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Functional Cumulation of the Influence of Vascular Peptide Bioregulator on Microcirculation in the Brain Cortex of Spontaneously Hypertensive Rats

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Abstract—We investigated the influence of the vascular peptide bioregulator on microcirculation in the brain cortex of spontaneously hypertensive rats of different ages and determined whether there was functional cumulation during two applications of the drug Slavinorm by above-mentioned animals. It was shown that a single course treatment with vascular peptide bioregulator had increased the density of the microvascular network of the pia mater in young animals by ~1.2 times and did not affect the perfusion and oxygen saturation of sensorimotor cortex. The second course treatment with Slavinorm was given in six months. Functional cumulation was revealed in 12-month-old rats that had two course treatments with vascular peptide bioregulation: the density of the microvascular network of the pia mater was increased by ~1.6 times; the perfusion level was increased ~15% in comparison with intact animals of the same age. These animals were more tolerant to cerebral vasospasm (the application of vasoconstrictor on the brain surface): the highest level of tissue oxygen saturation was remained at fairly constant perfusion in comparison with other animals.

Keywords: vascular peptide bioregulator, brain, hypertension, density of the microvascular network, perfusion, oxygen saturation, young and old animals

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INTRODUCTION

The development of new methods of the treatment for vascular hypertension is one of the most relevant problems in modern physiology science and healthcare. Persistent growth of arterial blood pressure (ABP) significantly impairs blood circulation in the brain. In experiments and clinical studies, it is shown that the development of vascular hypertension causes a reduction of the microvascular network in brain tissue [8, 13], remodeling of the vasculature [11, 13], and decelerated blood flow [7, 12].

In earlier works, we proved that one course of treatment with the vascular peptide bioregulator Slavinorm led to significant growth of microvascular network density in the pia mater of the brain cortex in old rats (22–24 months old) [2] and improved microcirculation in the sensorimotor cortex [4]. The peptide bioregulator Slavinorm represents a complex of polypeptides with a molecular mass of 72–678 Da extracted from calf vessels.

The goals of the presented research were to study the influence of the vascular peptide bioregulator Slavinorm on microcirculation in the brain cortex in spontaneously hypertensive rats of different ages (6 and 12 months) and to reveal whether there is functional cumulation in them if this drug is administered in two courses.

MATERIALS AND METHODS

This work was performed with the use of animals from the biological collection of the Pavlov Institute of Physiology of the Russian Academy of Sciences. The experiments were run on spontaneously hypertensive male rats of the *SHR* strain. The rats were managed in the standard conditions for a vivarium, with natural lighting and free access to water and food. The research was performed in accordance with the guidelines of the European Convention for the Protection of Vertebrate Animals Used in Experiments (Strassbourg, 1986).

For the above experimental study, four groups of *SHR* rats (spontaneously hypertensive rats) were created (see Table 1).

As a control group, we used intact normotensive rats of the *Wistar–Kyoto* strain aged 12 months.

All of the Slavinorm treatment courses (0.25 mg) were administered intramuscularly according to the following schedule: one injection per day for 5 days,

Table 1. Experimental groups of spontaneously hypertensive rats of the *SHR* strain

Group	ABP, mm Hg	Oxygen saturation in the sensorimotor brain cortex tissue, %
First—intact 6-month-old male rats, $n = 6$	185.6 ± 5.2	97.8 ± 0.4
Second—6-month-old male rats that received one course of Slavinorm treatment 2 months before the study (at the age of 4 months), $n = 14$	182.6 ± 3.4	96.5 ± 0.3
Third—intact 12-month-old male rats, $n = 6$	190.7 ± 3.3	96.1 ± 0.3
Fourth—12-month-oldmale rats that received two courses of Slavinorm treatment: the first at the age of 4 months and the second at the age of 10 months (2 months before the study), $n = 14$	191.1 ± 3	97.8 ± 0.3

2-day timeout, and one injection per day for the next 5 days—a course of ten injections in total.

The body temperature of the rats was maintained at the level of 37°C for the entire experiment.

The microvascular network was visualized and monitored, and perfusion and oxygen saturation in the sensorimotor cortex tissue were measured two months after the Slavinorm course treatment. The rats were anaesthetized intraperitoneally by Zotelil at a dosage of 20 mg/kg (Virbac, France). The parietal bone and pachymeninx were removed to enable visualization of the pia mater of the sensorimotor cortex. The surface of the brain was continuously irrigated by sodium chloride solution at a temperature of 37°C.

In order to determine the density of the microvascular network, the animals were placed under the lens of a television unit (with a general 40× magnification). With Photo M software (by A. Chernigovskii), static images were used to count the total amount of vessels per unit area.

