CHANGES IN THE LACTATE DEHYDROGENASE ISOZYME SPECTRUM IN EPILEPTIC FOCI INDUCED BY PENICILLIN IN THE RAT CEREBRAL CORTEX

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A powerful focus of epileptic activity in the cerebral cortex has been shown [4, 5] to perform the function of a determinant [4], which determines the character of activity of other foci and unites them into an epileptic complex, which can be used as a model of a pathological epileptic system. The simplest form of such a system is a complex consisting of primary (determinant) and secondary (mirror) foci. The study of the coupled changes in enzyme activity in the determinant and mirror foci is interesting in order to explain the mechanisms of epileptogenesis and, in particular, to determine the specific importance of the action of epileptogens (primary focus) and of synaptic activation (mirror focus).

The aim of this investigation was to study the isozyme spectrum of lactate dehydrogenase (LDH), which plays an important role in the energy supply of the nerve cell, including during seizures [1] in determinant and mirror epileptogenic foci.

EXPERIMENTAL METHOD

Experiments were carried out on 50 noninbred albino rats of both sexes weighing 180-200 g. A focus of epileptic activity in the region of the sensomotor cortex of the left hemisphere was created by application of a piece of filter paper measuring 2 × 3 mm, soaked in penicillin solution (sodium salt, 15,000 I.U./ml physiological saline). A piece of filter paper soaked in physiological saline was applied to the surface of the right cerebral cortex. Global electrical activity was recorded in the region of the sensomotor cortex of both hemispheres by means of cotton wick electrodes soaked in physiological saline, on a 4ÉÉG-3 electroencephalograph. The animals were then decapitated and the brain was removed for histological or biochemical study of LDH isozymes. Five LDH isozymes were detected in the tissues (LDH_1, LDH_2, LDH_3, LDH_4, and LDH_5) by the method in [3], and its H- and M-forms were found by the method in [8]. The H-form of LDH (LDH-H) is known to correspond to the rapidly migrating fractions of the enzyme (LDH_1, LDH_2), whereas the M-form (LDH-M) corresponds to slowly migrating fractions (LDH_5, LDH_4, LDH_3).

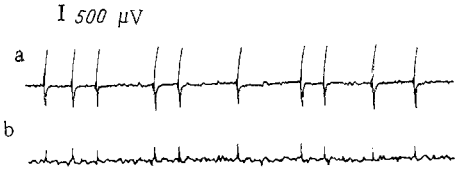

Fig. 1. Seizure activity in response to penicillin application to sensomotor cortex: a) left, b) right hemisphere.

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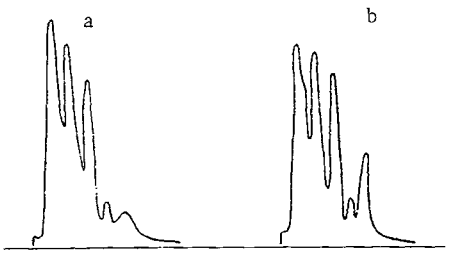

Fig. 2. LDH isozyme spectrum of rat sensomotor cortex: a) control, b) determinant epileptic focus.

TABLE 1. LDH Isozyme Spectrum in Rat Brain during Creation of Epileptic Focus by Application of Penicillin to Sensomotor Cortex (in % of total activity, M ± m)

Isozyme	Intact rats		Experimental rats			
	sensomotor cortex		epileptogenic focus in sensomotor cortex		system of two epileptogenic foci in sensomotor cortex	
	right hemisphere (n = 5)	left hemisphere (n = 5)	determinant focus (n = 6)	symmetrical focus (n = 6)	determinant focus (n = 6)	mirror focus (n = 6)
LDH_1	39,2±3,46	37,0±1,40	30,6±1,65*	33,1±3,04	36,6±2,94	31,8±2,36
LDH_2	28,8±1,74	23,4±1,7	26,9±1,33	33,4±1,92	30,3±1,95*	28,8±2,52
LDH_3	17,0±2,42	25,5±3,5	21,6±2,78	15,6±1,51	14,0±2,21*	18,8±1,34
LDH_4	12,5±2,22	11,8±2,95	14,7±1,58	14,5±1,29	14,0±2,25	18,6±1,30
LDH_5	2,4±0,57	2,4±0,33	7,4±1,30*	2,8±0,88	5,8±1,24*	4,6±0,77*

*P < 0.05 compared with control.

