EXPERIMENTAL BIOLOGY

Expression of mGluR5 and Synaptophysin Genes after Injury to the Dorsal Hippocampus, Inflicted by Cainic Acid

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The expression of synaptophysin (vesicular protein) and mGluR5 (metabotropic glutamate receptor) genes was studied 3, 7, and 20 days after cainic acid injury of the dorsal hippocampal area in Wistar rats. The expression of both genes was characteristically reduced in the hippocampus. Twenty days after the exposure the expression of mGluR5 in this brain area reached the control level, while synaptophysin expression remained low. An opposite trend was observed in the frontal cortex: synaptophysin expression 20 days after exposure did not differ from the control, while mGluR5 expression was reduced. The peculiar time course of both genes' expression in the hippocampus and frontal cortex indicates the involvement of the frontal cortex in mechanisms of functional recovery after hippocampal injuries.

Key Words: *hippocampus; frontal cortex; gene expression; synaptophysin; mGluR5 metabotropic glutamate receptor*

We demonstrated previously an energetic metabolic relationship between the hippocampus and frontal cortex of the rat brain. This relationship manifests in response to hippocampal injury inflicted by cainic acid (CA) [1]. Mitochondrial function undergoes significant changes during several days after the injury not only in the hippocampus, but in the neocortex as well. The mechanisms leading to these changes in brain structures and maintaining them for a long time remain unknown. It is obvious that they involve different stages of cell-to-cell communication. The signal pathways modulating the mitochondrial function include the cellular receptor system. One of its important components is glutamate receptors, as virtually all brain cells contain some of receptors to this stimulatory neuromediator [8]. Metabotropic glutamate receptors occupy a special place among these receptors; through a system of intracellular signals they regulate the cell status for a long period (intracellular processes). It is known that one of these receptors (mGluR5) participates in regulation of energy balance in the brain structures [6] and is involved in realization of cognitive functions, depending on the hippocampus [4].

Hippocampal injury leads to manifest mnestic disorders [5], which can be compensated for with time in some cases. The frontal cortex is presumably involved in the mechanisms of compensation for the functional defects in the hippocampal system [10,13]. The knowledge of these mechanisms will help develop new approaches to repair of im-

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paired cognitive functions. Presumably, damage to the dorsal hippocampus in rats activates the mechanisms promoting recovery in parallel with modulation of the mitochondrial function not only in the hippocampus, but also in the neocortex [1]. We evaluated the expression of metabotropic glutamate receptor and synaptophysin (a protein of the synaptic vesicular membranes [12,15] in order to detect the participants in the cell-to-cell communication, responsible for regulation of the mitochondrial function in the frontal cortex in hippocampal injury.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (180-200 g; *n*=20). The animals were kept under standard conditions with special feeding schedule: the rats received fodder during behavioral tests. Training was carried out in a 60×80×60 cm cage with a starting plane and several target shelves at different heights, with support on one of them. The habit was trained during 5 days. The animals performed 10 running episodes during each day. The methods for studies of the rat behavior have been described in detail previously [1,3]. Stereotaxic bilateral injection of CA (Sigma) was carried out under pentobarbital narcosis (30 mg/kg). Cainic acid (0.2 μg) was injected with Hamilton's syringe (1 μl solution) into the left and right dorsal hippocampus (coordinates: AP: -3.0; ML: ±3.0; V: -3.0). Transitory limbic convulsions of stages 1-2 were observed during 2-4 h after injection. Later no convulsions were observed, this fact indicating that the convulsive activity was located in the limbic system and did not manifest after 48 h according to electrophysiological values [2]. Controls were injected with isotonic NaCl in the same volumes during the same periods as the experimental rats.

