CHANGES IN DEHYDROGENASE ACTIVITY IN THE HIPPOCAMPUS DURING METRAZOL-INDUCED KINDLING

I. N. Moiseev, V. F. Pchelyakov,
A. A. Shandra, R. F. Makul'kin,
and L. S. Godlevskii

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The writers showed previously that the development of metrazol kindling is accompanied by changes in dehydrogenase activity in neurons and glial cells of the cerebral cortex [3]. Since the formation of epileptic activity in metrazol kindling is connected with the formation of a hyperactive determinant structure in the hippocampus [2], it was decided to study energy metabolism in this part of the brain during kindling.

Activity of enzymes of glycolysis and the Krebs' cycle in hippocampal slices was studied during metrazol-induced kindling.

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C56B1/6 × BALB/c)F_1 hybrid mice weighing 18-22 g. Kindling was induced by daily (for 1 month) intraperitoneal injections of metrazol in a dose of 30 mg/kg in a volume of 0.1 ml. The metrazol solution was made up ex tempore. Animals of the control group received an injection of the same volume of physiological saline. At the end of the experiment the animals were decapitated, the brain was removed, and the hippocampus separated from it and frozen in liquid CO_2. Slices 10 μ thick were cut in a cryostat. Activity of α-ketoglutarate, succinate, and malate dehydrogenases (KDH, SDH, and MDH, respectively) was determined histochemically by the method in [5], and activity of the H and M isozymes of lactate dehydrogenase (HLDH and MLDH, respectively) was determined by the method in [8]. Enzyme activity was determined on an MTsFV-1 cytophotometer by a two-wave method at 550 and 640 nm [4]. The target for cytophotometric study was the pyramidal cells of the hippocampus and gliocytes of the stratum radiatum of sectors CA_1 and CA_2. In some cases, when the boundaries of the glial cells could not be clearly distinguished against the background of the surrounding neuropil, it was assumed that their optical density was about equal, and an over-all parameter of optical density was determined.

EXPERIMENTAL RESULTS

In the experiments of series I enzyme activity was studied in mice with kindling seizures in the postictal period (30 min after the last injection of metrazol). A tendency was observed for SDH activity to decrease in the neurons and in the neuroglia under these circumstances (Fig. 1). Conversely, MDH and KDH activity in the gliocytes rose to 11 and 9%, respectively ($P < 0.001$), and a tendency was noted for activity of these enzymes in the nerve cells to increase. MLDH activity in the neurons was increased by 35% ($P < 0.001$) and in the glia by 30% ($P < 0.001$), and HLDH activity increased by 15 and 16%, respectively ($P < 0.001$). Changes in SDH activity 24 h after the last injection of metrazol (series II) were opposite in direction. For instance, in the neurons a tendency was found for it to rise, but in the neuroglia, to fall (Fig. 1). During investigation of MDH and KDH a tendency was observed for activity of both enzymes to increase in the neurons and in the neuroglia activity increased by 8 and 7%, respectively ($P < 0.05$). MLDH activity was reduced by 12% ($P < 0.001$) in the neurons and by 8% ($P < 0.001$) in the glia. HLDH activity showed a tendency to rise in cells of both types.

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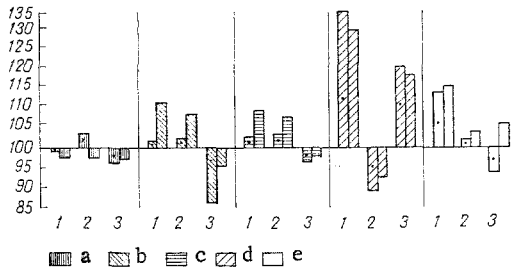

Fig. 1. Trend of changes in dehydrogenase activity in hippocampal neurons and glia cells during kindling. Ordinate, activity (in % of control). 1, 2, 3) 30 min, 24 h, and 30 days, respectively, after last injection of metrazol. a) SDH; b) MDH; c) KDH; d) MLDH; e) HLDH. Columns marked by a dot represent neurons, columns without a dot represent glia.

A tendency for SDH and KDH activity to fall in the neurons and gliocytes and for MDH activity to all in the gliocytes was observed 30 days after discontinuation of metrazol, i.e., on the formation of a kindling state (series III), whereas MDH activity in the neurons fell by 15% ($P < 0.001$). These changes were accompanied by an increase in MLDH activity by 21% ($P < 0.001$) in the neurons and by 19% ($P < 0.001$) in the glia, and also by a decrease in HLDH activity by 7% ($P < 0.05$) in the neurons and by an increase in its activity in the glial cells by 6% ($P < 0.05$).

