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Possible Involvement of Lactate in Neuroglial Interaction through Nicotinic Cholinergic Synapses in the Cranial Cervical Sympathetic Ganglion

P. L. Gorelikov and S. V. Savelyev

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Activities of LDH and its H- and M-isoforms in neurons and satellite gliocytes of the cranial cervical sympathetic ganglion in rabbits under normal conditions and during nicotinic cholinergic synapse blockade were evaluated by integral cytophotometry in tissue sections. Normally activity of H-isoform predominates in neurons and M-isoform in satellite gliocytes. Blockade of the cranial cervical sympathetic ganglion significantly decreased LDH activity (H- and M-isoforms) in neurons in direct proportion to the number of blocked nicotinic cholinergic receptors. Activity of M-isoform in satellite gliocytes decreased with increasing the degree of blockade, while activity of H-isoform did not change. The isoenzyme profile of LDH in satellite gliocytes reached the level of intact neurons. Presumably, lactate production in satellite gliocytes is regulated by sympathetic neurons through nicotinic cholinergic synapses.

Key Words: *blockade; nicotinic cholinergic synapses; lactate dehydrogenase; neuron-glia; ganglion*

Among numerous aspects of the neuron-glia cooperation [7,9] the so-called astrocyte-neuron lactate shuttle hypothesis (ANLSH) presenting a new viewpoint on the mechanisms of energy supply to the brain [13-15] attracts special interest. According to this hypothesis, astrocytes play the major role in compensation for neuronal energy expenditures; these cells deliver lactate as the energy substrate to neurons through the gliosynaptic contacts. Lactate supply is regulated by synaptic activity of neurons. This explanation of the cerebral energy supply mechanism often reflects the assumptions of the concept [6,8] on the universal role of lactate as an additional energy source for many tissues and organs. According to ANLSH, the role of the neuroglial complex and CNS synapses is exceptional. On the other hand, it is important to investigate the possible existence of a similar mechanism for energy homeostasis maintenance in the system of nicotinic cholinergic synapses and satellite gliocytes of the peripheral nervous system, differing from and similar to the corresponding CNS structures by many features [3,9]. A possible method for this evaluation is an experimental model of dosed synaptic blockade with nicotinic cholinergic receptor antagonists and analysis of LDH isoenzymes regulating various processes of lactate transformation in energy metabolism [2,8] under these conditions.

Activities of LDH and its H- and M-isoforms in sympathetic neurons and adjacent satellite gliocytes in the cranial cervical sympathetic ganglion (CCSG) were evaluated under conditions of partial

Institute of Human Morphology, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** petr_gorelikov@mail.ru. P. L. Gorelikov

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and maximum blockade of nicotinic cholinergic transmission.

MATERIALS AND METHODS

Experiments were carried out on 9 adult Chinchilla rabbits (3 animals per experimental series). CCSG blockade was induced by nicotinic receptor antagonist dimecolin. The drug was injected subcutaneously with consideration for its pharmacodynamics in rabbits [4] in doses of 10 and 50 mg/kg, inducing partial (series I) and maximum (series II) blockade of synaptic transmission, respectively. The material for the analysis was collected during the period of maximum manifestation of blocking effect (1 h postinjection). Activities of LDH, its H- and M-isoforms were evaluated in neurons and adjacent satellite gliocytes by integral cytophotometry [1] on a MIF-1 cytophotometer on cryostat sections after histochemical staining with NBT [2] allowing quantitative evaluation of the enzyme. Only glial cells located no farther from the neuron perikaryon than the longest diameter of the glial cell nucleus, were analyzed. In each experimental and control series, 150 neurons and 210-300 satellite gliocytes were analyzed. LDH isoenzyme profile (H/M) was determined as the ratio of H- to M-isoform activities. Significance of differences was evaluated using Student's t test; the differences were considered significant at $p \leq 0.05$. Approximation of the data was carried out by the method of least squares.

RESULTS

The neurons and gliocytes in intact ganglion are characterized by different LDH profiles (Fig. 1). Activity of H-isoform predominated in neurons (H/M=1.38), while M-isoform predominated in satellite gliocytes (H/M=0.69). H-isoform is involved in lactate utilization, while M-isoform in its accumulation, and hence, CCSG was assumed to possess a gradient of lactate distribution between the sympathetic neurons and the adjacent satellite gliocytes (an obligatory condition for realization of intercellular lactate transport) [6,8]. The same differences in isoenzyme distribution of LDH were detected for cerebral neurons and astrocytes [2,5,12], which supports ANLSH [13-15].

Blockade of nicotinic cholinergic transmission significantly ($p<0.05$) decreased activity of LDH and its H- and M-isoforms in neurons (Fig. 2, *a*). These changes increased with increasing the degree of blockade (number of blocked nicotinic cholinergic receptors) and were statistically significantly approximated (0.99 correlation in all cases) for LDH, H- and M-isoforms, respectively: y=48.73-12.95x, y=39.25-8.77x, and y=28.11-6.73x, where y is enzyme activity (arb. units), x is the factor, corresponding to the number of nicotinic cholinergic receptors not occupied by the ganglionic blocker in the control (x=1), in partial (x=2) and maximum (x=3) blockade. These regularities indicate that the formation of LDH system parameters directly depends on functional activity of the nicotinic cholinergic receptor system of intact sympathetic neurons. Activity of M-isoform decreased greater than of H-isoform, due to which the H/M ratio in the neurons in partial and maximum blockade was higher (1.61 and 1.51, respectively) than in the control (1.38; $p<0.05$; Fig. 1). It seems that the processes of lactate transformation into pyruvate were activated in neurons during blockade against the background of total reduction of LDH activities.