In order to measure the perfusion level and oxygen saturation (SO_2) in the sensorimotor cortex of the brain, a system of multifunctional laser diagnostics, LAKK-M (Research and Production Enterprise Lasma, Russia), was used. The given complex is used to measure the dynamic characteristics of blood microcirculation—perfusion, which is the change in the blood flow per unit time in the studied volume (around 1 mm^3) of tissue, in relative perfusion units, by laser Doppler flowmetry. SO_2 was measured by optic tissue oxymetry in the same volume of the brain cortex tissue. Initially, in standard conditions, the levels of perfusion and SO_2 were registered on the surface of each hemisphere at four measurement points with approximate coordinates $AP = 1, 2, 3, 4$ mm from the bregma; $SD = 1.0$ mm laterally from the biparietal suture. Also, the changes in perfusion and SO_2 were assessed in the conditions of the application of the noradrenaline vasoconstrictor on the brain surface (10^{-3} M).

During the statistical processing of all data, the reliability of differences was measured by the Mann–Whitney criterion; the level of significance of the differences $p < 0.05$.

RESULTS AND DISCUSSION

With sustainable growth of ABP, the vessel wall of arteries and arterioles in the brain are affected by modified hemodynamic factors (increased shear stress, enhanced laminar flow, etc.) and humoral factors typical for hypertension—particularly, an increased concentration of angiotensin II, which causes endothelial dysfunction and remodeling of the vasculature, in the blood [10, 11]. An ongoing course of the disease tends to cause complications such as the formation of vascular aneurisms, their rupture (hemorrhagic stroke), or thickening of the vessel wall and the formation of atherosclerotic plaques, leading to full blockage of brain vessels (ischemic stroke) [1, 15]. Slavinorm possesses angioprotective capabilities [14]. Its use in the treatment of vascular hypertension is aimed at regeneration of the brain vasculature and maintenance of the metabolism of endothelial cells of the vessel walls [6]. We propose that elimination of the dysfunction of the brain vasculature can allow recovery of the main microcirculation parameters to the level of normotensive animals.

In earlier works, we showed that remodeling of the microvasculature in the pia mater of the brain cortex in *SHR*-strain rats took place as early as at the age of 3–4 months, i.e., before the development of persistent hypertension.

These animals showed a significant (1.4- to 1.9-fold) decrease in the microvascular network density in the pia mater and perfusion level (by around 24%) [3] in the sensorimotor cortex of the brain as compared to normotensive rats of the *Wistar–Kyoto* strain of the same age. At the age of 4–6 months, *SHR* rats have already developed persistent hypertension [9]. Aging-related changes in animals of the *SHR* strain are less pronounced than in rats with normal ABP [5]. As intact *SHR* rats age from 6 to 12 months, their ABP does not notably change (see Table 1).

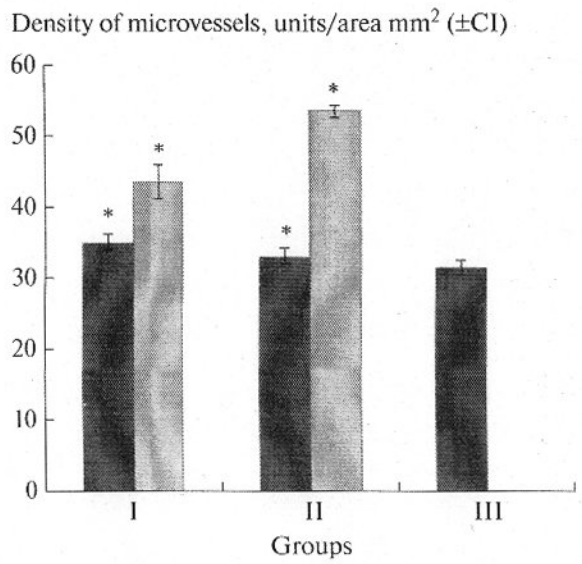

Fig. 1. Density of the microvascular network in the pia mater of the sensorimotor cortex in intact hypertensive rats of the *SHR* strain and after the use of the vascular peptide bioregulator. The dark columns represent the microvascular network density of the pia mater of the sensorimotor cortex in intact animals (* $p < 0.05$); the gray columns represent the same in animals after receiving the vascular peptide bioregulator ($p < 0.05$). Here and in Fig. 2 and 3, along the *x* axis, there are groups of animals: I—hypertensive rats of the *SHR* strain aged 6 months; II—hypertensive rats of the *SHR* strain aged 12 months; III—control-group rats of the *Wistar–Kyoto* strain aged 12 months.

