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Effect of Intracerebral Transplantation of Mesenchymal Stem Cells on Reactivity of Pial Arterioles in Old Rats

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Using a TV device for studying microcirculation (×160), we analyzed the responses of arterioles in the pia mater of the sensorimotor cortex in young (2-3 months) and old (22-24 months) rats after local application of a vasoconstrictor (norepinephrine, 10^{-6} M) or vasodilator (acetylcholine, 10^{-6} M). The responses of the arterioles were evaluated by changes in their diameter and by the number of responding vessels in the field of view. The constrictor responses of the pial arteries to norepinephrine did not significantly differ in intact young and old rats. The number and degree of dilatory responses to acetylcholine in old rats were lower than in young animals by 14 and 30%, respectively. Intracerebral transplantation of mesenchymal stem cells to old rats had practically no effect on reactivity of pial arterioles to acetylcholine, while the number of constricted vessels in response to norepinephrine increased by ~20%.

Key Words: *intracerebral transplantation; mesenchymal stem cells; reactivity; arterioles; brain*

Aging is associated with impairment of cerebral microcirculation due to decrease in the density of microcirculatory network [11] and elasticity and permeability of microvessels [5-7,12].

Arteriolar portion of the microvascular bed is of particular interest, because the smallest arterioles are responsible for ~30% gas exchange between the blood and brain tissue [13] and are involved in the regulation of cerebral blood flow. However, this portion of the vascular network in the brain is least studied. Experimental data on age-related changes in reactivity of the brain arterioles are contradictory: some authors reported impairment of arteriolar responses to vasoconstrictors and vasodilators [9,10], while other revealed no age-related differences [8,9].

In previous experimental studies, we have demonstrated that intracerebral transplantation of syngeneic mesenchymal stem cells (MSC) to old rats significantly increased the density of the microvascular network, in particular its arteriolar compartment, in the pia mater of the sensorimotor cortex.

Here we analyze the effects of MSC on reactivity of pial arterioles in old rats.

MATERIALS AND METHODS

Experiments were carried out on 2-3- and 22-24-month-old male Wistar–Kyoto rats (n=42). The animals were kept under standard vivarium conditions at natural illumination and free access to food and water. The study was performed in accordance with European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasburg, 1986).

Isolation of MSC from the bone marrow (BM) of syngeneic rats, MSC culturing/expansion *in vitro*, and evaluation of their viability were performed at the Institute of Molecular Biology and Genetics using routine methods [1]. Passage 2-3 MSC were transported to the Laboratory of Physiology of Circula-

Laboratory of Physiology of Circulation, I. P. Pavlov Institute of Physiology, Russian Academy of Sciences; *Institute of Molecular Biology and Genetics, V. A. Almazov Federal Heart, Blood, and Endocrinology Center, Ministry of Health of the Russian Federation, St. Petersburg, Russia. ***Address for correspondence:*** sib@kolt.infran.ru. I. B. Sokolova

tion, I. P. Pavlov Institute of Physiology, for further physiological experiments within the framework of scientific collaboration.

The animals were divided into 4 groups: intact 2-3-month-old rats (group 1), intact 22-24-month-old rats (group 2), 22-24-month-old rats with intracerebral transplantation of MSC (group 3), and 22-24-month-old rats with intracerebral administration of stem cell culture medium of α-MEM (control group).

We have previously demonstrated [4] that administration of pure α-MEM did not statistically increase or decrease the density of microvascular network in the pia mater in neither young nor old animals and did not affect reactivity of pial arterioles in 2-3-month-old rats. Similar results were obtained in this series in group 4 animals.

Intracerebral transplantation. The rats were intraperitoneally narcotized with zoletil (20 mg/kg; Virbac). A hole (1 mm) was drilled in the parietal area of the skull alternately above the right or left hemisphere with a dental drill without damaging the dura mater. MSC suspension (200,000 cells in 20 µl α-MEM) or 20 µl pure α-MEM was injected into the cerebral cortex to a depth of 2 mm with an insulin syringe. The skin on the head was sutured.

Visualization and monitoring of the microvascular network. In 3 weeks after intracerebral transplantation, the animals were narcotized and the parietal bone and the dura mater were removed to visualize the pia mater of the sensorimotor cortex. The brain surface was continuously sprayed with physiological saline (37°C). During the experiment, the body temperature was maintained at 37°C and mean blood pressure was 105-120 mm Hg.

The animals were placed under the objective of TV device (×160) and changes in the diameter of pial arterioles in response to application of norepinephrine (NE) or acetylcholine (ACh) to the brain surface were observed. The concentration of the applied agents was 10^{-6} M and the temperature was 37°C.

The number of arterioles responding by constriction or dilatation or not responding to drug application and changes in microvessels diameter were measured on static images using PhotoM software.

The significance of differences was evaluated using Mann–Whitney test at $p \leq 0.05$.

RESULTS

We have previously demonstrated that the density of microvascular network in the pia mater considerably decreased during aging by on average 1.2 times [2]. Intracerebral transplantation of MSC led to an increase in the density of arteriolar compartment of the microvascular bed in both young (by ~2.4 times in comparison with intact rats) [3] and old animals (by ~2.3 times) [2].

In the present study, the constrictor responses of the pial arteries to NE did not significantly differ in intact young and old rats (Fig. 1). The number and degree of dilatory responses to ACh in old rats were lower than in young animals by 14 and 30% ($p \leq 0.05$), respectively (Fig. 2).

