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The Effect of Vascular Peptide Bioregulator on Microcirculation in the Brain Cortex of Old Rats

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Abstract—At a video facility for the study of intravital brain microcirculation, we found that, after a course treatment with vascular peptide bioregulator, the microvasculature density of the pial tunic in old (22–24 months) rats increased by about 2.5–2.8 times; the constriction and dilative reactions of the pial arterioles increased after the application of noradrenaline and acetylcholine onto the brain surface, respectively. However, perfusion into brain tissues did not increase, but the degree of oxygen saturation in microvessels of this tissue region increased.

Keywords: vascular peptide bioregulator, old animals, density of microvascular network, arterial reactivity, brain

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Due to the increased life span in economically developed countries, the development of new drugs to extend the body's vital resources and improve human wellness in elderly age is one of the most topical tasks faced by modern medicine and biology. The vascular peptide bioregulator Slavinorm was developed at the St. Petersburg Institute of Bioregulation and Gerontology. It is a complex of polypeptides with a molecular weight 72–678 Da extracted from calf vessels. Slavinorm is expected to be used in medical practice as an angioprotector. Our earlier results from in vitro studies demonstrated that Slavinorm acts tissue specifically, stimulating the growth of vascular tissue explants in young and old rats in organotypic cultures [6, 8, 10].

The goal of the work we present is to study the influence of the peptide bioregulator Slavinorm on cortical microcirculation in old rats.

MATERIALS AND METHODS

The experiments were conducted on male Wistar-Kyoto rats. The animals were kept in standard vivarium conditions with natural lighting and free access to water and food. The studies were conducted according to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

For the presented experimental research, we formed three groups of animals: the first included intact rats aged 2–3 months, $n = 20$; the second included intact rats aged 22–24 months, $n = 13$; and the third included rats aged 22–24 that received the peptide bioregulator Slavinorm two months before the study, $n = 12$. The administration of Slavinorm (0.25 mg) to animals was conducted intramuscularly according to the following pattern: one injection per day for 5 days, a pause of 2 days, and one injection per day for 5 days. Visualization and monitoring of microvasculature, the study of reactivity of pial arteries, and measurement of the circulation time and oxygen saturation in sensomotor cortex vessels were conducted two months after the course of Slavinorm administration. The rats were narcotized with zoletil, 20 mg/kg (Virbac, France), intraperitoneally. The bregma bone and the dura mater were removed, thereby making it possible to visualize the pial brain tunic of sensomotor cortex. The brain surface was uninterruptedly irrigated with saline at 37°C.

To estimate the microvasculature density, animals were placed under the lens of the video facility (at ×40 magnification). Using Photo M software (developed by A. Chernigovski), we calculated the total number of vessels and, separately, the number of arteries per unit of area from static images. The reactivity of pial arteries and changes of the microvascular diameter before and after the action were studied with the application of noradrenaline (NA, vasoconstrictor) and acetylcholine (ACH, vasodilator) solutions in a concentration of 10^{-6} M onto the brain surface with a general 160-fold amplification of the optical system.

To measure the tissue circulation time in the sensomotor cortex and blood oxygen saturation (SO_2) in the microcirculatory bed, we used a LAKK-M multifunc-