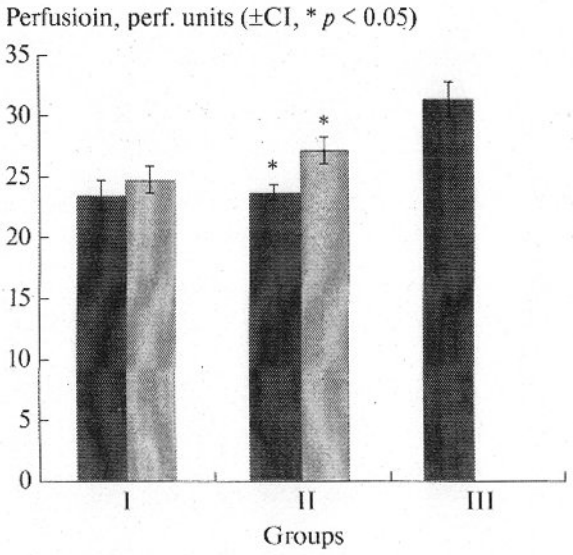

Fig. 2. Perfusion in the tissue of the sensorimotor cortex of the brain in rats. The dark columns represent the perfusion of the tissue of the sensorimotor cortex in intact animals; the gray columns represent the same in animals after receiving the vascular peptide bioregulator.

One-course treatment with Slavinorm caused the microvascular network density in the pia mater of the sensorimotor cortex in the brain of *SHR* rats to grow by 1.2 times (statistically significant) at the age of 6 months (Fig. 1). As such, the perfusion level in the sensorimotor cortex grew from 23.8 ± 1.3 to 25.1 ± 1.1 perf. units (statistically insignificant, Fig. 2), and the SO_2 level in the tissue remained unchanged (see Table 1). Therefore, one course of Slavinorm treatment for young *SHR* rats leads to the activation of angiogenesis in the pia mater of the sensorimotor cortex but does not affect the dynamic characteristics of microcirculation in the brain.

In order to regenerate microcirculation in the brain cortex to the level of normotensive animals, a repeated course of vascular peptide bioregulator was administered. Two months after the second course of Slavinorm, the *SHR* rats (fourth group) showed a 1.6-fold increase in the density of microvascular network in the pia mater of the sensorimotor cortex (statistically significant, Fig. 1). The perfusion level in the rats of the fourth group was around 15% higher than in intact *SHR* rats of the same age but lower than in intact normotensive rats at the age of 12 months (see Fig. 2). The SO_2 level in the brain tissue of animals in all groups was approximately equal (see Table 1).

Thus, when the course of the vascular peptide bioregulator was received twice by spontaneously hypertensive rats at the interval of 6 months, a functional cumulation was revealed; it manifested itself as increases in the microvascular network density and perfusion level in the sensorimotor cortex of the brain. All parameters of the *SHR* rats at the age of 12 months that received two treatment courses of Slavinorm matched normotensive rats of the same age.

People who suffer from hypertension, especially the elderly, respond to external adverse factors (such as hypoxia, angst, fatigue, weather changes, etc.) with spasms of brain vessels. In view of this, it is important to identify how Slavinorm influences microcirculation in the brain cortex in hypertensive rats under the effect of a vasoconstrictor. In extreme conditions, autoregulation is aimed at maintaining the oxygen supply to the brain tissues at a constantly high level. As is seen from Fig. 3a, when the noradrenaline (NA) vasoconstrictor is applied to the brain surface in young intact *SHR* rats, a high level of oxygen saturation of the tissues was maintained, and intact normotensive and hypertensive rats at the age of 12 months showed a 5% lower SO_2 level. When the vasoconstrictor is introduced to the brain surface, the animal age is probably of a primary importance: old rats endure a spasm of brain vessels harder that young ones.

Under the effect of NA at different measurement points, the perfusion level could decline or rise. In rats from the control group (*Wistar–Kyoto* rats), the value of decline (26.8 ± 2.7%) and growth (26.4 ± 4.2%) of the perfusion level under the effect of NA was approximately the same (Fig. 3b). Therefore, in order to

