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Energy Metabolism in Rat Brain Structures after Injections of Kainic Acid into the Frontal Cortex

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We studied behavioral reactions of rats after injection of subconvulsive dose of kainic acid into the frontal cortex and mitochondrial respiration in the hippocampus and frontal and temporal cortex 17-20 days after administration of kainic acid. Retention of acquired habit and the dynamics of its extinction in experimental rats were close to those in the control group. Changes in mitochondrial function were observed only in the region of kainic acid injection: activation of phosphorylating respiration during oxidation of succinate. Presumably, the detected activation of energy metabolism in the frontal cortex indicates functional restructuring in mitochondria, aimed at compensation of disorders caused by the neurotoxin.

Key Words: *kainic acid; mitochondria; oxidative phosphorylation; frontal cortex*

Energy metabolism in brain cells determines the potentialities of brain function normally and during exposure to adverse factors [10]. Study of these processes during exposure to neurotoxins is particularly interesting for the search of effective neuroprotectors. Kainic acid (KA) widely used in experimental studies is a selective agonist of kainate receptors (a subtype of receptors for glutamate, the main cerebral stimulatory neuromediator). Kainate receptors are located pre- and postsynaptically; in addition, they are expressed by the glial cells [4]. Kainic acid is used in experiments as an epileptogenic and proapoptotic agent, but the mechanisms of the effects of its low subconvulsive doses on brain structures are not yet sufficiently studied; low-dose KA does not enhance epileptiform activity largely determining the effects of its high doses.

Subconvulsive dose of KA (0.2-0.25 μg) injected into the hippocampus modulated energy metabolism in brain structures not only at the site of injection, but also in the neocortex [1]. These data indicate close metabolic relationships between brain structures, universally reacting to injury. Study of these "metabolic" interrelationships between the structures can be useful for the development of methods for neuroprotection of the hippocampal system, which suffers in many neurodegenerative diseases and during normal aging.

We studied the effects of KA injection into the frontal cortex.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (180-200 g). The animals were kept under normal conditions with special feeding protocol: fodder was given during behavioral experiments (during adaptation to experimental setting, training, and retrieval tests).

After training, the animals were divided into 2 groups: control (n=5) and experimental (n=5). Stereotaxic bilateral injection of KA (0.25 μg; Sigma) was

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performed with a microsyringe 1 μl into the left and 1 μl into the right frontal cortex (AP=3.5; L=1; H=3) under pentobarbital narcosis (30 mg/kg). Controls were injected with isotonic saline in the same volumes during the same time.

The rats were trained in a 60×80×60 box with a starting area and target shelves positioned at different heights; the reinforcement (bread ball soaked in milk) was placed on one of them. The animals were trained for 5 days; 10 rounds during each training sessions. Seven days after KA injection, retention of the habit trained before KA treatment was tested. The latency of finding the target shelf was recorded. During testing the animals performed 10 rounds daily. When the habit was well reproduced, its extinction was performed for 2 days (the animals received no reinforcement) [1].

Mitochondrial (MC) function in the studied brain structures was evaluated after training and 17-20 days after KA injection. For better preservation of native MC characteristics, tissue homogenates for the experiments were prepared by modified method described previously [7]. Due to this approach, changes in energy metabolism of neuronal and glial elements could be ruled out, which is particularly important for characterization of brain structures. Homogenates of the frontal and temporal cortex and the hippocampus were prepared.

After decapitation, the brain was rapidly removed, plunged in ice-cold saline, washed from blood, and the studied brain structures were isolated. Brain tissue was weighed, washed in cold isolation medium containing 125 m KCl, 10 mM HEPES-KOH, 0.5 mM EDTA, 0.5 mM EGTA, pH 7.4, and homogenized (1 g tissue: 2 ml isolation medium). In order to preserve acceptor control of respiration in brain homogenate, chelators in the minimum possible concentrations were added to the isolation and incubation media. Each brain structure from each animal was homogenized separately. All procedures were carried out at 4°C.

