Cytochemically Determined Activity of Glucose-6-Phosphate Dehydrogenase in Morphochemical Characteristics of the Brain of Wistar Rats Differing by Locomotion Parameters

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Activity of histochemically determined glucose-6-phosphate dehydrogenase, a key enzyme of the pentose phosphate pathway, was qualitatively determined in layer III and V neurons of the sensorimotor cortex and neurons of the caudate nucleus, nucleus accumbens, and hippocampus (CA3) in mature male Wistar rats with high and low locomotor activity in an open field. A negative correlation was revealed between locomotion of Wistar rats in the open field and activity of glucose-6-phosphate dehydrogenase in the sensorimotor cortex, especially in efferent layer V neurons and neurons of the caudate nucleus and nucleus accumbens, which attested to different capacity of the brain in Wistar rats with high and low open-field locomotion to regeneration of phosphopyridine nucleotides ($NADP^+$) and production of pentoses via the pentose phosphate shunt.

Key Words: *Wistar rats; locomotion; brain neurons; activity of glucose-6-phosphate dehydrogenase; quantitative cytochemistry*

CNS is characterized by high plasticity of its functions, which correlates with morphochemical specifics of some CNS structures involved into multilevel adaptive, compensatory, and regeneratory reactions of the brain. In recent studies, the individual and topological approach is becoming dominant in comparison of the initial parameters of functional state of the organism with those observed during adaptation or under pathological conditions. For instance, it was shown that the severity of experimental brain ischemia in the same model varies in Wistar rats differing by their behavior, primarily, by locomotor activity in the open field. Wistar rats demonstrating passive behavior in the open field were characterized by more severe brain ischemia, which is probably related to initial insufficiency of macroergic compounds and to more rigid organization and coordination of different pathways of generation and utilization of macroergic compounds compared to active animals [1]. There are also published data on negative correlation between individual sensitivity to brain ischemia and emotional resistance in rats. A negative effect of emotional stress on subsequent course of brain ischemia eliminating individual differences and sensitivity to this condition and determining 90% mortality was also demonstrated [2]. We previously showed that long-term administration of L-DOPA (Madopar-125) to rats with low locomotor activity in the open field determined more pronounced changes in cytochemical parameters of protein metabolism (inhibition of aminopeptidase activity) in the sensorimotor cortex, caudate nucleus, and nucleus accumbens

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[4]. The hippocampus plays a role in triggering the brain response to L-DOPA treatment, because activity of glutamate dehydrogenase, the enzyme involved into not only oxidative processes in cells and protein metabolism, but also into metabolism of glutamate as an excitatory neurotransmitter, locally increases in hippocampal neurons in rats with low locomotor activity in the open field.

Another oxidative enzyme, glucose-6-phosphate dehydrogenase (G-6-PDH) is a key enzyme of the pentose phosphate shunt and participates in the synthesis of specific precursors of RNA (pentoses) and in the regeneration of reduced NADP (NADPH). G-6-PDH is primarily localized in the cytosol, but 20% total activity of this enzyme is present in mitochondria.

We performed a comparative cytochemical study of G-6-PDH activity in neurons of some brain structures in mature Wistar rats differing by locomotor activity in the open field test.

MATERIALS AND METHODS

The study was performed on more than 25 mature male Wistar rats weighing 200-250 g. The rats were tested in an open field in a noiseless dim room. Rat locomotion (number of crossed squares) was determined visually over 5 min. Rats crossing more than 140 squares and less than 70 squares were used in the experiment. The animals demonstrating medium parameters of locomotor activity were not included in the experiment. Bearing in mind that Wistar rats is a population, we performed the following experimental series: rats with high open-field locomotion served as intact animals in series I, and rats with low open-field locomotion served as intact animals in series II. The objects of the study were sensorimotor cortex (layers III and V), caudate nucleus, nucleus accumbens, and hippocampus (CA3), the structures participating in the formation and realization of targeted behavior in animals.

The rats were decapitated under light ether narcosis. The brain was frozen at -8°C in a Cryo-Cat microtome for 10-15 min and 20-μ sections were prepared. Activity of G-6-PDH in the cytoplasm and processes of neurons was detected by the tetrazolim method (blue diformazan granules) [5] using $NADP^+$ and substrate sodium glucose-6-phosphate (Serva). Quantitative evaluation of enzyme activity was performed on a LYUMAM-IZ microscope (LOMO) at λ=589 nm using a probe with a diameter of 2.5 μ. In each subgroup in series I and II, 150 neurons in the studied brain structures were measured. One-way dispersion analysis (one-way ANOVA) was performed using Statistica software.