Intracerebral transplantation of MSC to old animals had practically no effect on the response of pial arterioles to ACh (Fig. 2), while after NE application, the number of constricted arterioles increased by ~20% and the number of dilated vessels decreased by 18% (Fig. 1).

These results demonstrate the absence of age-related differences in the reactivity of arterioles in the pia mater of the sensorimotor cortex of Wistar–

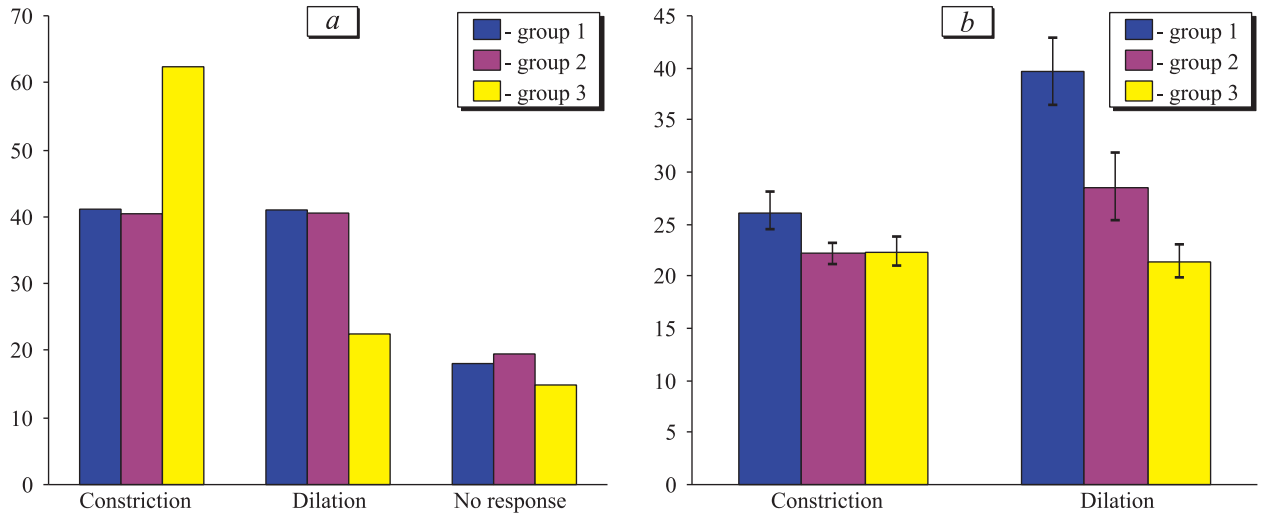

Fig. 1. Changes in reactivity of pial arterioles of rat sensorimotor cortex to NE application. *a*) Pial arterioles responding to NE, %; *b*) changes in the diameter of pial arterioles (%) in response to NE.

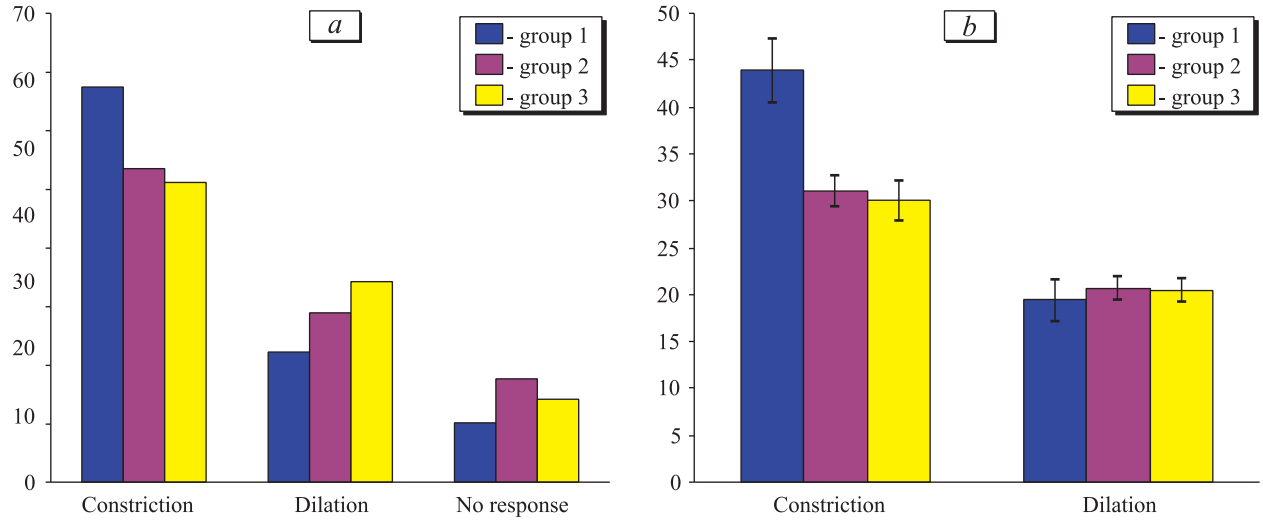

Fig. 2. Changes in reactivity of pial arterioles of rat sensorimotor cortex to ACh application. *a*) Pial arterioles responding to ACh, %; *b*) changes in the diameter of pial arterioles (%) in response to ACh.

Kyoto rats. Intracerebral transplantation of MSC did not significantly modulate the reactivity of arterioles in the examined area. We believe that transplantation of MSC into the cerebral cortex of old animals had a positive effect on the microcirculation in this area, because it significantly increased the density of the entire microvascular network, and especially its arteriolar compartment, while the responses of pial arterioles to vasoconstrictors and vasodilators did not practically differ from the reactions of native vessels.

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