EXPERIMENTAL RESULTS

After application of penicillin to the surface of the sensomotor cortex of the left hemisphere, epileptiform discharges (200-300 μV) began to appear within 3-5 min in the zone of application. Spike potentials reached their maximal amplitude 2-4 min after the beginning of the discharges. Synchronously with them epileptic potentials also appeared in the right hemisphere, but their amplitude was much less (Fig. 1). Changes in the ECoG and LDH isozyme activity were studied in the primary and mirror foci of the sensomotor cortex in the period preceding appearance of the mirror focus, and on synchronization of electrical activity in the two foci.

LDH_1 had the highest activity in the cerebral cortex of the intact animals, activity of LDH_2 and LDH_3 was a little lower, and activity of LDH_4 and, in particular, of LDH_5 was considerably weaker (Table 1). Histochemical investigation showed that LDH-H was much more active than LDH-M. Spatial dissociation also was found between H- and M-subunits of the enzyme in different structures of brain tissue. Activity of LDH-H was highest in the neuron bodies and was much lower in the neuropil. LDH-M, on the other hand, exhibited high activity in the neuropil but comparatively average activity in neuron bodies.

A change in the LDH isozyme spectrum, consisting of a significant decrease in LDH_1 activity in the tissue, very small fluctuations in the relative percentages of the hybrid forms (LDH_2, LDH_3, LDH_4), and a significant increase (threefold compared with the control) in LDH_5 activity (Fig. 2), was observed in the determinant epileptic focus.

Weakening of LDH-H activity was found in the neurons and an increase in LDH-M activity was observed in cells of the oligodendroglia. In layers I-II of the cortex there were no significant changes compared with the control animals, but in layers III, V, and VI neurons with high LDH-M activity appeared, and they were particularly numerous in layers V and VI. These neurons were found in the focus as groups numbering from three to five cells. Their bodies and nuclei appeared compressed from the sides and they were considerably elongated. The effect of lengthening of these neurons was due to precipitation of formazan (the marker of the location of the enzyme) in axon hillocks and in apical dendrites, and in the latter, moreover, at considerable distances from the soma. In the neuropil no significant changes

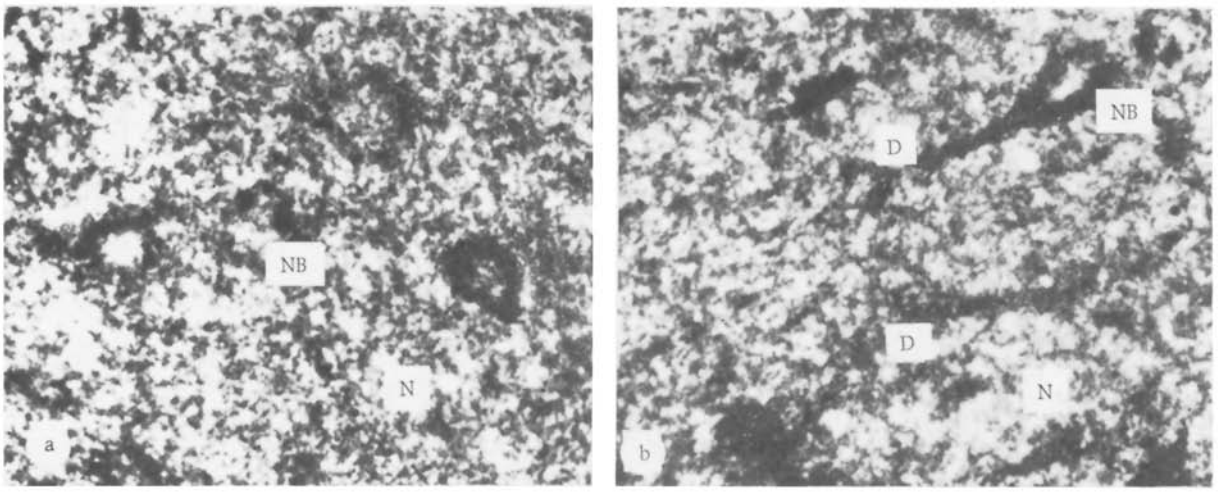

Fig. 3. Sensomotor cortex of control (a) and experimental rats with a determinant epileptogenic focus (b). Formazan in neuron bodies (NB), apical dendrites (D), and neuropil (N) of layer V. Reaction for LDH. 280 ×.

were observed compared with the control (Fig. 3). The microscopic changes described in the focus were diffuse in character; no visible differences were present between the central and peripheral regions of the focus.