RESULTS

In Wistar rats with high open-field locomotion, G-6-PDH activity in layers III and V of the sensorimotor cortex and in the hippocampus was lower by 21, 15, and 11%, respectively, than in animals with low locomotor activity. In the caudate nucleus, enzyme activity was similar in animals of both types, while in the nucleus accumbens this parameter was higher by 21% in animals with low locomotor activity. In Wistar rats with low locomotion, G-6-PDH activity was appreciably lower in all studied brain structures: in the sensorimotor cortex by 30-45%, in the caudate nucleus and nucleus accumbens by 38 and 34%, respectively, and in the hippocampus by 10% (Table 1). Hence, stable changes in G-6-PDH activity in brain structures of Wistar rats are normally absent, at the same time their variability attests to plasticity of the studied parameters depending of a number of factors not related to locomotor function of animals, *e.g.*, with seasonal fluctuations in metabolism. It can be hypothesized that rats with high locomotor activity are characterized by more pronounced variability of parameters of the studied brain structures compared to rats with low locomotion, which can contribute to individual and topological characteristics of CNS metabolism in these animals.

In series I, G-6-PDH activity was higher in layers III and V of the sensorimotor cortex, in the caudate nucleus, and nucleus accumbens in Wistar rats with low locomotion compared to rats with high locomotion (Table 1). In series II, G-6-PDH activity in layer III neurons of the sensorimotor cortex was similar in rats with high and low locomotion, but in layer V neurons (primarily efferent neurons) of the sensorimotor cortex it was considerably higher (by 9.7%) in rats with low locomotor activity. G-6-PDH activity in neurons of the caudate nucleus (dorsal part of the striatum) and nucleus accumbens (ventral part of the striatum) was similar in rats with high and low locomotion. There were no differences in G-6-PDH activity in CA3 hippocampal neurons in series I and II. These results attest to negative correlation between locomotor activity of Wistar rats and G-6-PDH activity in layer V (efferent) neurons of the sensorimotor cortex (higher enzyme activity in rats with low locomotion). G-6-PDH activity in associative layer III neurons of the sensorimotor cortex and neurons of the caudate nucleus and nucleus accumbens varies, but on the whole it was higher in rats with low locomotion, because in these animals, the demands in phosphopyridine nucleotide regeneration and pentose formation are higher than in rats with

TABLE 1. Cytochemically Determined Activity of G-6-PDH in Brain Structures of Wistar Rats Differing by Locomotor Activity in the Open Field Test (*M*±*m*)

Brain structure	Series I			Series II		
	HLA	LLA		HLA	LLA	
		abs.	%		abs.	%
Cortex layer III	0.715±0.009	1.034±0.009*	144.6	0.564±0.005	0.571±0.004	101.2
layer V	0.793±0.009	1.115±0.011*	140.6	0.673±0.004	0.738±0.005*	109.7
Caudate nucleus	0.544±0.007	0.947±0.008*	174.1	0.556±0.004	0.585±0.004	105.2
Nucleus accumbens	0.480±0.006	00.916±0.007*	190.8	0.581±0.005	0.604±0.004	104
Hippocampus	0.915±0.011	0.916±0.013	100	0.813±0.014	0.826±0.011	101.6

Note. The data are presented in optical density units at λ=589 nm. 100% corresponds to enzyme activity in rats with high locomotion; enzyme activity in rats with low locomotion was compared to this level. HLA and LLA are high and low locomotor activities, respectively. *$p<0.05$ compared to rats with HLA (according to one-way ANOVA).

high locomotion, which probably can determine more severe course of brain ischemia detected by the open field test [1]. We revealed no correlation between enzyme activity in the hippocampus and locomotion in these rats in both experimental series.

The protective mechanisms promoting survival of the organism under conditions of hypoxia and other types of stress include activation of the synthesis of proteins and nucleic acids in systems experiencing maximum load during adaptation to the damaging factor [3]. Overstrain of these mechanisms leads to failure of individual resistance. A product of the reaction catalyzed by G-6-PDH participates in the synthesis of a universal regulatory factor, NO, involved in these mechanisms.

Thus, peculiarities of oxidative metabolism in the pentose phosphate shunt in some structures of the brain involved into the formation and realization of targeted behavior were revealed in Wistar rats differing by locomotor activity in the open filed test. A negative correlation was revealed between locomotor activity of Wistar rats in the open field test and activity of G-6-PDH in brain neurons, especially in layer V neurons of the sensorimotor cortex, which probably attests to the existence of some differences in the regeneration of phosphopyridine nucleotides ($NADP^+$) and formation of pentoses via the pentose phosphate shunt in their brain. It can be concluded that the initial state of oxidative processes, in particular, pentose phosphate shunt, should be taken into account when describing brain reaction to ischemia.

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