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Age-Related Changes in Microcirculation in the Cortex of Hypertonic Rats

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Abstract—We have studied the density of the microvascular system of the pia matter and perfusion and oxygen saturation of the sensorimotor cortex in hypertonic rats of different ages. We have found that the density of the microvascular system did not decrease, but increased, with age. The perfusion of the cortex decreased, and oxygen saturation of brain tissues increased. By the age of 12 months, the exploratory behavior of rats in an open-field test worsened significantly by all parameters.

Keywords: brain, aging, hypertension, microcirculation, perfusion, behavior

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More than half of elderly people experience a persistent increase in arterial pressure. Aging and arterial hypertension are two systems of factors impairing the blood circulation in the brain. In both cases, thickening of brain arteriole walls, constriction of their openings, obliteration, and plasmorrhagia occur, leading to changes of various degrees in vascular walls, up to the point of necrosis [7, 9, 11, 13]. Both with age and during hypertension, a decrease in the circulation rate was found in different brain structures [6, 9, 12].

The aim of this study was to investigate age-related changes in the main parameters in spontaneously hypertensive rats.

MATERIALS AND METHODS

Experiments were carried out on 40 spontaneously hypertensive male SHR rats at the ages of 3–4 and 12 months. Wistar–Kyoto male rats with normal blood pressure at the ages of 3–4, 12, and 24 months were used as controls. Animals were housed under standard conditions with natural illumination and free access to food and water. All animal procedures were carried out according to international regulations (European Communities Council Directives of November 24, 1986, 86/609/EEC). The behavioral testing of animals aged 3–4 and 12 months was carried out in an open-field apparatus according to a standard protocol [2]. The locomotion (the number and duration of "locomotion" acts), exploratory activity ("hole," "locomotion," "rearing," "rearing with support," "stationary movement," and "sniffing" acts), emotional behavior ("grooming," "stationary movement," "rearing"), nonspecific activation (change in the total number of acts), or inhibition of behavior (a significant decrease in the number and duration of acts). The number, duration, and order of acts in the test were recorded using original "open field" software [2]. The statistical analysis of differences between two samples was carried out using the nonparametric Wilcoxon–Mann–Whitney U test (Statistica 8 program) at the significance level $p < 0.05$.

Visualization and monitoring of blood vessels of the pia mater was carried out in anesthetized animals (20 mg/kg Zoletil, Virbac, France) using a television device for in vivo microscopy. Rats were put under the lens of the device after removing the parietal bone and dura mater. The density of the microvascular system was studied on the whole surface of the sensorimotor cortex at 40-fold magnification. During the experiment, the brain surface was continuously watered with saline at 37°C. The body temperature of rats during the experiment was maintained at 37°C; the arterial pressure was 176 ± 2.4 mmHg in young hypertensive rats and 191 ± 2.1 mmHG in old hypertensive rats and 120–140 mmHg in the control group.

The total number of vessels and arterioles per unit of the surface were calculated on static images. During the statistical analysis, the significance of differences was evaluated using the Mann–Whitney test at the significance level $p < 0.05$.

Changes in the perfusion (P) and the oxygen saturation (SO_2) in the sensorimotor cortex were evaluated in the experimental animals using the LAKK-M multifunctional laser diagnostics complex (Lasma, Russia). P and SO_2 were recorded from the whole surface

