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AGE CHANGES IN ENZYMES OF NEURONAL AND MICROVASCULAR ENERGY METABOLISM IN THE BRAIN OF SPONTANEOUSLY HYPERTENSIVE RATS

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One of the most important risk factors of cerebrovascular disturbances is arterial hypertension (AH), as epidemiologic studies in man [7, 12] and experimental models [14] have demonstrated. The brain of hypertensive animals is more vulnerable to hypoxia and ischemia [2, 9]. The traditional explanation of these facts is to attribute them to disturbances of regulation of the cerebral circulation under conditions of AH [4, 10]. Yet there have been few investigations into the metabolism of brain tissue and of the microvessels, although there is evidence [1, 13] that they may play a decisively important role in the severity of brain damage when the circulation in them is disturbed.

The aim of this investigation was to compare some aspects of metabolism of neurons and microvessels in the brain of spontaneously hypertensive (SH) rats at different age periods.

EXPERIMENTAL METHOD

Experiments were carried out on SH rats and on control normotensive Wistar—Kyoto rats. The experimental animals were obtained from the Experimental Biological Models Group (Director, Yu. S. Dmitriev) of the I. P. Pavlov Institute of Physiology, Academy of Sciences of the USSR. Three groups of control animals and three of SH rats aged 1.5, 3, and 6 months were studied (six, eight, and seven animals in each group respectively). All the animals were kept under identical conditions: the systolic blood pressure (SBP) was measured in the caudal vessels by an indirect method in rats of groups 2 and 3 several times in the course of 3 weeks. Material for investigation was obtained after decapitation of the animals. A piece was excised through a parasagittal incision from the right cerebral hemisphere, measuring 0.6 × 0.4 × 0.3 cm, corresponding to the sensomotor cortex,

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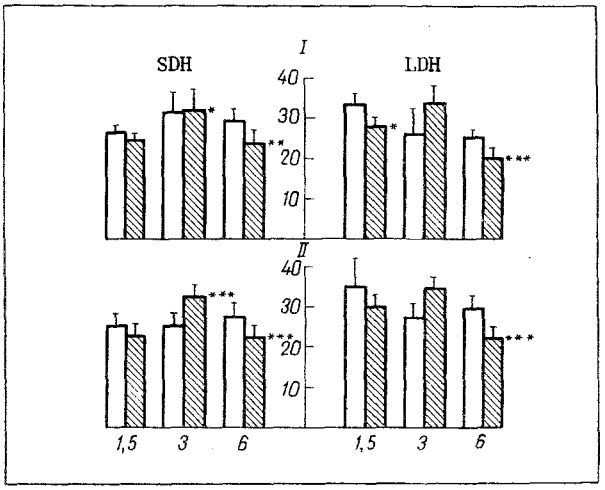

Fig. 1. SDH and LDH activity in cortical and hippocampal neurons of control animals and SH rats of different ages. Unshaded columns — control, shaded — SH rats. Here and in Fig. 2: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. The significance of differences was calculated relative to the previous control or SH group respectively. I) Cortex, II) hippocampus. Abscissa, age of animals (in months); ordinate, optical density (in conventional units).

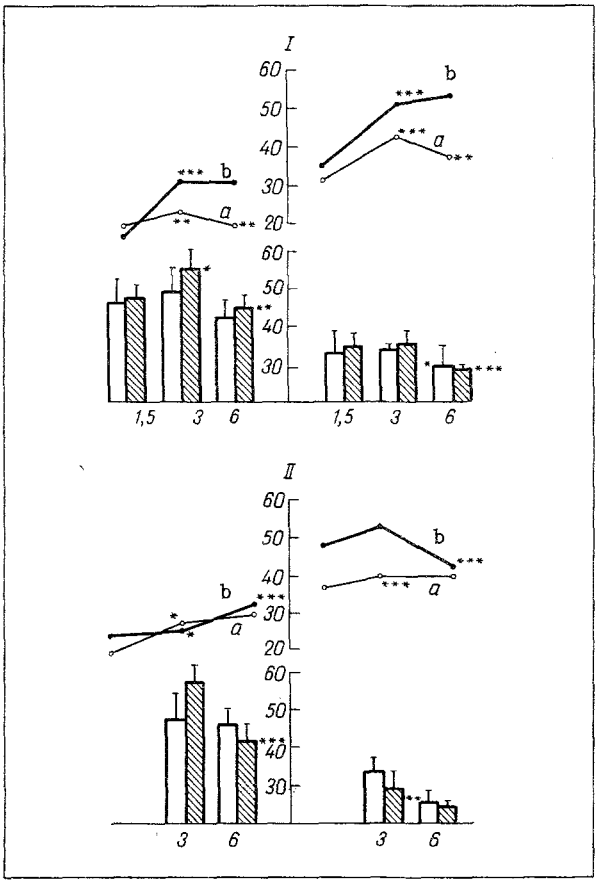

Fig. 2. Number of microvessels of different diameter, and their AP activity, in sensomotor cortex and hippocampus of control rats and SH rats of different ages. Top graphs: specific volume (in v); bottom graphs: optical density (conventional units). a) Control, b) SH rats. Remainder of legend as to Fig. 1.

with the adjacent hippocampal structures. After immersion in cold iso-octane the material was frozen in liquid nitrogen. In the Laboratory of Pathomorphology, Central Research Laboratory, I. P. Pavlov First Leningrad Medical Institute, frozen sections 10 41 thick were stained by the usual histochemical methods [3, 5] to demonstrate activity of the following enzymes in neurons of the cerebral cortex and hippocampus: succinate and lactate dehydrogenase (SDH and LDH), GABA transaminase (GABA-T), and of the following enzymes in the endothelium of the microvessels: alkaline phosphatase (AP) and L-glycerophosphate dehydrogenase (GPDH).

