EFFECT OF AUDIOGENIC SEIZURES ON ACTIVITY OF H AND M FORMS OF LACTATE DEHYDROGENASE IN NEURONS AND NEUROGLIA IN VARIOUS PARTS OF THE CENTRAL NERVOUS SYSTEM

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Cytospectrophotometric analysis showed that the ratio between activities of aerobic H forms of lactate dehydrogenase (LD) and the activity of its anaerobic M forms in cerebral cortical neurons and cerebellar Purkinje cells and also in their glial satellite cells of rats of the Krushinskii–Molodkina and Wistar strains is higher than in motoneurons in the anterior horns of the spinal cord and their perineuronal glia. In Krushinskii–Molodkina rats (with genetically determined high sensitivity to audiogenic seizures) epileptiform audiogenic seizures were accompanied by marked activation of both H and M forms of LD in the cortical neurons but by absence of changes in the perineuronal glia. In the spinal motoneurons, on the other hand, the activity of all forms of LD was unchanged, whereas in the neuroglial cells surrounding these neurons activity of the H forms of LD was definitely increased. During the seizures an increase in activity of M forms of LD was found in the cerebellar Purkinje cells but a decrease in their activity in the glial satellite cells.

KEY WORDS: neuron; neuroglia; audiogenic seizures; lactate dehydrogenase isozymes.

The object of the present investigation was to determine the effect of audiogenic seizures on the ratio between the basic forms of lactate dehydrogenase (LD) in various neuron–neuroglia systems, using a quantitative cytochemical method.

EXPERIMENTAL METHOD

Epileptiform fits, the severity of which was assessed by means of Krushinskii's 4-point scale [6], were induced in adult male rats of the Krushinskii–Molodkina strain with genetically determined increased sensitivity to audiogenic seizures, by exposure of the animals for 2 min to sound with an intensity of 96 dB. The animals were quickly decapitated immediately after the end of acoustic stimulation, at the height of the seizures. The motor cortex, the cerebellar cortex, and the lumbar enlargement of the spinal cord were taken for analysis. Rats of the same strain, not exposed to sound, acted as the control. Male Wistar rats of the same weight also were used for comparison.

The activity of LD isozymes migrating quickly and slowly during electrophoresis (predominantly H and M forms of LD respectively) in the neurons and neuroglial cells surrounding them was determined by Brody's cytochemical method [8, 9] in Gerebtzoff's modification [10]. The reaction was carried out with substrate (0.1 M sodium lactate) in the presence of NAD (0.01 M) with tris-HCl buffer, pH 7.4, on cryostat sections cut at −26 to −28°C; tetranitro-BT, dissolved in dimethylsulfoxide, was used as the stain. To detect H forms of LD, urea (2.61 M) was added to the medium; to detect M forms the substrate was added in an excessive concentration (0.72 M), The optical density of the diformazan (end product of the LD reaction

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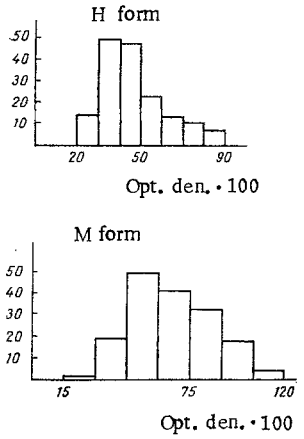

Fig. 1

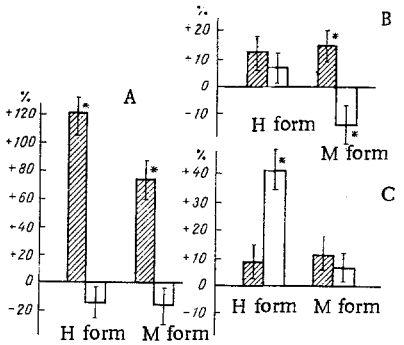

Fig. 2

Fig. 1. Distribution of H and M forms of LD in rat spinal motoneurons. Abscissa, activity of forms of LD (in optical density units); ordinate, number of cells in each range.