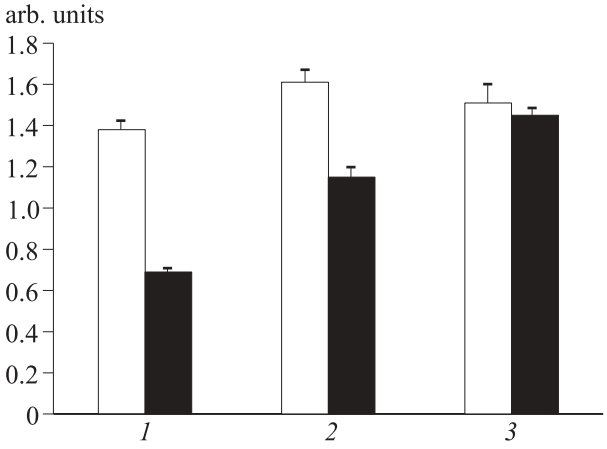

Fig. 1. Ratio of active H and M isoenzymes in sympathetic neurons (light bars) and satellite gliocytes (dark bars) in the control (*1*), in partial (*2*) and maximum (*3*) blockade.

For satellite gliocytes adjacent to the neurons blockade (Fig. 2, *b*) led to most pronounced changes only for M-form (similarly as in neurons), its activity decreasing significantly ($p<0.05$) as the blockade effect augmented (Fig. 2, *b*), while activity of H-form did not change ($p>0.05$). A significant decrease in total LDH activity was observed only in maximum blockade and seemed to be due to changes in the M-form activity. The H/M ratio in gliocytes shifted towards the neuronal (Fig. 1). It was more than one in partial blockade (1.15) and statistically did not differ from the neuronal in maximum blockade (1.45). Hence, the production of intracellular lactate in satellite gliocytes notably decreased as a result of nicotinic cholinergic blockade of synaptic transmission, and the LDH system in general started functioning similarly as in intact neurons. It seems that lactate production process in satellite gliocytes is regulated by the sympathetic neurons through the nicotinic cholinergic synapses.

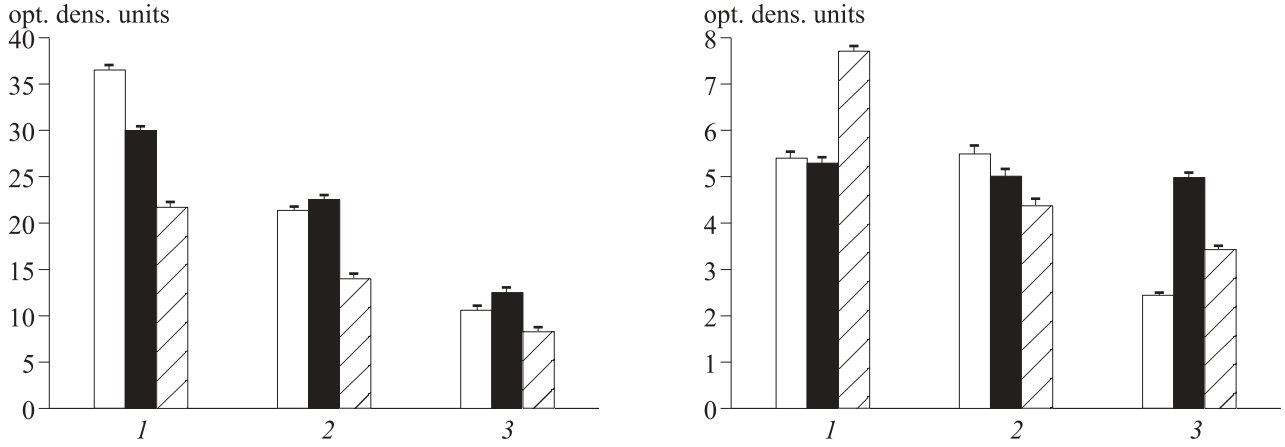

Fig. 2. Time course of total LDH activity (light bars), isoenzymes H (dark bars) and M (cross-hatched bars) during synaptic blockade in sympathetic neurons (*a*) and satellite gliocytes (*b*). *1*) control; *2*) partial blockade; *3*) maximum blockade.

The presence of neuron-gliocyte gradient in lactate production under normal conditions and its subsequent characteristic alteration caused by suppression of nicotinic cholinergic synaptic activity suggests that satellite gliocytes, similarly as astrocytes for the CNS neurons, can serve as lactate sources for CCSG sympathetic neurons in the presence of actively functioning nicotinic cholinergic synapses. The data indicating that lactate (but not glucose) is primarily utilized as energy substrate in stimulation of another sympathetic (lumbar) ganglion and lactate is completely oxidized during this process [10,11] indirectly confirms this hypothesis.

It is highly possible that the sympathetic ganglia possess the mechanism of energy homeostasis described by the ANLSH.

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