Morphometry of all the microvessels was carried out by a stereologic method [8], using two vascular markers (AP and GPDH); the number of microvessels 4-20 μ in diameter and under 4 μ was determined separately. The material was subjected to statistical analysis by Student's and Wilcoxon's tests.

EXPERIMENTAL RESULTS

In animals of the control group at the age of 3 months SBP was 130.6 mm Hg, compared with 169.1 mm Hg in SH rats; at the age of 6 months the values were 140.7 and 178.6 mm Hg respectively (at both ages $p < 0.001$). At the age of 1.5 months differences in the brain tissue as regards activity of the various enzymes were small: in SH rats LDH activity (Fig. 1) was lower in the cortical neurons ($p < 0.01$) and GPDH activity was lower in the smaller microvessels ($p < 0.05$). A larger number of large and small microvessels showing AP activity (Fig. 2) was clearly visible in the hippocampus of the SH rats ($p < 0.01$-0.001), with a tendency for the number of these microvessels in the cortex to decrease ($p < 0.05$).

At the age of 3 months differences were found in the time course of the markers of metabolism in the two groups of animals. SDH, GPDH, and GABA-T activity in cortical and hippocampal neurons in the control remained the same as at the age of 1.5 months, with only a tendency noted for LDH activity in the cortex to decrease (Fig. 1). In SH rats SDH activity in the hippocampus was clearly increased, and SDH and LDH activity in the cortex showed a smaller increase. At this age SDH activity in the hippocampus was higher in SH rats than in the control animals ($p < 0.001$), but LDH activity in the cortex and hippocampus was weaker ($p < 0.05$).

AP (Fig. 2) and GPDH activity in microvessels of different diameter in the control remained at the previous level in rats aged 3 months, whereas in SH rats AP activity increased in the larger vessels and GPDH activity in the smaller vessels ($p < 0.005$). Nevertheless, differences in activity of the microvessels between the two groups of 3-month-old rats were small and were varied in direction. The clearest changes occurred in the number of vessels with activity of these marker enzymes. It was increased, mainly highly significantly, both in the control (except GPDH activity in the small vessels) and in SH rats (not significantly only in the small hippocampal vessels). A more significant increase in the number of "active" microvessels was observed in SH rats, in which at the age of 3 months AP activity was significantly higher in the cortical vessels ($p < 0.01$) and in the small hippocampal vessels ($p < 0.001$), whereas in the control high GPDH activity was observed in the large cortical vessels ($p < 0.001$).

In control rats aged 6 months no significant changes were found in SDH, LDH (Fig. 1), GPDH, and GABA-T activity in cortical and hippocampal neurons. On the contrary, activity of each of these enzymes decreased significantly in SH rats compared with that observed at the age of 3 months, and it was also significantly lower than in control rats aged 6 months.

Compared with rats aged 1.5 months, in animals aged 6 months LDH activity in the cortical and hippocampal neurons and GPDH activity in the cortex were significantly lower, especially in SH rats.

AP activity (Fig. 2) and GPDH activity in the microvessels of the control rats were reduced, but not significantly, at 6 months. A marked decrease in activity, not significant only for small hippocampal vessels, was discovered in the SH rats. Meanwhile vascular activity in the control rats and in SH rats at the age of 6 months did not differ. The number of microvessels of different diameters at the age of 6 months, compared with their number at 3 and 2.5 months, showed different changes. On the whole, however, in SH rats at 6 months significantly more of the different microvessels, in which AP and GPDH activity was identified, were discovered as before than in the control (the only exception was a decrease in the number of small vessels with GPDH activity in the cortex, $p < 0.05$).

Thus the level of activity of these marker enzymes of neuronal and microvascular metabolism and the specific volume of the microvessels undergo age changes in SH rats, which differ distinctly from those in the control. It can be concluded on the whole that in SH rats at the age of 3 months there is a clear increase both in the corresponding metabolic activity in the neurons and microvessels and in the number of microvessels "active" with respect to these markers. At the age of 6 months there is a marked decrease in activity of all the marker enzymes of metabolism without any reduction in the number of "active" microvessels. At the age of 3 months, greater activity of these marker enzymes in neurons and microvessels is observed in SH rats than

in the control animals, together with a larger number of microvessels. At 6 months, on the other hand, activity of these enzymes in neurons of SH rats is lower than in the control, whereas the number of "active" microvessels was on the whole greater than in the control.

The results suggest correlation between morphological and functional features of the brain in SH rats and its greater vulnerability when exposed to ischemia and hypoxia. The familiar concept that explains the decrease in density of the microcirculatory network and worsening of the oxygen supply to the neurons and chronic hypoxia [6] was not confirmed by our data, as also was the case in rats with renal AH [1]. In SH rats aged 3 months, activity of the marker enzymes revealed a more intensive energy metabolism in the neurons and greater activity of the microvessels, together with an increase in their number. The "stressed" functioning of these systems can perhaps be explained by the greater degree of their damage when the blood supply is disturbed. However, at the age of 6 months the "metabolic" situation in the neurons changes to the opposite in SH rats. This may be both the general pattern of age changes in the animals of this group and also the result of disturbances of brain function due to AH itself [9, 10]. This latter hypothesis seems more convincing, if the moderately high AH in these animals, the absence of cerebrovascular accidents in them, and the presence of a larger number of microvessels in SH rats aged 6 months than in the control are taken into account. On this plane it would be interesting to compare the degree of hypoxic brain damage in SH rats at different ages.

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