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CYTOPHOTOMETRIC STUDY OF CHANGES IN GLUTAMATE DEHYDROGENASE AND GABA TRANSAMINASE IN THE CEREBRAL CORTEX DURING METRAZOL KINDLING

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Kindling is a model of epilepsy created by repeated weak electrical stimulation of brain structures [13-15]. It has been shown that kindling can also be induced by repeated injections of subthreshold doses of various chemical convulsants [7, 10]. The pathogenetic mechanisms of kindling have not been adequately studied. It was decided to investigate enzyme activity of neurons and glia in animals with developed kindling.

The aim of this investigation was to study activity of glutamate dehydrogenase (GDH) and GABA transaminase (GABA-T) in the cerebral cortex of mice during metrazol-induced kindling.

EXPERIMENTAL METHOD

Experiments were carried out on inbred F_1 hybrid mice (CBA × C57BL/6, BAC/C) and on non-inbred albino mice weighing 18-22 g. Each group consisted of at least 15 animals. Metrazol was injected intraperitoneally in a dose of 30 mg/kg daily for three weeks in a volume of 0.1 ml under the same conditions, at the same time of day, in a room with the same intensity of illumination and with the same noise effect. After injection of metrazol the animals were kept in a glass chamber and were under observation for 30-40 min. Behavior seizures were assessed in points, as follows: 0) no seizure; 1) shaking of the head or twitching of individual trunk muscles; 2) repeated clonic spasms of the trunk; 3) clonic spasms of the forelimbs; 4) clonicotonic convulsions with the animal falling on its side, followed by postictal depression; **and 5) repeated** severe tonicoclonic convulsions or lethal convulsions. Animals of the control group received injections of physiological saline under the same conditions. The animals were decapitated 24 h after the last injection of metrazol and the brain was re-

TABLE 1. Enzyme Activity in Sensomotor Cortex of Control Mice and during Kindling (in conventional optical density units, M ± m)

Experimental conditions	Enzyme	Neurons	Glia
Control	GDH	39,45±0,89	22,59±0,59
Experiment		33,41±0,72*	18,03±0,44*
Control	GABA-T	49,47±0,9	35,78±0,65
Experiment		44,27±0,95*	36,99±0,81

Legend. *P < 0.05 compared with control.

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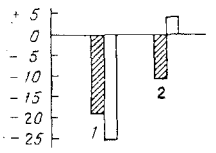

Fig. 1. GDH (1) and GABA-T (2) activity in neurons and neuroglia of sensomotor cortex of mice during kindling. Ordinate, deviations from control level (in %), taken as 100. Shaded columns — neurons; unshaded — neuroglia.

moved and a block of tissue cut from it. Control and experimental material was placed together on the stage of an object holder and frozen in liquid carbon dioxide, after which sections 10 μm thick were cut in a cryostat at −20°C. The sections were mounted on coverslips and incubated in a constant-temperature chamber at 37°C for 15-20 min. Activity of GDH (E.C. 1.4.1.2) was determined histochemically by the method in [3] and GABA-T (E.C. 2.6.1.19) by the method in [12] in the modification in [1]. As a result of these reactions, formazan, a marker of enzyme activity, is formed in the tissues. Photometry of the glial cells of the sensomotor cortex was carried out by a two-wave method on the MTsFV-1 cytophotometer at wavelengths of 550 and 640 μm, with a probe with an area of 0.2 μm^2, enlargement of the objective 20, and on the photometer tube 2.4. Photometry of neurons was carried out under the same conditions with a 10× objective. The results were subjected to statistical analysis of the Élektronika BZ-21 microcomputer [5].

EXPERIMENTAL RESULTS

After repeated injections of subthreshold doses of metrazol the animals developed seizures which progressed from single myoclonic spasms to marked tonicoclonic generalized convulsions. Microscopic study of histochemical preparations of the cerebral cortex of animals of the control and experimental groups showed the presence of formazan granules in various structures of nerve tissue: neurons, gliocytes, and neuropil. In the upper layers (II-IV) the formazan content was greater in the neuropil than in corresponding structures of the underlying layers V-VI. In the latter, by contrast with the upper layers, nerve cells were more active.

Meanwhile, despite the apparent similarity in the distribution of these enzymes in microstructures of nerve tissue, GDH was connected more with the bodies and apical dendrites than GABA-T. The results of the cytophotometric study of microstructures of brain tissues are given in Table 1. They show that GDH and GABA-T activity in the nerve cells of the experimental animals was lower by 18.1 and 11.7%, respectively, than the activity of these enzymes in the control. GDH activity in the glial cells of the experimental animals was reduced by 25.3% compared with the control, whereas GABA-T activity, on the contrary, showed a tendency to rise, and exceeded the control level by 3.4% (Fig. 1). The fall in GDH activity found in these experiments indicates a decrease in the relative contribution of glutamate to the energy supply of nerve and, in particular, glial cells. It has been shown [4] that after a single subcutaneous injection of metrazol in a dose of 60 mg/kg rats develop convulsions; after the eighth convulsion, moreover, as the results of biochemical tests have shown, cortical GDH activity is 19% lower than in the control. In metrazol kindling, incidentally, GDH activity in the neurons, according to the results of cytophotometry, also was reduced by 18%. It is an interesting fact that disturbance of GDH metabolism in gliocytes was on a more substantial scale than in neurons. Considering that activity of enzymes of the tricarboxylic acid cycle in the glia is relatively low [11], the possibility cannot be ruled out that these changes in GDH could lead to a deficiency of high-energy compounds. Confirmation that cortical gliocytes are more sensitive than neurons to subconvulsant doses of metrazol is given by the results of an electron-microscopic investigation [16], which showed that disturbances of the ultrastructure of the neuroglial cells were much more severe than those in neurons. As a result of glutamic acid accumulation due to a decrease in GDH activity, other alternative pathways of glutamic acid metabolism may be activated, such as the decarboxylation reaction with the formation of increased quantities of GABA. During subsequent transamination GABA is transformed into succinic semialdehyde. By oxidation, the latter is converted into succinic acid. GABA-T catalyzes transamination of GABA and α-ketoglutaric acid, and also takes part in the regulation of their content in nerve tissue.

Information on the effect of metrazol on the level of GABA and activity of enzymes concerned in its metabolism is contradictory and is based mainly on the results of biochemical investigations, in which no attempt was made to determine these substances differentially in individual microstructures of nerve tissue. After a single intraperitoneal injection of

metrazol in a dose of 20 mg/kg, the brain GABA level was unchanged in the rats at different times after the end of the convulsions (maximum after 15 min) [2]. With an increase in the dose of metrazole to 60 mg/kg some decrease was found in the GABA content, but GABA-T activity did not differ from the control values [6].

According to the results of these experiments, in metrazol kindling GABA-T undergoes changes in different directions: Its activity in the neurons falls whereas in the glia it tends to rise, and this can be explained on the grounds of compartmentalization of GABA in these structures. According to data in the literature [9, 11], the basic pool of this acid is linked with presynaptic nerve endings, which contain up to 50% of the total quantity of GABA formed in the brain. On release into the synaptic space much of it diffuses into glial cells. It can therefore be postulated that the tendency for GABA-T activity to increase in the gliocytes may be due to an increase in GABA uptake by cells of the neuroglia.

After injection of subthreshold doses of metrazol, animals thus developed kindling, accompanied by a progressive increase in severity of the convulsions and by changes in activity of enzymes GDH and GABA-T, which are more marked in the neuroglia than in neurons.

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