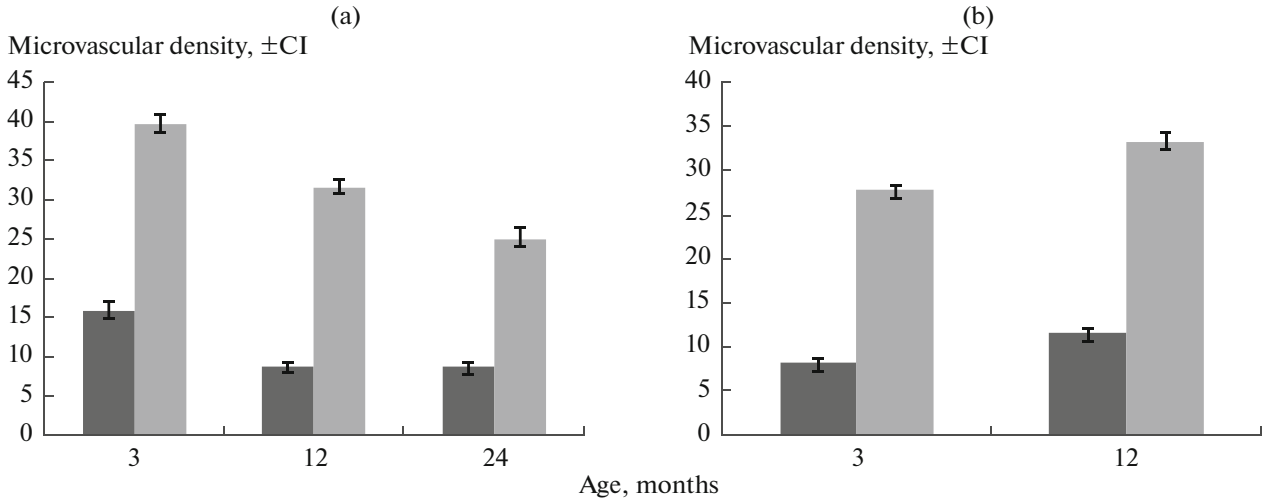

Fig. 1. The microvascular density in the pia mater of the sensorimotor cortex in rats.
(a) Wistar–Kyoto rats with normal blood pressure; (b) hypertonic SHR rats; dark columns show the arteriole density in the pia mater of the sensorimotor cortex ($p < 0.05$); light columns show the density of the whole microvascular system of the sensorimotor cortex ($p < 0.05$).

of the sensorimotor cortex [3] in rats at an initial state without any impacts, after the application of a vasoconstrictor noradrenaline (NA, at the concentration 10^{-3}), and during the acute ischemia. The latter was modeled by the clamping of both carotid arteries for 5 min. The statistical analysis of differences was carried out using Student's *t*-test at the significance level $p < 0.05$.

RESULTS AND DISCUSSION

Results of the study of the microvascular density in the pia mater of the sensorimotor cortex in hypertensive rats at the ages of 3–4 (young) and 12 months (old) are shown in Fig. 1.

We have previously shown that the density of the microvascular system in young SHR rats is 1.4 times lower than in young Wistar–Kyoto rats with normal arterial pressure; the density of arteries and arterioles was 1.9 times lower at the same part of the pia mater [4]. By this parameter, young SHR rats were not different from the 24-month-old Wistar–Kyoto rats (very old) (Fig. 1). The aging of the microvascular system in hypertensive rats was different from this process in the control group. The microvascular density in the pia mater decreased with age in rats with normal arterial pressure (Fig. 1a) and increased in SHR rats (Fig. 1b). The microvascular density in old SHR rats was 1.2 times higher (no statistical significance) than in young SHR rats; the density of arterioles was 1.4 times lower ($p < 0.05$) respectively.

We suggest that the persistent increase in blood pressure in SHR rats resulted in the development of ischemic areas since a young age. Brain arteries and arterioles are known to play an important role in the tissue oxygen supply, and the distribution of 30% oxygen between blood and tissues occurs at the level of these vessels. In addition, the oxygen partial pressure (ρO_2) in the arteriole blood is higher than in capillaries, the ρO_2 gradient is more prominent in tissues near arterioles than capillaries, and the brain artery supplies s larger volume of tissue in comparison to capillaries [1]. The development of hypertonia leads to a decrease in the blood flow in different parts of the brain [6, 8–10]. A decrease in the microvascular density, the percentage of arterial vessels, and the rate of cerebral blood flow can result in the formation of regions with insufficient blood supply. Tissue hypoxia is known to stimulate angiogenesis [14]. The insufficient oxygen supply in hypertonic rats starting at a young age may contribute to the compensatory increase in the density of the arterial part of the cerebral microvascular system.