The animals were decapitated 3, 7, and 20 days after injection of CA. Brain structures (frontal cortex, hippocampus) were isolated and plunged in denaturing buffer, in which they were homogenized. The homogenate was stored at -20°C. Total RNA was isolated from homogenated brain structures using guanidine isothiocyanate (Sigma) by the phenol-chloroform extraction. This method provides isolation of pure non-degraded RNA, fit for analysis of gene expression. The concentration of total RNA was measured by spectrophotometry, the quality of the resultant RNA was evaluated by electrophoresis in 0.8% agarose gel. The primers were selected using the gene nucleotide sequence from the GeneBank database. Primers for synaptophysin: F — TTCTGGCTACAGCCGTGTT; R — ACAGG GTCCCTCAGTTCCTT. Primers for mGluR5: F — AGTTCGTGAGCAATATGGGATT; R — GATC CATCTACACAGCGTACCA. Reverse transcription reaction was carried out by the standard protocol developed by the firm manufacturing reverse transcriptase (Fermentas). The real time PCR was carried out in a DT-322 detecting amplifier (DNA-Technologies). The amplification protocol was the same for both genes and differed by the melting stage (56°C for Syp, 58°C for mGluR5). Results of real time PCR were evaluated using software developed by the DNA-Technology Firm. The results were statistically processed using Statistica software.

RESULTS

Behavioral tests were used for evaluating the effect of CA injected into the hippocampus. This hippocampal injury led to disorders in the reproduction of food-getting habit, trained before its injection; in addition, CA-inflicted injury induced the liability to persevering and reduction of the inhibitory processes [1,3]. These cognitive disorders were observed in the animals 1-2 weeks after CA injection, and hence, this period was the most interesting for us. Genes expression was evaluated in rats demonstrating disorders of this kind.

Evaluation of synaptophysin gene expression showed that it changed in response to hippocampal injury not only in the hippocampus, but also in the frontal cortex. The level of mRNA reduced in the hippocampus after CA injection in comparison with the level in control animals, and low level of mRNA was retained during subsequent days (Fig. 1). Various changes in synaptophysin expression were observed in the frontal cortex in response to CA: activation during the initial period, reduction of mRNA level after 1 week, and normalization after 20 days.

Synaptophysin is the first cloned synaptic vesicular protein. It is widely represented in all brain compartments, its content constituting about 7% of all vesicular membranous proteins [12]. Synaptophysin became the first vesicular protein, to which antibodies were obtained, therefore, it was used as a marker for studies of the pre- and postsynaptic processes before other proteins. High expression of synaptophysin indicates plastic restructuring processes in the brain. For example, vibriss stimulation in rats is associated with a local increase of synaptophysin gene expression in the respective barrels of the somatosensory cortex [15]. Presumably, the detected increase in mRNA level in the frontal cortex 3 days after hippocampal injury indicates plastic restructuring involving the hippocampus