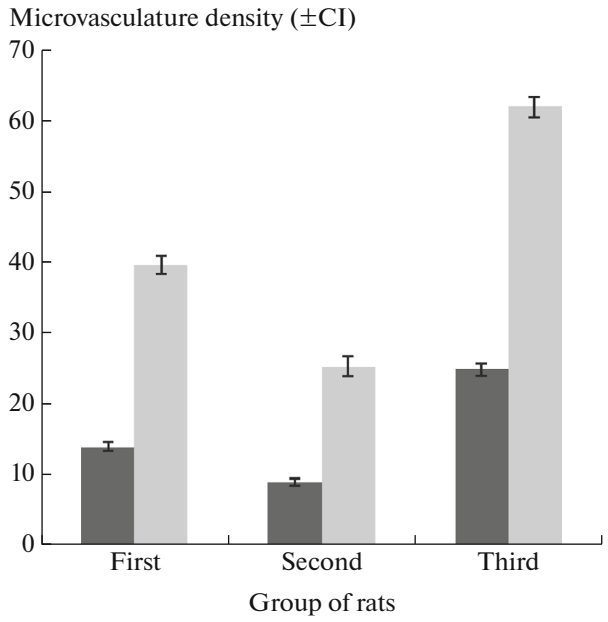

Fig. 1. Microvasculature density of sensomotor cortex pial tunic in young and old rats of intact groups and in rats of the third group after a course of administration of the peptide vascular bioregulator Slavinorm. Dark bars—microvasculature density of arterial area of sensomotor brain cortex pial tunic ($p \le 0.05$); gray bars—density of all microvasculature of sensomotor brain cortex pial tunic ($p \le 0.05$).

tional laser diagnostic complex (Scientific Production Enterprise Lazma, Russia). This complex evaluates dynamic blood microcirculation characteristics (microcirculation index) and the change of blood flux per time unit in the studied tissue volume of about 1 mm^3 in relative perfusion units by laser Doppler fluorometry. With the optical tissue oxymetry method, we estimated SO_2 in the same volume of cortical tissue. In standard conditions, the microcirculation index and SO_2 were initially registered on the surface of each hemisphere at four points with the approximate coordinates AP = 1, 2, 3, 4 mm from the bregma; SD = 1 mm laterally from the sagittal suture. The blood temperature of animals during all the experiment was kept at 37°C; the systolic blood pressure in old rats were on average 120–140 mm Hg.

In the statistical processing of all data, the significance of differences was estimated by the Mann–Whitney U test, and the confidence level of differences was $p \le 0.05$.

RESULTS AND DISCUSSION

We demonstrated earlier that the density of microvasculature in the cortex of Wistar-Kyoto rats decreases by approximately 1.6–1.8 times during aging [3]. The course of Slavinorm application resulted in an increase in the density of whole microvasculature of pial tunic in 22- to 24-month-old animals on average by 2.5 times as compared with old rats in intact groups. The density of the arterial area of pial tunic microvasculature increases on average by 2.8 times (Fig. 1). The significant activation of angio- and arteriogenesis in the brain of old animals compensated for the vascular desolation of brain tissue observed in old rats [7, 11]. The high density of the microvascular bed makes it possible to keep the gaseous exchange between blood and tissue at a level preventing the formation of an ischemic zone in the brain and the development of such diseases as dyscirculatory encephalopathy.

As is known, after the application of any vasoreactive agent onto the brain surface, some pial arteries have a direct reaction: their diameter decreases under the action of a constrictor and increases when a dilator is used; the diameter of other microvessels, on the contrary, increases under the action of constrictor and decreases under the action of a dilator; and a small group of pial arteries does not react to the exposure at all [1, 4, 9]. We detected significant differences in the reactivity of pial arteries in young rats (the first group), old rats of the intact group (the second group), and in animals receiving Slavinorm (the third group). Thus, in the third group as compared with other groups, the number of pial arteries responded to NA with constriction increased by 30%. Correspondingly, the number of vessels with the paradoxical reaction, dilation, decreased (Fig. 2a). In animals under the action of NA, not only the number of arteries, but also the degree of diameter decrease, reacted in one way or another. It is more informative to consider this index not as a whole by all vessels but separately in every branching order of the arterial tree (see Fig. 2b). In old animals, the constriction degree of the smallest arteries and 3rd- to 5th-order arterioles is more statistically significant than in young rats. In old animals receiving Slavinorm, the constriction increases as compared with intact rats of the same age and significantly exceeds this index in young animals (see Fig. 2c). These results should probably be considered negative. In old age, the predisposition to vascular spasms is rather high, which is confirmed by our results obtained from intact old animals. The application of Slavinorm increased spasms of pial arteries under the constrictor action.

Under the action of the vasodilator ACH, the number of arteries responding by direct paradoxical reaction or with no reaction to the exposure was approximately equal (Fig. 3a). At the same time, the degree of arterial dilatation in animals of different groups differs sufficiently significantly: in young rats, the large first-order arteries responded to ACH with bigger dilatation; in old rats, the small third- to fifth-order vessels did so. The same tendency remained in old animals receiving Slavinorm (see Fig. 3b). Moreover, the first- and second-order arteries responded with more significant dilatation as compared with rats of the second group.