Mitochondrial respiration was measured by the polarographic method using closed platinum Clark's electrode in a cuvette (1 ml) placed in a thermostat (26°C) at fixed stirring rate. The incubation medium contained 125 mM KCl, 10 HEPES-KOH, 2 mM KH_2PO_4, 0.25 mM EDTA (pH 7.2). Succinate (4 mM) or succinate (4 mM) and glutamate (2 mM) served as the substrates. ADP was added in a concentration of 0.2 mM, 2,4-dinitrophenol in a concentration of 0.025 mM. Protein was measured by the method of Lowry in each aliquot collected from the polarographic cuvette. Protein concentration in the cuvette was 3.0±0.5 mg/ml. Homogenate (0.1 ml) was added into the cuvette.

Oxygen consumption in the presence of oxidation substrate and phosphate without ADP (V_2); MC respiration rate during oxidative phosphorylation (oxygen requirement in the presence of oxidation substrate, orthophosphate, and ADP (V_3); MC respiration rate after ADP use in the presence of oxidation substrate and orthophosphate (V_4); phosphorylation rate of added ADP (ratio of added ADP (μmol) to time of its etherification (from the moment of ADP addition until its complete consumption; V_P); oxygen consumption by MC in the presence of oxidative phosphorylation dissociator 2,4-dinitrophenol (V_{DNP}) were evaluated.

The respiration (acceptor) control (RC) of MC status was estimated:

$$RC=V_3/V_4.$$

The results were processed using Excel software. Two-sample t test with different dispersions or paired two-sample t test for the means was used. The differences were significant at $p<0.05$.

RESULTS

Local injection of KA into the frontal cortex caused no changes in animal behavior. The habit retention and the dynamics of its extinction in experimental rats were close to the values in controls: in none tests appreciable deviations were noted.

Energy parameters of MC respiration at the site of KA injection in the frontal cortex changed under the effect of neurotoxin during succinate oxidation: V_3 and V_P increased in comparison with the control (Table 1). The MC respiration parameters in state 4 after Chance (V_2 and V_4) and parameters of uncoupled respiration (V_{DNP}) did not change. The differences in the studied parameters of MC respiration in the hippocampus and temporal cortex of control and experimental animals were insignificant. Oxidation of MC succinate is the main process, providing energy to the cell [3]. Glutamate participating in transamination reaction abolished oxaloacetate inhibition of SDH, thus making possible detection of latent inhibition of enzyme activity, which can develop in MC hyperactivity or damage [2]. Being a structural homolog of succinate, oxaloacetate inhibits SDH, thus acting as an endogenous normal regulator of the enzyme: its hyperproduction in the Krebs cycle inhibits SDH activity and hence, energy production in MC. This effect of glutamate was demonstrated in MC of the frontal and temporal neocortex of control animals and temporal neocortex of experimental animals and was virtually absent in MC of the frontal cortex and

TABLE 1. Parameters of MC Respiration in Rat Brain Homogenates after KA Injection into Frontal Cortex ($M \pm m$)

