

Correction of Experimental Hypertensive Neuroretinopathy with Semax

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ABSTRACT

Currently, the search for ways of specific pharmacological correction of hypertensive retinal changes is of great interest. The objective of this research is to study the correction possibility of retinal injuries with semax in the simulation of hypertensive neuroretinopathy in Wistar rats. This model was performed by daily injection of N-nitro-L-arginine methyl ester (L-NAME) at a dose of 12.5 mg/kg within 28 days and a single increase in intraocular pressure (IOP) to 110 mmHg for 5 min. The retinoprotective effect of semax at a dose of 72 µg/kg, in comparison with picamilon at a dose of 30 mg/kg, was estimated by laser Doppler flowmetry (LDF) and electroretinography (ERG). The use of semax led to increase in the retinal microcirculation level to 671.7 ± 22.1 perfusion units ($p < 0.05$, in comparison with the group with picamilon). In the group with semax, the b/a coefficient increased reliably by 14.6% ($p < 0.05$) in comparison with the group with picamilon. Thus, semax can be a promising agent in hypertensive neuroretinopathy treatment.

Keywords: Semax; Hypertensive Neuroretinopathy; Rats; Laser Doppler Flowmetry; Electroretinography.

INTRODUCTION

Hypertensive retinopathy results from pathological changes in the central retinal artery and its branches, as well as from hemodynamic changes in other vessels of the ophthalmic artery system.^{1, 2} The pathogenetic link of hypertensive retinopathy is retinal ischemia, which can lead to atrophy of the optic nerve.³⁻⁵ Acute occlusions of the retinal arteries in 91.2% of cases occur alongside cardiovascular diseases (60% – atherosclerosis and arterial hypertension).⁶

More and more attention is being paid to the development of short-chain peptide drugs for the cytoprotection.⁷⁻¹¹ In particular, neuroprotection.^{12, 13} The heptapeptide semax (Met-Glu-His-Phe-Pro-Gly-Pro) is a synthetic analogue of ACTH₍₄₋₁₀₎, which exerts marked nootropic and neuroprotective activities,¹⁴ protects effectively brain against ischemic stroke.¹³ Studies have shown that semax promotes the survival of neurons during glutamate excitotoxicity,¹⁵ protects against atrophy of the optic nerve and optic neuritis of inflammatory or toxic-allergic etiology.¹⁶

Studying the new perspective ways of neuroprotection in retinal injuries, in particular, developing in hypertension, is an actual problem of experimental and clinical pharmacology.¹⁷ Therefore, an important task is to find specific and highly effective means for correcting of hypertensive neuroretinopathy. To study the new pharmacological properties of drugs, it is necessary to conduct further studies *in vivo*.¹⁸⁻²¹ on the adequate experimental pathology models.²² In connection with the above, it is promising to study the possibility of

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pharmacological correction of hypertensive neuroretinopathy with semax in laboratory rats.

The objective of this research is to study the correction possibility of retinal injuries with semax in the simulation of hypertensive neuroretinopathy in Wistar rats.

MATERIALS AND METHODS

Animals. Experiments were conducted on 40 Wistar rats weighing 225-275 g, which were obtained from the Stolbovaya laboratory animal nursery in the Moscow region, Russia. For the study, the rats were taken with no external signs of disease, having passed the quarantine regime within 10 days. Ethical principles of conducting experiments on laboratory rats were observed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, CETS No. 123. All manipulations on animals were performed under general anaesthesia with intraperitoneal (i.p.) administration of chloral hydrate solution. The experiments were approved by the Local Ethics Committee of Belgorod State National Research University, Belgorod, Russia (Protocol#05/20).

Design of the Experiment. The following groups were included in the experiment:

- 1) a control (with i.p. saline in the equivalent volume for 28 days) (n = 10);
- 2) a group with simulated hypertensive neuroretinopathy (HNRP) (n = 10);
- 3) a group with semax at a dose of 72 µg/kg on the model of HNRP (n = 10);
- 4) a group with picamilon at a dose of 30 mg/kg on the model of HNRP (n = 10).

HNRP simulation was conducted by daily i.p. administration of a non-selective inhibitor of NO-synthases N-nitro-L-arginine methyl ester (L-NAME) (Sigma, Germany) at a dose of 12.5 mg/kg in solution form within 28 days and a single increase in intraocular pressure (IOP) to 110 mmHg by applying mechanical pressure to the anterior chamber of the eye for 5 min on the 26th day of the experiment.¹⁷ Increase in IOP was conducted under general anaesthesia (i.p. chloral hydrate at a dose of 300 mg/kg of rat body mass).