Age-related changes in other parameters of microcirculation P and SO_2, both initial and after a severe impact on SHR rats, were also different from the processes occurring in rats with normal blood pressure during aging. In Wistar–Kyoto rats, P and SO_2 decreased in the sensorimotor cortex with age (Fig. 2). In young SHR rats, the P level in this tissue was 1.2 lower ($p < 0.05$) than in young Wistar–Kyoto rats (Fig. 2a) and corresponded to the P values in very old rats with normal blood pressure. By the age of 12 months, P in the sensorimotor cortex of hypertonic rats significantly decreased (Fig. 2a) in comparison with young hypertonic rats and rats with normal blood pressure of the same age. Regarding the saturation of cerebral tissue with oxygen, Wistar–Kyoto rats did not exhibit significant changes in P and SO_2 until 12 months of age; by 24 months, SO_2 drastically decreased in the cerebral

cortex (Fig. 2a). In SHR rats, the SO_2 level significantly increased with age (Fig. 2b). An increase in the arterial density in the studied regions and a decrease in the brain-tisuue oxygen consumption contributed to an increase in the tissue SO_2. The LAKK-M device helps to estimate the level of oxygen consumption in the studied region. During aging up to 12 months, in both rats with normal blood pressure and hypertonic rats, the oxygen consumption in the cortex decreased 1.7 times (Fig. 2c). The results described above allow concluding that microcirculation in the cerebral cortex of hypertonic rats decreases with age.

However, it is important to consider which changes in the parameters of microcirculation take place under the impact of extreme factors: constriction of pia vessels and ischemia. Some pia arterioles constricted, some dilated, and others did not respond to the application of vasoconstrictor NA on the pia mater of both young and old animals [3]. This impact caused a decrease in P in some parts of the brain and an increase in the others. In Wistar–Kyoto rats at the ages of 3–4 and 12 months, the mean decrease in P (26.6 ± 2.5 and 26 ± 2.7%) and increase in P (23.4 ± 7.3 and 26.4 ± 4.2%), respectively, was approximately the same, and mean P did not change significantly. In SHR animals at the ages of 3–4 and 12 months, P decreased by 25.1 ± 2.0 and 15.1 ± 3%, respectively; in the other studied regions, it increased by 42.7 ± 6.4 and 38.1 ± 9.2% (Fig. 3a). Therefore, P increased by 17.6 and 23% in the brain under the effect of NA.

The 5-min occlusion of both carotid arteries led to a decrease in P in the sensorimotor cortex by 50–70% in all groups of rats. The decrease in SO_2 was approximately the same in young rats with normal blood pressure and with hypertonia (Fig. 3b). The global ischemia had the worst impact on 12-month=old Wistar–Kyoto rats and the mildest effect on hypertonic rats of the same age. Arterial hypertension may be some sort of natural preconditioning of ischemic states of the brain. Adaptation of the cerebral tissue to persistent hypoxia in hypertonic rats helps them to endure extreme conditions induced by cerebral-vessel spasms or acute oxygen deficiency more successfully.

The sensorimotor cortex (anterior parietal and parietal regions) is a region representing the afferents of the cutaneous-kinesthetic analyzer, which plays the leading role in rodent adaptive responses.

The microcirculation parameters of this region affect the formation of exploratory behavior in animals. Young SHR rats showed more active vertical behavior than did Wistar–Kyoto rats: the number of rearings in the open field test was seven times greater. Young SHR rats also inspected holes 1.5 times more frequently. Other test parameters did not differ between the groups.

