retinal layers the ImageJ 1.47 program was used. For calculations the program for statistical analysis Microsoft Excel 2003 was used.

FINDINGS

Figure 1: А – Example of electroretinography (ERG) of an intact animal; Б - Example of electroretinography (ERG) of a control group animal.

The ERG of retina of an intact rat and that from the control group is presented in the Fig. 1. After 72 hours since the pathology simulation the b/a radio was reduced to 1,2±0,04 relative units (32% of the reference values) against its normal values in the white laboratory rats 2,5±0,1 ($p<0,05$). With correction of the pathology DIP the b/a ratio was definitely increased up to 2,0±0,08 ($p<0,05$) as compared with the control group (Table 1). By the ischemia correction with Erythropoietin the b/a ratio within the group made 2,3±0,06 ($p<0,05$), by that with Nicorandil - 2,2±0,06 ($p<0,05$) which definitely differs from the values of the control group. In the groups of animals that received Glibenclamidum no increase in the b/a ratio was observed.

The microcirculation rate in the retina of intact rats made 743,9±5,0 perfusion units (p. u.), that in the control group made 353,3±11,7 p. u. ($p<0,001$) which proves the formation of the retinal damage after 72 hours since reperfusion (Table 1).

With the correction of pathology by DIP the microcirculation rate after 72 hours since reperfusion definitely increases to 638,5±15,8 p. u. ($p<0,05$) as compared with the control group. By correction of the retinal pathology with Nicorandil the rate of microcirculation in the group increases to 705,2±15,5 p. u. and definitely differs from the control group values ($p<0,001$). By correction of the pathology simulated with Erythropoietin the rate of microcirculation in the group increases to 724,0±4,1 $^{p.}$ u. which definitely differs from the control group values ($p<0,001$) and tends to the value in the intact animal group. Administration of Glibenclamidum in the groups with correction of ischemic damages prevented increase in the microcirculation rate.

The findings of the morphological examination of retina have proved the development of ischemia after 72 hours of reperfusion which also confirms the adequacy of the selected pathology model, protective features of DIP, pharmacological preconditioning with Erythropoietin in the dose of 50 IU/kg and pharmacological preconditioning with Nicorandil in the dose of 0,6 mg/kg by the correction of the retinal ischemia/reperfusion after 72 hours of reperfusion which was expressed in the lesser damage of the retinal layers and retention of the minimal structural changes. The inner nuclear layer including was subjected to morphological examinations bipolar, Muller's, horizontal cells and photoreceptor layer.

The results of morphometry are indicated in the Table 1. The thickness of the inner nuclear layer of the intact rats layer made 23,8±1,0 µm. This indicator after simulation of ischemia/reperfusion in the control group after 72 hours of reperfusion made 20,3±0,8 µm which definitely differs from the values in the intact animal group ($p<0,05$) and confirms the development of degenerative changes within this period. Against correction of pathology with DIP the thickness of the inner nuclear layer is increased to 21,7±0,4 µm ($p<0,05$) as compared with the control group. By correction with Erythropoietin this indicator increases to 23,3±0,7 µm ($p<0,05$) as compared with the control group and is comparable to the values in the intact rats group. Against correction with Nicorandil the protective effect of the retinal layers was also observed. Administration of Glibenclamidum in the groups with correction prevented the increase in the thickness of the inner nuclear layer due to elimination of the preconditioning effect (Table 1). The results of morphometry of the photoreceptor layer in all experimental groups have not revealed statistically relevant differences.

Table 1: Retinal-protective effects of Erythropoietin and Nicorandil by the example of the rat retinal ischemia/reperfusion (M±m; n=10)

Sequence No.	Experimental groups	b/a ratio, relative units	Microcirculation rate, p. u.	Thickness of the inner nuclear layer, µm
1.	Intact	2,5±0,1^{y}	743,9±5,0^{y}	23,8±1,0^{y}
2.	Control (IRS)	1,2±0,04*	353,3±11,7*	20,3±0,8*
3.	DIP	2,0±0,08*^{y}	638,5±15,8^{*y}	21,7±0,4*^{y}
4.	EPO	2,3±0,06^{y}	724,0±4,1^{y}	23,3±0,7^{y}
5.	Nic	2,2±0,06*^{y}	705,2±15,5^{y}	22,9±0,5^{y}
6.	IRS + Gl	1,2±0,05*	359,4±10,3*	20,5±0,4*
7.	IRS+DIP+ Gl	1,2±0,04*	361,5±14,3*	20,6±0,6*
8.	IRS+EPO + Gl	1,2±0,06*	372,7±9,6*	20,3±0,5*
9.	IRS+Nic + Gl	1,2±0,05*	365,5±11,3*	20,5±0,4*

Remarks: * - $p<0,05$ as compared with the intact animal group; ^{y} - $p<0,05$ as compared with the control group; IRS – retinal ischemia/reperfusion; EPO – Erythropoietin, 50 IU/kg; Nic - Nicorandil, 0,6 mg/kg; Gl -Glibenclamidum, 5 mg/kg.

SUMMARY

The model of the retinal ischemia/reperfusion selected by us has been proven by numerous studies [10, 11]. The use of the laser Doppler flowmetry, morphometry and electroretinography methods allows estimating the state of retina and confirming the protective properties of Erythropoietin and Nicorandil with the use of the selected pathology model. The protective features of the specified agents due to the preconditioning mechanism of action are confirmed by the levelling of the Erythropoietin and Nicorandil effects after administration of Glibenclamidum which proves that opening of the mitochondrial K+ATP channels acts as the driving mechanism in the pharmacologically induced preconditioning phenomenon [12].

CONCLUSIONS

The obtained results confirm the presence of protective effects of the recombinant Erythropoietin in the dose 50 IU/kg and Nicorandil in the dose of 0,6 mg/kg by simulation of the eye ischemia/reperfusion consisting in the definite increase in the b/a ratio of electroretinography, definite increase in the microcirculation rate and improvement of the histologic pattern of the retinal layers.

Administration of Glibenclamidum prevented correction of the ischemic retinal damages due to blockage of the ATP-sensitive potassium channels which confirms the preconditioning effect of Erythropoietin in the dose of 50 IU/kg and that of Nicorandil in the dose of 0,6 mg/kg by simulation of the retinal ischemia/reperfusion.

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