For the study, semax, nasal drops, 0.1% (PEPTOGEN Innovative Research and Production Center) were used. In diseases of the optic nerve, semax is instilled 2-3 drops in each nasal passage 2-3 times/day. The daily dose is 600-900 µg. The course of treatment is 7-10 days [https://www.vidal.ru/drugs/semax__28676]. 1 drop of the standard solution contains 50 µg of the active substance, 0.05 ml of the solution. The conversion factor for an adult with a body weight of 70 kg is 39. For a rat weighing 250 g, the conversion factor is 7.0. Thus, the estimated dose (ED) of semax was calculated:

$$ED = \frac{0,9 \cdot 39}{70 \cdot 7} = 0,072 \text{ ml/kg/day or } 72 \text{ µg/kg/day}$$

A solution of nasal drops was administrated into the nasal cavity using a micropipette once a day daily for 7 days, from the 22nd to the 28th days of the experiment.

The administration of picamilon (reference drug) at a dose of 30 mg/kg (Pharmstandard-UfaVITA JSC, Russia) was conducted 60 minutes before L-NAME administration, from the 22nd to the 28th days of the experiment, inclusive. Picamilon was daily administered intragastrically (i.g.).¹⁷

The effectiveness of the pharmacological correction of semax and picamilon in the experimental hypertensive neuroretinopathy was evaluated on the 29th day of the experiment by b/a coefficient and retinal microcirculation level.

Laser Doppler Flowmetry: 72 hours after increase in IOP, the retinal microcirculation level in rats was measured by LDF. The registration was performed using MP150 production Biopac System, Inc. (Goleta, USA), a computer-based data acquisition system with AcqKnowledge 4.2 software, and a TSD-144 needle-type sensor (Biopac System, Inc., Goleta, USA). After the rats were anaesthetized, the microcirculation level was measured at 10 points on the circumference of the eyeball.³

Electroretinography: The assessment of retinal functional activity was conducted with a- and b-wave amplitudes: a-wave is a negative wave reflecting the electrical activity of photoreceptors, and b-wave is a positive wave reflecting the activity of bipolar and Muller cells with the possible involvement of the horizontal and amacrine cells. ERG was performed in rats according to the method previously published by us [2, 4]. The ratio of the amplitudes of the b- and a-waves, the b/a coefficient, was calculated.

Statistical Data Processing: For all data, descriptive statistics were used, and the data were 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 a 0.05 significance level. Statistical analyses were performed using Statistica 10.0 software.

RESULTS

LDF results. Results of retinal microcirculation evaluation are presented in Figure 1. In the group with the HNRP simulation, the level of retinal microcirculation decreased by 44.3% (p < 0.05) in comparison with the control. When correcting HNRP by semax at a dose of 72 µg/kg, blood flow level increased by 62.7% (p < 0.05) in comparison with the group with no treatment and differed significantly (by 9.9%, p < 0.05) from the mean value of the group with picamilon at a dose of 30 mg/kg. When correcting HNRP by picamilon, the microcirculation level in retina increased by 48.0% (p < 0.05) in comparison with the group with no treatment.

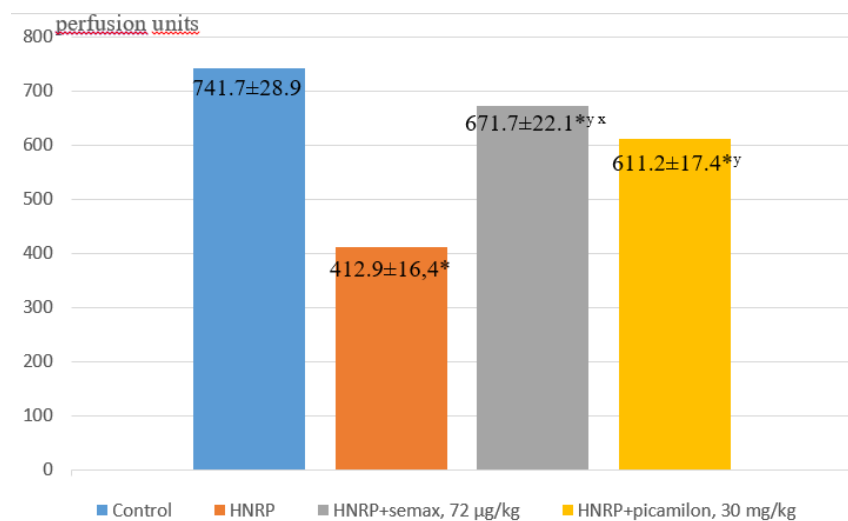
Results of the ERG, and b/a Counting. Influence of the studied drugs on the values of amplitudes of a- and b-waves in experimental groups is presented in Table 1. Further, the b/a coefficient was calculated, the values of which are presented in Table 2. In the group with HNRP simulation, the b/a coefficient decreased by 26.8% in comparison with the control group ($p < 0.05$). Against the background of semax, b/a increased by 31.4% in comparison with the group with no treatment ($p < 0.05$), and by 14.6% in comparison with the group with picamilon ($p < 0.05$). In the group with picamilon, the b/a coefficient did not reach the target values, but increased by 14.7% in comparison with group with no treatment ($p < 0.05$).