Group, brain structure		Oxidation substrates					
		V_2	V_3	V_4	V_{DNP}	V_P	RC
Frontal cortex							
control	succinate	13.0±4.7	27.7±1.7	13.2±1.1	39.6±5.7	55.8±8.8	2.1±0.3
	succinate+glutamate	11.6±1.6	38.0±2.8* (p=0.005)	13.5±0.4	41.4±2.2	89.7±3.6* (p=0.015)	2.8±0.2* (p=0.005)
experiment	succinate	15.0±2.4	33.9±5.0$^+$ (p=0.03)	14.0±1.0	42.1±3.7	74.1±7.1$^+$ (p=0.015)	2.4±0.5
	succinate+glutamate	14.7±2.9	38.4±4.0	13.6±1.9	42.8±3.4	85.3±9.9	2.8±0.5
Temporal cortex							
control	succinate	12.9±1.5	26.3±1.9	13.0±1.3	38.2±4.5	62.8±3.1	2.2±0.4
	succinate+glutamate	12.2±2.0	36.6±6.3* (p=0.02)	14.1±0.3	43.1±8.1	98.1±7.6* (p=0.002)	2.5±0.2
experiment	succinate	13.0±2.1	31.7±3.3	12.6±1.4	42.8±3.5	66.6±5.9	2.6±0.4
	succinate+glutamate	13.0±2.0	40.4±1.6* (p<0.001)	12.7±1.5	46.7±2.3	95.0±5.7* (p<0.001)	3.2±0.4
Hippocampus							
control	succinate	11.2±1.9	26.4±2.5	14.3±2.0	35.3±4.3	52.8±10.3	1.9±0.3
	succinate+glutamate	12.2±3.2	29.5±4.1	13.3±1.1	37.4±6.6	69.1±12.0	2.2±0.5
experiment	succinate	13.2±1.6	29.7±1.5	13.3±2.3	37.3±3.9	58.9±3.3	2.2±0.4
	succinate+glutamate	13.4±1.5	32.0±1.4	12.5±0.9	38.3±4.1	78.1±5.2* (p=0.005)	2.5±0.1

Note. Significant difference *between samples with and without glutamate; $^+$between control and experimental samples.

hippocampus of experimental animals. Hence, tests with glutamate showed that KA led to activation of energy metabolism at the site of injection and that additional activation of MC respiration cannot be attained after addition of glutamate. Energy metabolism in the hippocampus did not change. Moreover, glutamate test showed the absence of latent inhibition of MC respiration in this brain compartment in control and experimental animals. A trend to MC conjugation in the presence of glutamate was noted in control and experimental animals, and activation (32.6%) of phosphorylation rate was noted in experimental animals under these conditions.

Hence, our study detected the effect of subconvulsive dose of KA on energy metabolism at the site of injection 17-20 h after it. It is known that KA can cause both activation and inhibition of energy metabolism in the brain. Activity of cytochromoxidase C in the hippocampus and frontal cortex increases 1.2-1.3 times in comparison with the control 1 h after intraperitoneal injection of KA in a dose of 15 mg/kg to rats (this dose causes severe limbic convulsions and reduces the threshold convulsive activity) [8]. KA activates glutamate receptors and triggers numerous cascade changes in brain cells, which led to long-lasting neurochemical shifts [6]. Delayed reaction of the nervous system to KA is associated with the development of neurodegeneration and glial response [5]. However, these effects are typically produced by high KA doses inducing convulsive hyperactivity causing not only local injuries, but also injuries distant from the site of injection. One of the tasks of our study was to minimize damage to the brain, and we therefore used KA dose causing no significant injuries. Presumably, the effect of subconvulsive KA dose is due to local cell injuries inducing the compensatory processes, which require extra energy expenditures.

Changes in the behavioral parameters and MC function detected in our study differ significantly from previous results of KA injection into the hippocampus [1]. Injection of KA into the hippocampus of rats led to disorders in the cognitive functions along with changes in energy metabolism in the neocortex and hippocampus, while KA injection into the frontal cortex caused no disorders in the cognitive function and MC respiration was activated only in the neocortex, but not in the hippocampus. The differences can be explained by higher sensitivity of the hippocampus to KA and low convulsive threshold of this brain structure [4]. The

difference in the effects is presumably explained by different role of the studied brain structures in the cognitive functions, though they can mutually supplement and compensate for dysfunction of one structure by activity of another structure in response to brain injury [9].

Hence, injection of KA into the frontal cortex significantly modifies MC function in this brain compartment. Glutamate together with succinate used as the oxidation substrate led to activation of MC respiration in control and experimental animals, which indicated the involvement of oxaloacetate in the regulation of MC activity; endogenous glutamate regulated MC status *in vivo*. Activation of oxidative phosphorylation in the frontal cortex seems to reflect compensatory processes, supporting structural and functional integrity of this brain structure.

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