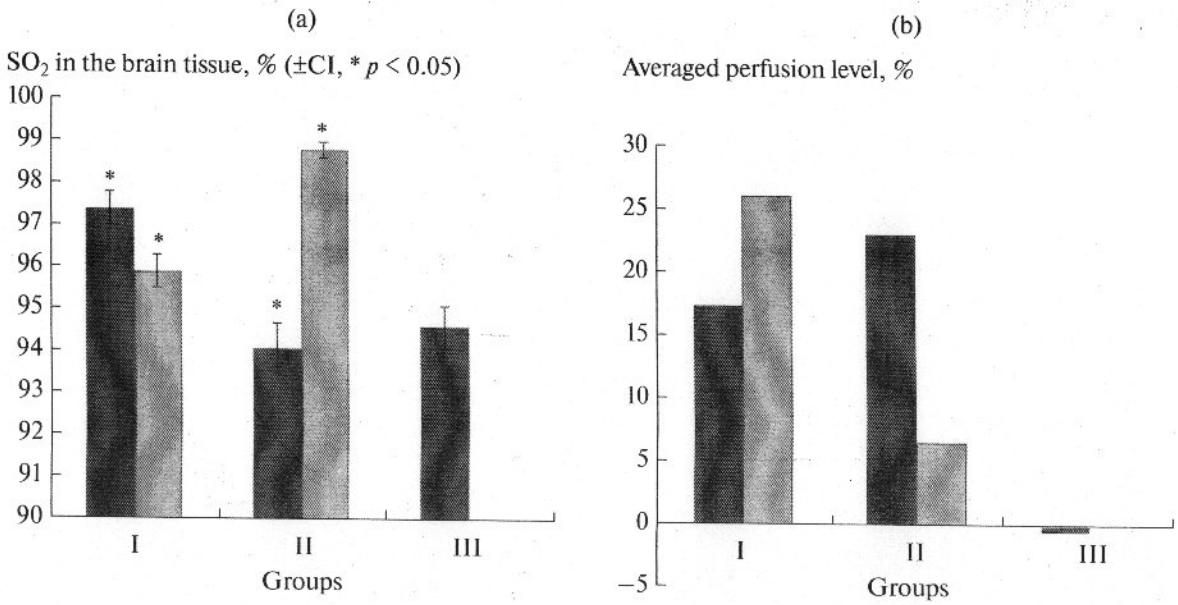

Fig. 3. Change in oxygen saturation and perfusion in the tissue of the sensorimotor cortex of rat brain under the effect of a vasoconstrictor (application of noradrenaline on the brain surface). (a) SO_2 in the tissue of the sensorimotor cortex of the brain in rats affected by the vasoconstrictor; the dark columns represent SO_2 in the tissue of the sensorimotor cortex in intact animals; the gray columns represent the same in animals after receiving the vascular peptide bioregulator; (b) averaged level of change in perfusion in the tissue of the sensorimotor cortex of the brain in rats affected by the vasoconstrictor; the dark columns represent the averaged perfusion level in the tissue of the sensorimotor cortex in intact animals; the gray columns represent the same in animals after receiving the vascular peptide bioregulator.

maintain the tissue SO_2 at the level of 94.6 ± 0.5% in the rats of this group, the average perfusion level does not rise. *SHR* rats show a somewhat different pattern. In intact hypertensive rats at the age of 6 and 12 months, the perfusion level grew by 46.7 ± 13 and 38.1 ± 9.2% on average at the majority of measurement points, correspondingly, whereas at other measurement points the perfusion level declined by 29.2 ± 4 and 15.1 ± 3%, correspondingly. Therefore, in order to preserve SO_2 at the level of 94–97% in the brain cortex tissue under the effect of NA (Fig. 3a), tissue perfusion in these animals increases by 17 and 23%, correspondingly (Fig. 3b). In *SHR* 6-month-old rats that received one course of Slavinorm treatment, the following similar changes were revealed under the effect of NA: the decrease was 31 ± 4.6%, and the increase was 57 ± 17.6%. Therefore, in order to maintain SO_2 in the brain cortex tissue at the level of 95.9 ± 0.4%, perfusion in these animals increases by 26% (Fig. 3b). The *SHR* 12-month-old rats that received two courses of treatment by the vascular peptide bioregulator, the perfusion level declined by 29.6 ± 3.8% at the majority of measurement points and increased by 36.3 ± 13.2% at other measurement points. As such, the given group of animals under the effect of the vasoconstrictor showed the highest oxygen saturation of the tissues, 98.8 ± 0.2%, and the averaged perfusion grew only by 6.7%.

CONCLUSIONS

In this study, we found that one course of treatment with the vascular peptide bioregulator Slavinorm received by young spontaneously hypertensive rats allowed an increase in the microvascular network density of the pia mater and did not affect other microcirculation parameters in the brain cortex. When Slavinorm was administered a second time, as a result of functional cumulation, the microvascular network density increased by approximately 1.6 times, and the perfusion level increased by 15% on average. The use of the vascular peptide bioregulator did not affect the level of oxygen saturation of the brain tissues or the ABP of rats of all ages. However, functional cumulation allowed spontaneously hypertensive old rats to endure the spasm of brain vessels more easily.

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