DISCUSSION

Based on literature data, tripeptide Pro-Gly-Pro (PGP) was predominant in a mixture of Semax derivatives in rat blood plasma and brain tissues just 1 h after intraperitoneal administration of Semax.²³ Independent effects of PGP were revealed recently, including an effect on cell culture survival in oxidative stress.²⁴ The effect of Semax and PGP previously was shown on the expression of genes that encode

neurotrophic factors and their receptors in an experimental model of brain ischemia in rats.²⁵ However, despite the advances described above, the molecular mechanisms underlying the Semax neuroprotective action and the degree of PGP participation in them remain obscure.¹³

In cerebral ischemia, semax showed neuroprotective, neurometabolic and antioxidant effects, and also promotes the synthesis of BDNF and nerve growth factor in the brain.¹² Semax has been employed successfully in clinical practice for treating patients with severe brain blood circulation disorders. In spite of numerous studies, many aspects of the therapeutic effects of this preparation remain unknown. The effects of Semax and its C-end tripeptide PGP on the functional morphology of nervous tissue cells were studied in the normal rat brain and in a model of incomplete global rat brain ischemia. In control animals, both peptides activated the capillary network and caused similar morphological changes to neurons and the neuropil regions. It was shown that Semax and PGP increased proliferation of the neuroglia, blood vessel endothelium, and progenitor cells in the subventricular zone. In these experimental conditions, only Semax abated the manifestation of ischemic damage to the nervous tissue.



HNRP – hypertensive neuroretinopathy; * $p < 0.05$ compared to the control; ^y $p < 0.05$ compared to the group with simulated hypertensive neuroretinopathy; ^x $p < 0.05$ compared to the group with picamilon

Fig. 1: Retinal microcirculation level in experimental groups.

Table 1: Influence of semax and picamilon on the a- and b- wave amplitudes when correcting experimental hypertensive neuroretinopathy ($M \pm m$; $n = 10$), mV

Experimental Groups	The a-Wave Amplitude ($n = 10$)	The b-Wave Amplitude ($n = 10$)
Control	0.36 ± 0.03	0.94 ± 0.07
HNRP	0.34 ± 0.02	0.65 ± 0.05 *
HNRP+semax, 72 µg/kg	0.35 ± 0.03	0.88 ± 0.07 y
HNRP+picamilon, 30 mg/kg	0.36 ± 0.04	0.79 ± 0.06 *y

HNRP – hypertensive neuroretinopathy; * $p < 0.05$ compared to the control; ^y $p < 0.05$ compared to the group with simulated hypertensive neuroretinopathy; ^x $p < 0.05$ compared to the group with picamilon.

Table 2: Influence of semax and picamilon on the value of the b/a coefficient when correcting experimental hypertensive neuroretinopathy (M ± m; n = 10), R.U.

Experimental Groups	b/a (n = 10)
Control	2.61 ± 0.08
HNRP	1.91 ± 0.06 *
HNRP+semex, 72 µg/kg	2.51 ± 0.12 yx
HNRP+picamilon, 30 mg/kg	2.19 ± 0.11*x

R.U. – relative units; * p < 0.05 compared to the control; y p < 0.05 compared to the group with simulated hypertensive neuroretinopathy; x p < 0.05 compared to the group with picamilon.

This was probably attributable to a decrease in vascular stasis symptoms as well as the trophic effect of the peptide [25].

In connection with the above, the protective effect of semax on the model of hypertensive neuroretinopathy in rats may be associated with the presence of neuroprotective, neurometabolic and antioxidant effects in semax. Based on the obtained data of the retinal microcirculation level in the experimental groups on the model of hypertensive neuroretinopathy, it follows that a positive effect on the state of the retinal blood flow in descending order has semax at a dose of 72 µg/kg, then picamilon at a dose of 30 mg/kg. Based on the obtained values of the b/a coefficient, it follows that a positive effect on the electrophysiological state of the retina in the correction of hypertensive neuroretinopathy in descending order has semax at a dose of 72 µg/kg, then picamilon at a dose of 30 mg/kg.

CONCLUSION

Thus, when correcting HNRP by semax at a dose of 72 µg/kg, retinal blood flow level increased by 62.7% (p < 0.05) in comparison with the group with no treatment and differed significantly (by 9.9%, p < 0.05) from the mean value of the group with picamilon at a dose of 30 mg/kg.

In the group with semax, b/a increased by 31.4% in comparison with the group with no treatment (p < 0.05), and by 14.6% in comparison with the group with picamilon (p < 0.05). Therefore, semax can be a promising agent in hypertensive neuroretinopathy treatment.

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