CHANGES IN GLUTAMATE DEHYDROGENASE ACTIVITY IN GANGLION NODOSUM NEURONS IN RABBITS WITH ACUTE EXPERIMENTAL EMOTIONAL STRESS

V. D. Goncharuk

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KEY WORDS: ganglion nodosium; neuron; glutamate dehydrogenase.

The results of numerous investigations have shown that an increase in functional activity of neurons is connected with regular changes in the activity of the enzymes of energy metabolism and in the concentrations of proteins, nucleotides, and other biologically active substances [1, 7]. Under these circumstances changes in chemical activity of intensively functioning nerve cells are combined with a change in their volume [2] and by an increase in the number of perineuronal satellites around them.

In rabbits predisposed to develop stress [6] the level of water-soluble proteins has been found to be lowered in neurons of the ganglion nodosum during the action of a stress-inducing factor [3].

There is extremely little information in the literature on the energy metabolism of nerve cells of ganglia of the autonomic nervous system. One of the few investigations [4] showed that during electrical stimulation of preganglionic nerves or during interoceptive stimulation, triggering the mechanism of a peripheral reflex, the concentration of high-energy compounds in some sympathetic ganglia undergoes significant changes. In the same investigation, during electrical stimulation of the preganglionic nerve to the perfused superior cervical ganglion of the cat, a considerable fall in the ATP concentration was observed even during brief stimulation if glucose was omitted from the nutrient solution.

In connection with the foregoing facts, the study of the activity of glutamate dehydrogenase (GDH), one of the most important mitochondrial enzymes, has become exceedingly interesting, for this enzyme participates in various conversions of glutamic acid and is responsible for linking carbohydrate metabolism, on the one hand, with nitrogen, amino acid, and protein metabolism, on the other hand [8].

EXPERIMENTAL METHOD

Neurons of the ganglion nodosum of the vagus nerve of rabbits predisposed to develop stress were used as the test object. Material for investigation was obtained in the Laboratory of Physiology of Emotions and Emotional Stresses (Director, Corresponding Member of the Academy of Medical Sciences of the USSR Professor K. V. Sudakov). Acute emotional stress was induced in immobilized rabbits by simultaneous stimulation of the ventromedial hypothalamic nuclei and the hind limb, using a specially developed stochastic scheme. Experiments were carried out on 12 animals.

GDH activity was determined by a histochemical method with nitro-BT, which gives deposits of formazan at sites of activity of this enzyme (a modified method of Rubinstein, 1962). Sodium glutamate was used as the substrate.

Sections 20 μ thick were cut on a Cryocut freezing microtome, incubated in medium for 20 min at 37°C, fixed in neutral formalin, dehydrated in a series of alcohols of increasing concentration, and mounted in Canada balsam. For greater reliability of the results, ganglia of experimental and control animals were processed simultaneously under absolutely identical conditions on the same slide.

B. I. Lavrent'ev Laboratory of Neurohistology, P. K. Anokhin Institute of Normal and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 10, pp. 494-496, October, 1981. Original article submitted April 10, 1981.

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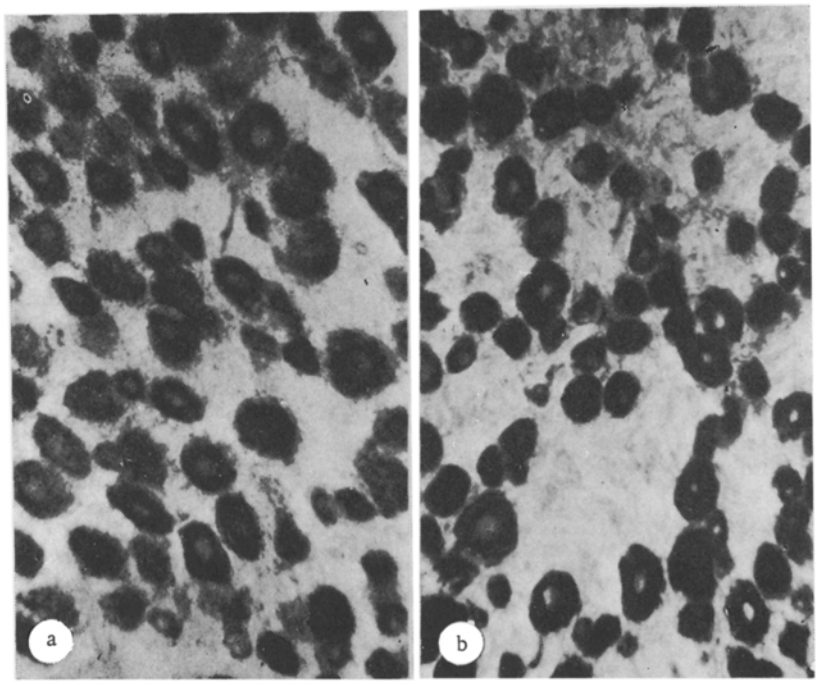

Fig. 1. Fragment of ganglion nodosum after staining reaction for GDH. a) Control: greatest activity in large cells, less in medium-sized, and least in small cells; activity of glial cells weak; b) experiment: enzyme activity rises and becomes about equal in all cells, neurons somewhat reduced in size, activity of glial cells increased. Fixation in neutral formalin. Objective OPA-1, photographic ocular 10 × P.

Some sections were fixed in Carnoy's fluid, stained with cresyl violet by Nissl's method, and used as the morphological control.

The optical density of the cytoplasm, depending on the quantity of precipitated reaction product, was measured with a Lyumam microscope equipped with a light probe.

