TIME COURSE OF BRAIN DEHYDROGENASE AND GABA TRANSAMINASE ACTIVITY DURING METRAZOL KINDLING

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Repeated injection of metrazol in subthreshold (not inducing seizures) doses causes the appearance of the kindling phenomenon, namely increased sensitivity to the action of the epileptogen, and progression of behavioral and electrographic seizure reactions. At the height of development of the process the state of enhanced susceptibility to seizures is manifested as the appearance of generalized seizures in response to injection of a subthreshold dose of metrazol. This state of affairs continues for a long time after administration of the epileptogens has ceased [10]. The pathogenetic mechanisms of kindling has not been adequately studied. It was accordingly decided to study the state of energy metabolism in the neurons and glia of animals with established kindling.

The aim of this investigation was to study activity of certain redox enzymes in the cerebral cortex during metrazol kindling in mice at various periods after seizures, and also 1 month after determination of metrazol ceased.

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57BL/6)F_1 hybrid mice weighing 18-22 g. Kindling was induced by daily intraperitoneal injection of metrazol in a subthreshold dose (30 mg/kg in a volume of 0.1 ml) for 1 month. The metrazol solution was made up immediately before injection. Animals of the control group received an injection of the same volume of physiological saline. Altogether three series of experiments were carried out on 36 mice. After decapitation of the mice the brain was removed, a block obtained from it, and control and experimental materials were mounted together on the specimen holder, and frozen in liquid CO_2. Sections 10 μ thick were cut in a cryostat. Activity of α-ketoglutarate dehydrogenase (α-KGDH), succinate dehydrogenase (SDH), and malate dehydrogenase (MDH), enzymes of the Krebs' cycle, and activity of glutamate dehydrogenase (GDH), which regulate interaction of protein and carbohydrate metabolism, were determined histochemically in the sensomotor cortex by the method in [7]. Isozymes (H- and M-forms) of lactate dehydrogenase (LDH), which catalyzes one of the central reactions of glycolysis, were determined by the method in [11] and GABA transaminase (GABA-T), a key enzyme of the GABA shunt, was demonstrated by the method in [12] in the modification in [1]. For the cytophotometric investigation a two-wave method, at wavelengths of 550 and 640 nm, was used on the MTsFV-1 cytophotometer. In some cases a single-wave method was used. The technique of the investigations was described in more detail previously [6].

EXPERIMENTAL RESULTS

In the experiments of series I activity of the above-mentioned enzymes was studied in animals with established kindling, in the post-seizure period (30 min after injection of the test dose metrazol). Cytophotometric investigation of the microscopic sections revealed changes in activity of GABA-T and also of certain dehydrogenases (Table 1; Fig. 1). GABA-T activity was depressed in neurons, GDH activity in neurons and gliocytes, and α-KGDH activity in the glia. A tendency was noted for MDH and SDH activity to fall, whereas LDH-M activity, on the contrary, increased considerably in the neurons and glia.

In the experiments of series II the same parameters were studied 24 h after cessation of

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

Enzyme	Series of experiments	Neurons		Glia	
		Control	Experiment	Control	Experiment
GDH	I	58,73±1,21	48,78±0,87***	38,36±0,92	30,02±0,78***
	II	39,45±0,89	33,41±0,72***	22,59±0,59	18,03±0,44***
	III	44,03±0,91	40,20±0,87**	26,20±0,76	25,31±0,80
GABA-T	I	12,94±0,32	11,79±0,31*	5,91±0,22	5,90±0,20
	II	49,47±0,90	44,27±0,95*	35,78±0,65	36,99±0,81
	III	37,60±0,77	38,16±0,73	20,00±0,70	19,87±0,57
SDH	I	74,37±1,67	73,68±1,23	49,97±1,58	48,60±1,21
	II	74,52±1,77	81,41±1,80**	47,36±1,42	55,34±1,46***
	III	83,85±1,66	86,91±2,31	56,42±1,37	55,38±1,47
MDH	I	62,18±0,82	60,65±0,88	32,17±0,58	30,95±0,63
	II	48,26±1,26	47,13±1,74	35,74±1,48	34,22±1,54
	III	54,12±0,88	53,18±1,01	33,94±1,11	32,39±0,93
α-KGDH	I	91,23±1,67	91,10±1,57	56,69±1,33	51,53±1,37*
	II	55,10±0,89	52,72±1,03	45,75±1,00	41,46±0,83*
	III	67,37±1,84	65,28±1,73	56,24±1,78	53,74±1,64
LDH-H	I	76,68±1,35	78,63±1,18	44,91±1,20	44,24±1,04
	II	95,51±1,73	107,60±1,72***	76,10±1,42	79,06±1,36
	III	104,76±1,60	114,98±1,66***	74,45±2,08	78,83±1,89
LDH-M	I	8,50±0,27	10,92±0,26***	4,87±0,24	6,65±0,20***
	II	130,76±1,72	122,27±1,79***	114,29±1,43	106,31±1,62***
	III	46,07±1,37	56,96±1,06***	33,18±0,96	39,09±0,81***