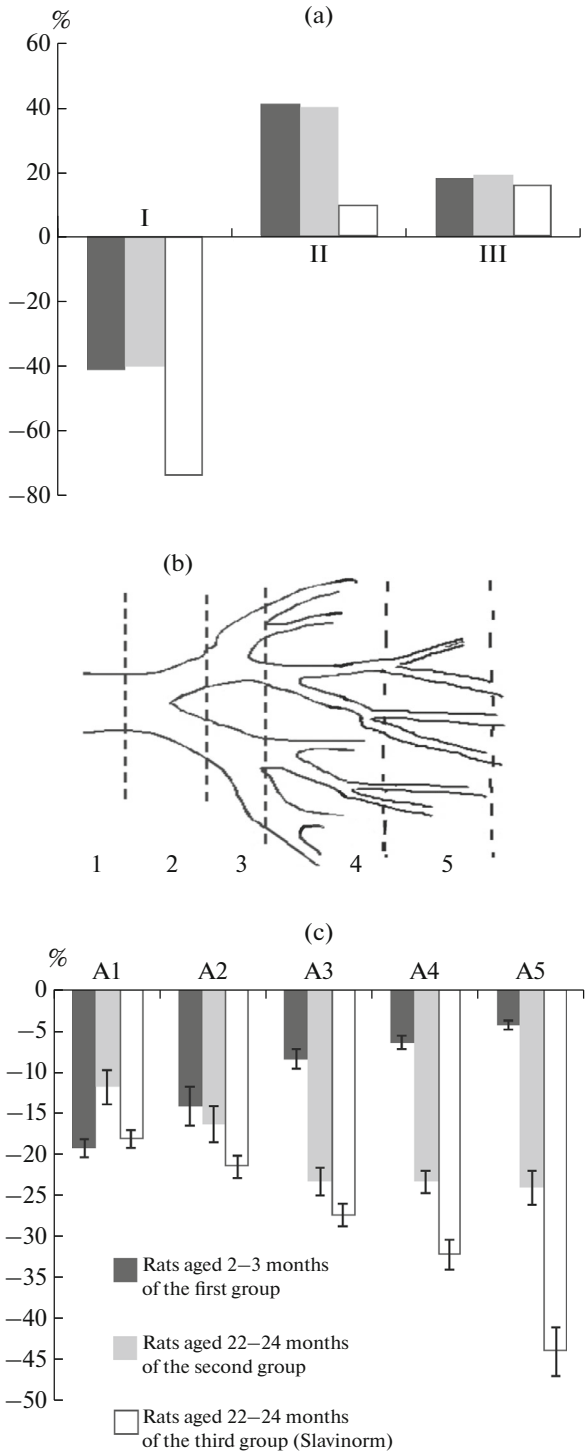

Fig. 2. Changes in the reactivity of pial arteries of sensomotor brain cortex in rats under the influence of noradrenaline (NA). (a) Percentage of pial arteries responding to NA in different ways: I—arterioles responded to NA with constriction; II—arterioles responding to NA with dilatation; III—arterioles that did not responded to NA; (b) schematic sketch of arterial vessel of pial tunic of brain cortex; the branching order of arteries is denoted by numbers; (c) degree of diameter change of pial arteries reacted on NA with constriction ($p \leq 0.05$); ordinate axis—degree of decrease in diameter of pial arteries (±CI); abscissa axis—branching order of pial arteries.