Old hypertonic rats at the age of 12 months showed a decrease in the locomotor and exploratory behavior in comparison with SHR rats at the age of 3 months

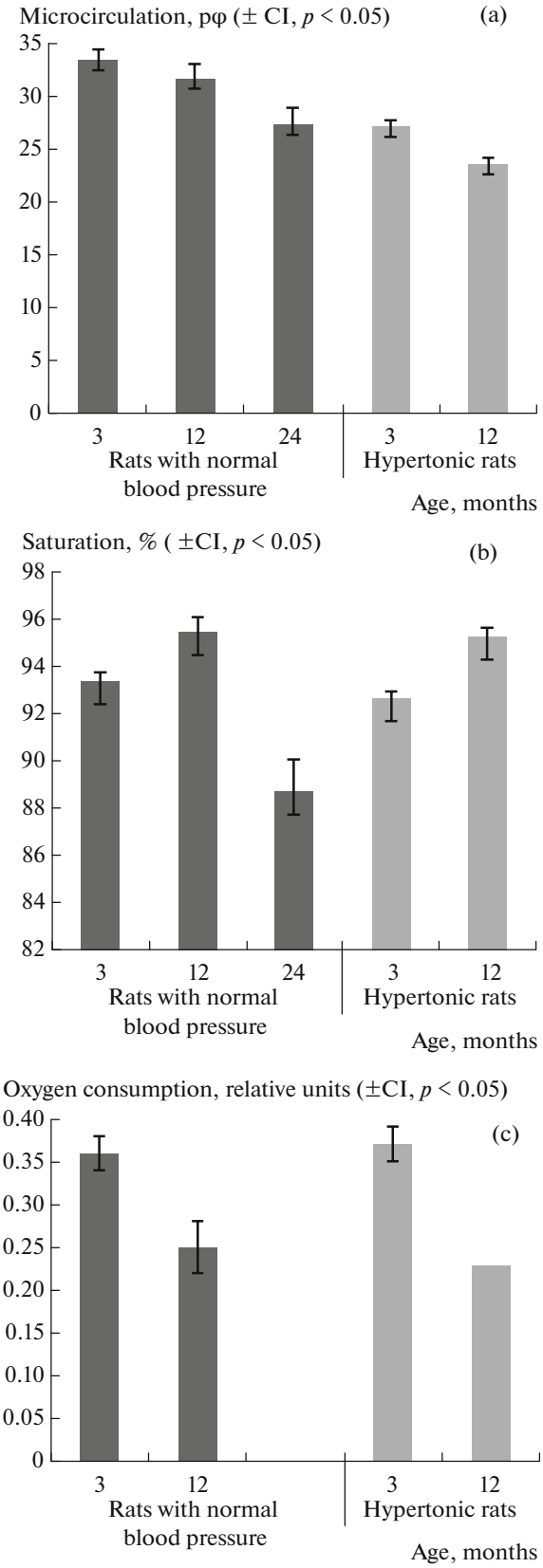

Fig. 2. Perfusion and saturation in the sensorimotor cortical tissue in rats.
(a) Perfusion in the sensorimotor cortex, (b) saturation (SO_2) in the sensorimotor cortical tissue in rats, and (c) oxygen consumption in the sensorimotor cortical tissue in rats.