Legend. *P < 0.05, **P < 0.01, ***P < 0.001

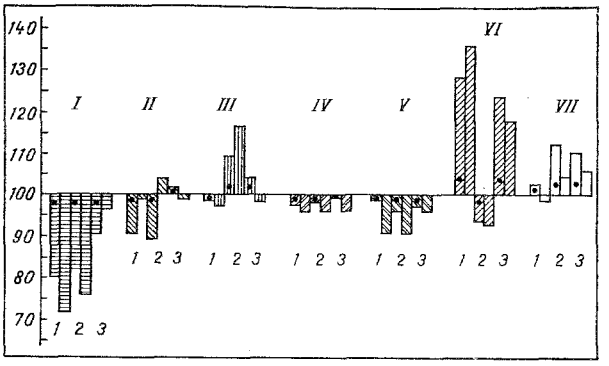

Fig. 1. Time course of brain dehydrogenase and GABA-T activity in mice during kindling (in % of control, taken as 100). I) GDH, II) GABA-T, III) SDH, IV) MDH, V) α-KGDH, VI) LDH-M, VII) LDH-H. First column represents post-seizure period; 2nd and 3rd columns — 24 h and 30 days respectively after last injection of metrazol. Columns with circles denote neurons, columns without circles — glia.

the metrazol injections, i.e., at the end of the time when the convulsant acted directly on the brain of the mice with established kindling. Changes in activity of certain enzymes also were observed in the animals of this series. GDH and LDH-M activity was depressed in neurons and neuroglia, α-KGDH activity in the neuroglia, and GABA-T activity in neurons. MDH activity showed a tendency to fall. Meanwhile SDH activity in the neurons and glia and LDH-H activity in the neurons increased.

In the experiments of series III enzyme activity was studied 30 days after the last injection of metrazol. Activity of most enzymes during this period closely approached that in the control animals. This was the case with SDH, α-KGDH-T, and GDH (for the last, only in glia). Meanwhile LDH-M activity increased significantly.

These investigations showed that metrazol kindling is characterized by increased susceptibility to seizures, by the onset of seizures, and also by disturbances of energy metabolism arising in nerve tissue becasue of inhibition of NAD-dependent dehydrogenase activity. The time course of the changes in activity of the redox enzyme is such that two phases in the

formation of the metabolic disturbances can be distinguished. In the first phase the disturbances are latent in character, when the increased energy expenditure due to seizure activity is compensated by increased oxidation of succinic acid, for which, according to data in the literature [2], there are no competitors in the task of maintaining a high level of high-energy compounds. The second phase of disturbance of energy metabolism is characterized by an increased intensity of anaerobic oxidation of carbohydrates which, under normal conditions is not characteristic of nerve tissue. Essentially, the stage of compensated disturbances of the energy metabolism of the cells during kindling is marked by lability, and it can be upset by injection of subthreshold doses of metrazol.

As regards inhibition of NAD-dependent dehydrogenases during the development of kindling, which is especially pronounced in the post-seizure state, it must be pointed out that, as our previous investigation showed [5], nicotinamide in fairly high doses can largely abolish the seizures and inhibit the development of kindling.

The results of these experiments show that the development of metrazol kindling, like the post-seizure state, is accompanied by contrary changes in enzyme activity. Besides changes which are the direct result of the pathogenic procedures and of the seizures themselves, or which may be of pathogenic significance for the subsequent development of kindling (enhanced susceptibility to seizures), processes arise which may play the role of factors capable of suppressing the seizures themselves (for example, inhibition of GABA-T). Thus the molecular mechanisms controlling function in this case are based on the principle of antagonism and work through the activation of antisystems [3].

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