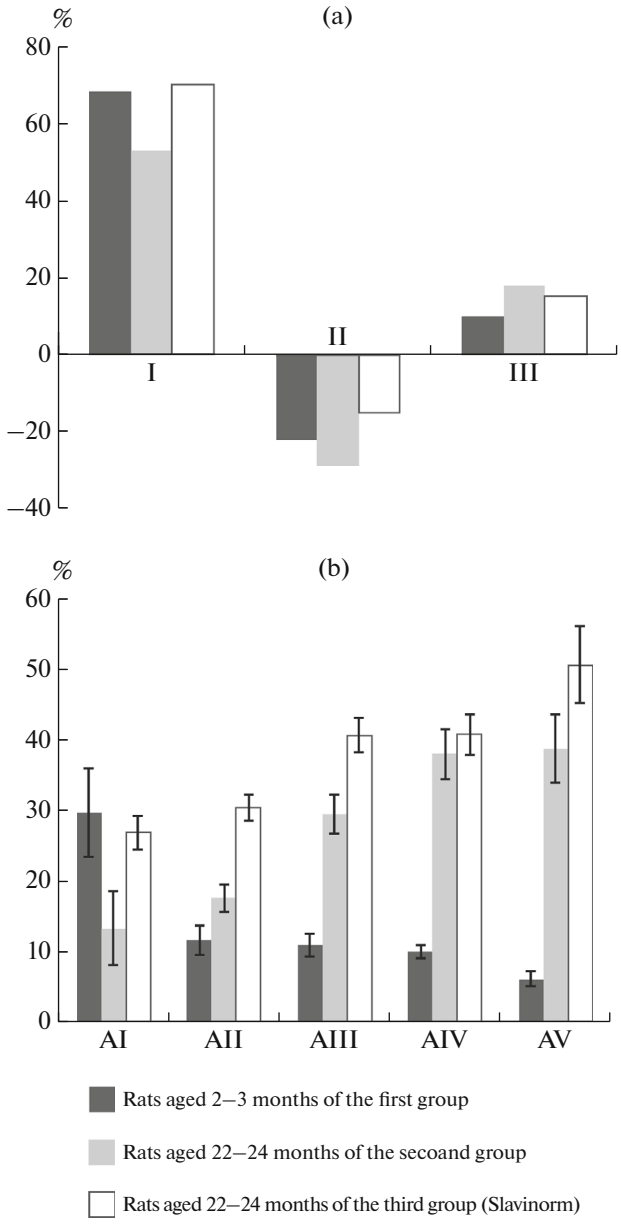

Fig. 3. Change of reactivity of brain sensomotor cortex pial arteries in rats under the action of acetylcholine (ACH). (a) Percentage of pial arteries responding to ACH in different ways: I—arterioles responding to NA with dilation; II—arterioles responding to ACH with constriction; III—arterioles that did not responded to ACH; (b) degree of diameter change of pial arteries reacting to ACH with dilation ($p \leq 0.05$); ordinate axis—degree of increase in the diameter of pial arteries (±CI); abscissa axis—branching order of pial arteries.

The increase in the microvasculature density of the pial tunic in old animals after the application of the peptide vascular bioregulator did not influence the microcirculation index in the brain cortex (Fig. 4a). As can be seen from the figure data, the microcirculation index in old rats of the second and third groups is statistically significantly lower than in young animals.