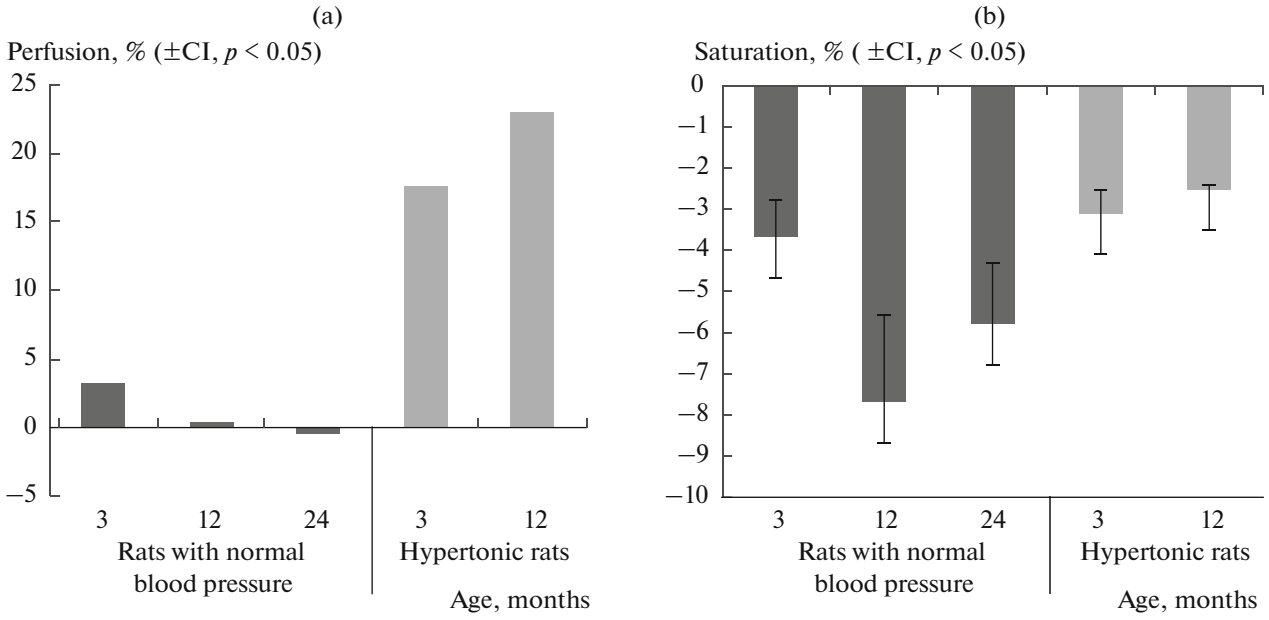

Fig. 3. Changes in perfusion and saturation in the sensorimotor cortex in rats under extreme conditions.
(a) changes in perfusion in comparison with the initial level after the application of a vasoconstrictor on the brain surface; (b) changes in saturation in comparison with the initial level during the global ischemia.

(Fig. 4). We should note that, in old hypertonic rats, vertical behavior was impaired: rearing with support (a 4.3-times decrease) and vertical rearing (a 5.9 times decrease). A significant decrease in hole inspection (1.5 times) was also found. These results demonstrate that exploratory behavior and the investigatory reflex decreased with age, while locomotor activity remained intact. Locomotion and sniffing decreased 1.3 times.

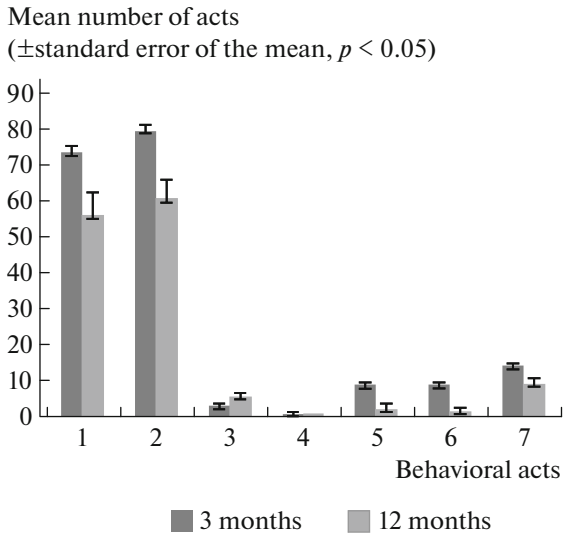

Fig. 4. Exploratory behavior of hypertonic rats of different ages in the open-field test.
(1) Locomotion, (2) sniffing, (3) stationary movement, (4) grooming, (5) rearing with support, (6) rearing, and (7) hole.

For comparison, in aging rats with normal blood pressure, these parameters more than halved [5].

Here is one more interesting comparison. In old Wistar–Kyoto rats, the duration of all acts was significantly greater than in young rats; i.e., old rats carried out a smaller number of behavioral acts, and it took them more time to realize them. Such behavior is evidence of slowing responses, fast tiring, and emotional suppression. Old SHR rats carried out fewer vertical acts and took less time to carry them out. The duration of locomotor activity and stationary movement increased significantly (the latter increased 4.6 times). This is evidence of a high level of anxiety and arousal, but not emotional suppression.

CONCLUSION

Hypertonic rats do not have such a significant impairment of microcirculation with age as do rats with normal blood pressure.

ACKNOWLEDGMENTS

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