GDH activity, equal to the density of formazan deposition in the sections, was expressed in relative units and calculated by the equation:

$$E = \frac{F_0}{F_0},$$

where F_0 and F denote the photic flux incident on and transmitted through the preparation.

Altogether 720 cells were studied. Only those cells in which the section passed through the middle of the nucleus were examined; cell ghosts and small fragments were disregarded.

The numerical results were subjected to statistical analysis by Student's t test on the Nairi-K computer.

EXPERIMENTAL RESULTS

Nerve cells of the ganglion nodosum of various sizes in the control animals differed in their density of deposition of the reaction product. Maximal enzyme activity was observed in large cells, rather less in medium-sized cells, and less still in small cells. The large cells measured 30 μ or more in diameter, the medium-sized cells 20-30 μ, and the small cells under 20 μ. GDH activity in the glial cells was low (Fig. 1a).

Some decrease in size of the nerve cell bodies was observed in the experimental material, accompanied by an increase in GDH activity, which became about equal in all neurons; the number of active glial cells also increased (Fig. 1b).

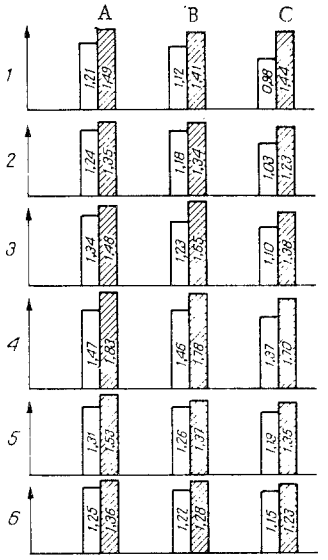

Fig. 2. Histograms of distribution of GDH activity depending on size of neurons for each of six experiments separately. Ordinate, enzyme activity (in conventional units). A) Large cells; B) medium-sized cells; C) small cells. Unshaded columns — control, shaded columns — stress. Absolute values of activity may be influenced by reaction conditions, but differences within the limits of each experiment can be judged with a high degree of reliability — 99.9% by Student's t test.

Histograms of distribution of GDH activity depending on size of the cells, based on the results of six experiments, reveal individual differences between the control animals. However, the general tendency is maintained: in the experimental series GDH activity was highest in the medium-sized and small cells (Fig. 2).

Evidently, during stress, nerve cells of the ganglion nodosum begin to operate at a higher, possibly maximal, level of energy expenditure. Several amino acids become involved in the tricarboxylic acid cycle with the aid of GDH, leading to decomposition to carbon dioxide and water. It can be tentatively suggested that under the influence of an extreme stimulus increased breakdown of amino acids is observed, with liberation of the necessary energy, and as a result, in animals predisposed to developing stress, the level of water-soluble proteins is lowered.

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MORPHOMETRIC DETECTION OF SPECIALIZED INTERNODAL CONDUCTING PATHWAYS OF THE HEART

E. R. Pavlovich and I. A. Chervova

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KEY WORDS: specialized internodal pathways of the heart; morphometry.

Three basic hypotheses are constantly discussed in the literature on conduction of the impulse from the cardiac pacemaker — the sinus node (SN) to the atrioventricular node (AVN): 1) There are no specific conduction pathways in the atria and the impulse spreads over the whole working myocardium [1, 8]; 2) selective conduction pathways exist, i.e., pathways spreading the impulse more rapidly than the surrounding myocardium but without any specialized morphological substrate [7, 11-13]; 3) specialized pathways exist, i.e., pathways differing in structure from the working myocardium and conducting the impulse more rapidly than the surrounding myocardium of the right atrium (RA) [4-6, 9, 10]. It is difficult to give an unequivocal answer to this question because of the absence of a common nomenclature in investigations at different structural levels of organization (anatomy, histology, cytology) [5, 9, 10], and also the absence of morphofunctional correlations [4, 7, 13]. The technical difficulties encountered in previous investigations resulted from the study of hearts of relatively large animals: rabbit, monkey, dog, man [6, 9, 12], in which it is difficult to detect components of the intracardiac conduction system because of constraints imposed by the methods of light and electron microscopy and microelectrode techniques for such material. Furthermore, accurate quantitative methods have not been adequately used in the search for specialized conduction pathways [6] and most workers have contented themselves with qualitative, descriptive and, consequently, subjective criteria of identification.

The object of this investigation was to attempt to obtain quantitative morphological evidence in support of the existence of specialized conduction pathways in RA of the rat heart, using morphometric methods.

EXPERIMENTAL METHOD

Mature noninbred male rats weighing 250-300 g were used. The animals were anesthetized by intraperitoneal injection of pentobarbital (0.05 mg/g body weight) after which the heart was perfused with 2.5% glutaraldehyde in phosphate buffer (pH 7.4). Samples were taken from the region of the superior vena cava with the adjacent myocardium of RA and, after standard processing for electron microscopy, they were embedded in one block. Tissues of SN and AVN were identified among the working myocardium in semithin sections [3]. Quantitative data on the tissue composition of the regions chosen for study were obtained by the dot method from negatives [2], with a final magnification of 7500 (the area of one field of vision was 640 μ^2). The material was subjected to statistical analysis and differences were evaluated by Student's t test.

EXPERIMENTAL RESULTS

Quantitative analysis of regions of SN and the adjacent working myocardium of RA revealed a significant difference ($P < 0.001$) in the content of muscle, connective-tissue, and nerve cells in these regions, but differences in the relative proportions of vascular struc-

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