The results of these investigations showed that disturbances of activity of the enzymes regulating energy metabolism in hippocampal structures of animals with kindling are characterized by the following features: 1) the changes mainly affect NAD-dependent enzymes (MDH, KDH, and LDH); 2) changes in enzyme activity in the neurons and glia are phasic in character; 3) changes in enzyme activity in neurons and gliocytes are usually in the same direction. In the postictal period (30 min and 24 h after injection of metrazol) the role of NAD-dependent enzymes of the Krebs cycle increased, but later (30 days after the last injection of metrazol) their activity was lower than in the control.

By contrast with the NAD-dependent dehydrogenases, changes in SDH activity were of a mild degree at all stages of the investigation. Hippocampal neurons in general have a comparatively low level of SDH activity [9]. Changes in activity of the NAD-dependent enzymes of the Krebs cycle and of MLDH 30 min after the last injection of metrazol were in the same direction (Fig. 1). Later, however, after 24 h, the increase in activity of enzymes of the Krebs cycle was accompanied by a decrease in MLDH activity, and vice versa after 30 days. The results may be evidence that in the early postictal period the levels of both respiration and glycolysis are raised in the glial cells and, to a lesser degree, in the pyramidal cells of the hippocampus, and after 24 h activity of respiration is virtually unchanged, but glycolysis is significantly reduced. After 30 days, on the other hand, the intensity of anaerobic glycolysis is increased whereas the level of respiration falls (especially in neurons). The increase in the contribution of anaerobic processes to the energy production of the cells, which is not characteristic of nerve tissue under physiological conditions, may also have its negative aspects. It has been shown, for instance, that during convulsions the increase in LDH activity leads to lactic acid accumulation in nerve tissue [1, 7], and to changes in the intercellular pH [10], as a result of which activity of enzyme systems may be disturbed [6] and activity of hippocampal pyramidal neurons increased [11]. During the study of activity of these enzymes in the cerebral cortex [2] the present writers observed that disturbances of energy metabolism in nerve cells of the sensomotor cortex were more marked than in the hippocampal pyramidal neurons.

Data in the literature [3] on the functional heterogeneity of the various nerve structures in the formation of epileptic activity during metrazol kindling, together with analysis of the results of the present investigation, thus reinforce the arguments in support of a special role of the hippocampus in the formation of a hyperactive determinant structure.

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ROLE OF MONOAMINES IN RECOVERY OF CONDITIONED REFLEX ACTIVITY AFTER FRONTAL LOBECTOMY IN RATS

T. V. Grekhova, V. S. Kudrin, I. I. Miroshnichenko, and G. A. Romanova

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The role of the monoaminergic system of the brain in integrative processes in the CNS — behavior, learning, memory — is widely known [4, 5, 7, 8]. At the same time, it has been shown that most diseases of the CNS are based on a disturbance of neurochemical processes in the brain, in conjunction with other mechanisms [1, 2, 12-15]. An essential condition for the successful compensation of these disturbances by drugs is knowledge of the mechanisms of the pathological changes in neurotransmitter systems.

The writers showed previously [3, 9] the injury to the frontal cortex of the rat brain is accompanied by changes in serotonin (5-HT) metabolism in the cerebral cortex and deep brain structures.

It was decided to study the character of changes in monoamine — 5-HT, noradrenalin (NA), dopamine (DA) — levels in the development of compensation recovery after bilateral injury to the frontal cortex.

EXPERIMENTAL METHOD

Chronic experiments were carried out on 23 noninbred male albino rats weighing 180-200 g. The functional state of integrative activity of the brain was assumed on the basis of conditioned reflex parameters. Conditioned motor feeding reflexes were formed in the animals in a specially equipped chamber, with two-way reinforcement in response to photic and acoustic stimuli. Conditioned relfexes were considered to be formed if 80-100% of correct responses were obtained on each of 3 successive days.

At the end of conditioning the animals were divided into two groups: 1) rats undergoing a mock operation; 2) animals undergoing frontal lobectomy. The state of conditioned reflex activity was tested daily after the operation. On the 9th day after lobectomy the rats were decapitated and the brain removed for biochemical investigation.

Concentrations of NA, DA, 5-HT, dihydroxyphenylacetic acid (DHPAA), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) were determined in the cerebral cortex, hypothalamus, corpus straitum, hippocampus, and brain regions including the nuclei raphe and locus coeruleus [6, 10]. The results were subjected to statistical analysis by Student's test. Monoamine levels in structures of the rat brain were determined by high-performance liquid chromatography with electrochemical detection [11].

Laboratory of Pathophysiology of Neurohumoral Regulation, Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR. Laboratory of Neurochemical Pharmacology, Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 1, pp. 12-14, January, 1987. Original article submitted March 13, 1986.

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