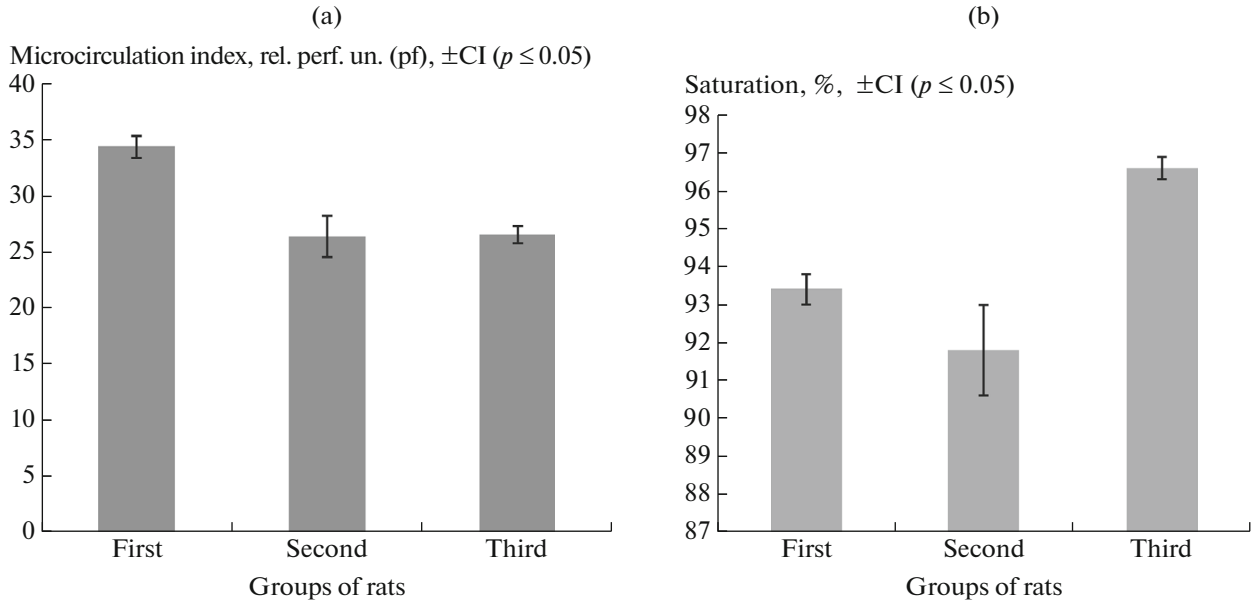

Fig. 4. Microcirculation in brain sensomotor cortex in rats of different age. (a) Perfusion into brain sensomotor cortex; (b) saturation (SO_2) of mixed blood in microvasculature of brain sensomotor cortex.

The circulation time in the brain cortex is probably determined less by the number of microvessels and their reactivity but, to a greater extent, by the cardiac output, pulmonary circulation time, etc. Since the number of arteries increases significantly in measured tissue volume, the arterial blood fraction with high SO_2 also increased and, consequently, the tissual SO_2 also increased (see Fig. 4b).

With the LAKK-M multifunctional laser diagnostic complex, we managed to estimate another important parameter of microcirculation, the rate of oxygen use (in relative units) [2]. This index was 0.36, 0.47, and 0.21 rel. un. in young and old rats of the intact groups and in old rats receiving Slavinorm, respectively. These data show that the highest amount of oxygen was utilized in old rats of the intact group. This probably occurred due to the decrease in tissual circulation time and the increase in distance between arteries. In these conditions, the pO_2 gradient between blood and tissue increased, and, per time unit, more oxygen diffused from microvessels than in young animals. Hence, the oxygen-depleted blood was delivered to the deeper brain structures. A significant increase in microvasculature density substantially decreases the pO_2 gradient between blood and tissue, and, at the same microcirculation index, the oxygen utilization in old animals of the third group decreased by almost 2 times. Correspondingly, high-oxygen blood was delivered to the deeper brain structures.

CONCLUSIONS

Thus, as a result of one course application of the peptide bioregulator Slavinorm, the following changes in the main microcirculation parameters were obtained in the brain cortex.

The density of the microvasculature and its vascular area in the pial brain tunic increased by more than in 2.5 times; we consider this result undoubtedly positive.

The small pial arteries and arterioles had increased constrictory reaction with exposure to the vasoconstrictor and the dilatatory reaction exposed to the vasodilator; this "readiness" of the vessels to change their diameter probably possesses, more likely, a negative character. The pial vessels are immediately involved in brain circulation time regulation; their task is to maintain an invariable level of blood circulation against changes in endogenous and exogenous condition. With an increased reactivity of pial arteries, any action can result in an inadequate shift in blood velocity.

No increase in the level of perfusion in brain tissue was detected. The decrease in microcirculation indicator by itself is a pathological phenomenon suggesting deteriorating oxygen support of the brain. However, the increase in microvasculature density, at the expense of which the whole field of tissual pO_2 is maintained at a high level, can compensate for the age-related decrease in microcirculation index. In regard to this parameter, we find it difficult to positively or negatively estimate the influence of Slavinorm on microcirculation.

We estimate the increase in mixed blood saturation in microvessels as a positive result: owing to this "oxygen reserve," old animals better survive ischemic attacks and brain vascular spasm [5].

The above changes in microcirculations resulted in reduced oxygen utilization from microvessels on cortical tissue level. Hence, more oxygen-saturated blood was delivered to the deeper brain structures; this is a positive result.

The obtained data indicate the potential of studying the peptide bioregulator Slavinorm as an angioprotector for patients with age-related brain microcirculation impairment.

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