Investigation of the symmetrical area of the cortex showed that no significant changes in the LDH isozyme spectrum occurred in it, compared with that in intact animals, in the period preceding formation of the mirror focus. Enzyme changes were usually found after formation of the mirror focus: The content of LDH_1 was slightly reduced and, because of this, there was a small increase in LDH_4 activity, whereas activity of LDH_5 rose to almost twice the control level.

Comparison of LDH-H activity in the mirror and determinant foci showed that the histochemical changes in them were similar in character. Similar patterns also were observed when LDH-M were studied. Changes in the LDH spectrum arising in the determinant focus in the period of synchronization of electrical activity in the two foci deserve attention. They were manifested as normalization of LDH_1 activity, a significant increase in the relative percentage of LDH_2, and a significant decrease in the LDH_3 level compared with the control. LDH_5 activity was almost 2.5 times higher than the corresponding levels in intact animals.

In the modern view H-subunits of the enzyme are responsible to a greater degree for the oxidation of accumulated lactate into pyruvic acid, and they thus stimulate the initial stages of aerobic conversion of carbohydrates. The M-subunits of LDH catalyze chiefly the final stage of anaerobic oxidation of carbohydrates, namely reduction of pyruvate, which leads to accumulation of lactic acid [2]. According to data in the literature, during electrical stimulation of the cortex [9], and also during the formation of a cobalt [7] or penicillin epileptic focus [6] the lactic acid concentration in the brain tissues rises.

During the formation of epileptic foci the predominant LDH_1 fraction of the enzyme thus decreases in activity whereas activity of LDH_5 rises considerably. This fact points to intensification of anaerobic processes. Histochemical data on the increase in LDH-M activity coupled with a decrease in the LDH-H content are in good agreement with the biochemical data and support them. For instance, the most marked changes in enzymic activity take place in efferent layers of the cortex (V-VI); these changes, moreover, are found to a greater degree in neuron bodies than in gliocytes. Increased epileptic activity in the focus is evidently coupled with intensification of anaerobic processes in both nerve and glial cells.

Studies of the symmetrical area of the cortex of the contralateral hemisphere showed that during the formation of a dependent, mirror focus, just as in the determinant focus anaerobic processes in it are intensified. The presence of this similarity in the changes in electrical activity and isozyme distribution in the determinant and mirror foci indicate the formation of a single system with similar electrophysiological and enzymic changes.

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EFFECT OF THYMALIN AND HETEROLOGOUS TRANSFUSION ON BLOOD CLOTTING AND FIBRINOLYSIS IN THYMECTOMIZED RATS

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The writers showed previously that in thymectomized rats 10 min after transfusion with heterologous (human) blood hypocoagulation develops rather more intensively but fibrinolysis is activated to a lesser degree [13, 14]. These findings can be explained to some extent on the grounds that after removal of the thymus in rats destruction of heterologous erythrocytes takes place less intensively and, consequently, the process of intravascular blood clotting, which arises in heterologous transfusion shock, follows a milder course [7, 8, 13]. A low-molecular-weight factor, with a modulating action on the state of cellular immunity, has been isolated from the thymus [9, 10]. This compound, to which the name "thymalin" has been given, subsequently has achieved wide application in experimental and clinical medicine [2, 5, 10]. It has been shown, in particular, that thymalin restores adequate responses of the hemostasis system in thymectomized animals to adrenalin [5], thrombin [3], and histamine [4].

In the investigation described below the state of the blood clotting system and of fibrinolysis was studied in thymectomized animals with heterologous transfusion shock treated beforehand with thymalin.

EXPERIMENTAL METHOD

Experiments were carried out on 46 rats: 28 experimental (thymectomized at the age of 1.5 months) and 18 control. All the experiments were carried out 3 months after removal of the thymus, when a frank deficiency of cellular immunity had developed.

For a period of 1 week 10 thymectomized rats were given intramuscular injections of 1 mg thyamlin (Lenmyasoprom Medical Preparations Factory) in 0.5% procaine solution intramuscularly in a dose of 1 mg. The control animals, both intact and thymectomized, received a corresponding dose of procaine. The jugular vein was exposed in all the animals under superficial ether-halothane anesthesia and 2-3 ml blood was withdrawn into 0.2-0.3 ml of 3.8% sodium citrate solution. The intact animals (control) and thymectomized rats not receiving (experiment 1) and receiving (experiment 2) thymalin, were all given an injection

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