Fig. 2. Changes in activity of H and M forms of LD in neurons and perineuronal neuroglia of different parts of the nervous system in Krushinskii–Molodkina rats under the influence of audiogenic seizures: A) cerebral cortex (layers II-III of the motor area); B) cerebellar cortex (layer of Purkinje cells); C) spinal cord (anterior horns). Shaded columns represent neurons, unshaded columns glial satellite-cells. Vertical lines indicate standard error. Deviations significantly ($P < 0.05$) different from the control are marked by asterisks. Ordinate, deviations (in %) from corresponding LD activity in Krushinskii–Molodkina rats in a state of relative rest.

for tetrazolium) was measured on the MUF-5 double-beam recording cytospectrophotometer [1] at 560 nm. Preliminary trials showed that the cytochemical reaction for LD satisfied the Bouguer–Lambert–Bier law, and it was therefore possible to carry out the cytospectrophotometric determination of all components of LD in separate cells [2, 4]. The intensity of the color reaction for LD was regarded as a quantitative indicator of the enzyme activity, as judged by the total quantity of diformazan (in relative units) calculated per cell, allowing for the volumes of the cells determined by measuring their linear dimensions under the microscope with the MOV-1-15 ocular micrometer. Each mean value was found after investigation of a group of eight rats and 20-25 neurons and neuroglial cells in the immediate vicinity of the bodies of the corresponding neurons were measured in each animal. All calculations and statistical analysis by Student's method were carried out with the Minsk-22 digital computer.

EXPERIMENTAL RESULTS AND DISCUSSION

The results of photometry indicated substantial individual differences between the cells of each type. However, analysis of histograms of the H and M forms of LD in the motoneurons (the most heterogeneous cell structures of the CNS) points to a monomodal, near-normal distribution (Fig. 1). This meant that later all that was necessary was to compare the mean values and their standard errors.

Neurons of layers II-III of the motor area were investigated in the cerebral cortex, Purkinje cells in the cerebellar cortex, and anterior horn motoneurons in the spinal cord (in each case, together with cells of the perineuronal glia), for previous investigations had shown (by analysis of RNA and proteins) the high functional lability of metabolism of these cell structures [3, 7].

Interlinear, regional, and intercellular differences in the ratio between H and M forms of LD are shown in Table 1.

Audiogenic seizures led to a sharp increase in activity of both H and M forms of LD in the cortical motoneurons, in the absence of any changes in the surrounding neuroglia (Fig. 2). A very small increase in activity of M forms of LD was found in the Purkinje cells and a decrease in the glia. No changes could be found in the spinal motoneurons, but in their glia the H forms of LD were appreciably activated (Fig. 2).

TABLE 1. Ratio between Activity of H Forms and Activity of M Forms of LD in Neurons and Their Glial Satellite Cells in Different Parts of the Rat Nervous System (M±m)

Part of nervous system and rats	Ratio	
	neurons	neuroglia
Cerebral cortex (layers II-III of motor area)		
Wistar	0,66±0,04	0,69±0,03
Krushinskii–Molodkina strain	0,74±0,04	0,70±0,03
Cerebellar cortex (layer of Purkinje cells)		
Wistar	0,61±0,03	0,61±0,03
Krushinskii–Molodkina strain	0,85±0,05	0,75±0,04
Spinal cord (anterior horn cells of lumbar enlargement)		
Wistar	0,47±0,03	0,49±0,03
Krushinskii–Molodkina strain	0,55±0,04	0,52±0,03

Compared with Wistar rats, in Krushinskii–Molodkina rats direct correlation has been described between the level of predisposition to seizures and the overall mobility of the nervous processes, their imbalance, and the more rapid production and modification of conditioned reflexes [5, 6]. The higher mobility of the nervous processes (connected in particular with the more effective learning in rats of the Krushinskii–Molodkina strain) requires greater expenditure of energy by the nervous structures of these animals. The explanation of this fact could be that the phylogenetically younger, higher levels of the CNS possess, according to the writers' observations, higher activity of aerobic forms of LD, which catalyze phylogenetically more promising and energetically more effective aerobic reactions aimed at stockpiling high-energy substances.

Marked changes in LD activity in the present experiments were found as early as 2 min after the beginning of acoustic stimulation. This result must certainly have been due to short-term regulation of isozyme activity rather than to changes in the rate of protein synthesis. This short-term adjustment of the LD system in nerve tissue, to judge from the results described above, takes place during audiogenic seizures mainly in neurons of higher, phylogenetically younger levels of the CNS.

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