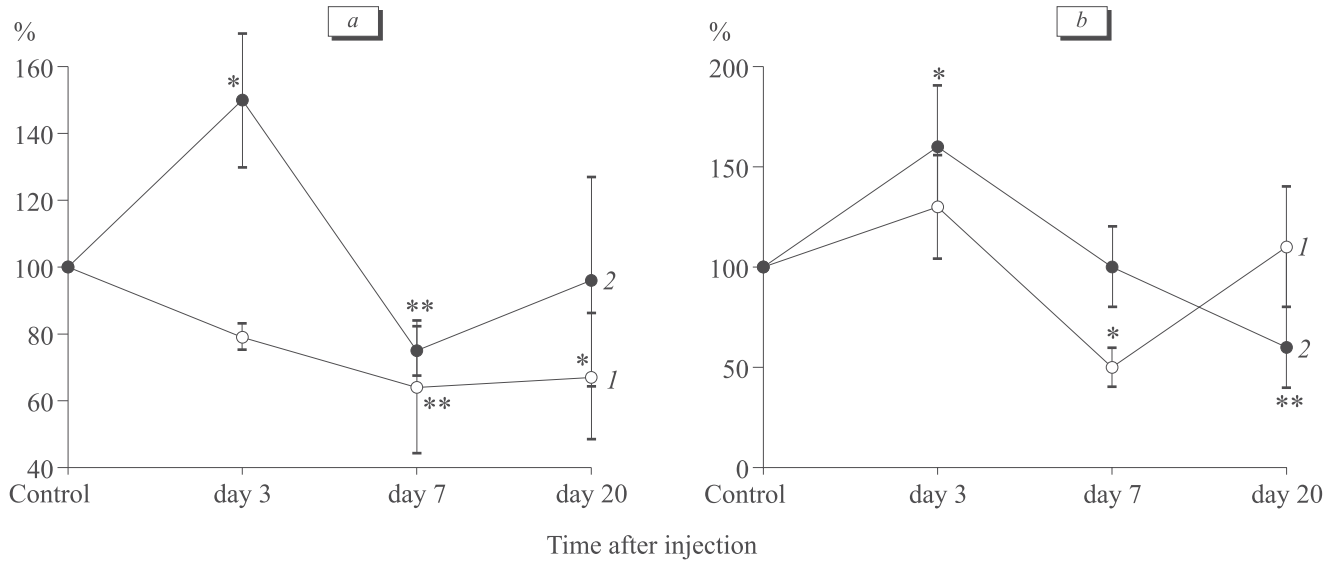

Fig. 1. Time course of genes expression in the hippocampus (1) and frontal cortex (2) after injury inflicted to the dorsal hippocampus by CA in a dose of 0.2 μg. a) expression of synaptophysin gene; b) of mGluR5 gene. *p<0.05, **p<0.005 compared to control.

and neocortex. Involvement of the frontal cortex in the reparative processes, initiated by hippocampal injury, is confirmed by our previous results, when we detected activation of mitochondrial processes in the frontal cortex 1-2 weeks after injection into the hippocampus of the same CA dose as in the present study [1].

Reduced expression of synaptophysin in the hippocampus and frontal cortex 1 week after CA injection does not mean reduction of the synaptic function of this protein, regulated by tyrosine kinase phosphorylation [12]. Reduced expression can mean that an alternative pathway of this function realization is switched on (through other proteins). It is really possible, as mice with knocked out synaptophysin gene were viable, which led to discovery of synaptophysin homologues in different animal tissues [12].

Expression of mGluR5 metabotropic glutamate receptor also changed after hippocampal injury. The level of mRNA in the hippocampus reduced 7 days after CA injection, while after 20 days it normalized. In the frontal cortex the expression of mGluR5 gene 3 days after CA injection was significantly higher than in control animals; 7 days post-injection mRNA level was normal, while 20 days after the injury it was low.

Metabotropic glutamate receptors are involved in fine "tuning" of excitability of selective neuronal chains of the brain. Stimulation of mGluR5 causes activation of many intracellular messengers (protein kinase C, MAP kinase, Ca^{2+}/calmodulin-dependent protein kinase, *etc.*), which, in turn, can activate DNA transcription factors [7]. The mGluR5 are located in the hippocampus on postsynaptic membranes; their important role in mechanisms of plasticity and memory, epileptogenesis [9,14], regulation of energy balance [6] has been shown. Blocking of mGluR5 promotes neuron survival [11], while their activation promotes epileptogenesis [14]. These facts indicate that the intricate time course of mGluR5 gene expression in the hippocampus and frontal cortex, detected in this study, confirms the active participation of this brain structure in the morphofunctional restructuring after hippocampal injury. However these changes do not correlate with mitochondrial function in the neocortex and hippocampus [1]. We conclude from this that mutual regulation of energy metabolism in brain structures is not directly mediated through mGluR5 and is realized with involvement of some other, heretofore unknown messengers.

Hence, study of the expression of synaptophysin and mGluR5 expression showed that hippocampal injury by CA caused lasting changes of different direction in the synaptic processes, located not only in the hippocampus, but also in the frontal cortex. It is noteworthy that not all neurochemical processes in the frontal cortex normalized 20 days after hippocampal injury, which was seen by the time course of mGluR5 expression: this gene's expression was reduced during this period. Activation of synaptophysin and mGluR5 expression 3 days after neurotoxin injection can be caused mainly by active plastic restructuring, induced by the destructive effect of CA and convulsive activity in the hippocampus, induced by this treatment. Synaptophysin can really reduce the excessive acti-

vation of the targets through modification of the vesicular cycle of synaptic processes. The mGluR5 is also involved in the neuron protection from hyperactivation, as reduction of this metabotropic glutamate receptor expression has a neuroprotective effect [11]. Later the effect of convulsive activity is less pronounced, and changes in mRNA level reflect the compensatory processes, initiated by local hippocampal injury.

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