Expression of Mitochondrial Uncoupling Protein UCP2 in the Brain of Rats after Hippocampal Injury Inflicted by Kainic Acid

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The expression of mitochondrial protein UCP2 and cytochrome C-oxidase subunit III genes in the hippocampus and prefrontal cortex was evaluated by real-time PCR 3 and 7 days after microinjection of kainic acid into the dorsal hippocampus. In contrast to cytochrome C-oxidase subunit III mRNA, the level of UCP2 mRNA in the hippocampus increased 1 week after microinjection of kainic acid. The expression of both genes in the prefrontal cortex did not differ from the control. Presumably, activation of UCP2 synthesis in hippocampal injury indicates the neuroprotective effects of this protein.

Key Words: *hippocampus; kainic acid; gene expression; mitochondrial uncoupling proteins*

Mitochondrial uncoupling proteins (UCPs; 31-34 kDa) were detected in mammals, fishes, birds, and plants [8]. The function of one of them, UCP1, is well known. This protein catalyzes proton conductivity in mammalian adipocytes participating in thermogenesis mechanisms. The functions of other UCPs representative remain unknown, but, presumably, they are different in different tissues. It is known that bird, fish, and plant UCP2, UCP3, and UCPs catalyze proton conductivity, similarly as UCP1. Some UCPs reduce the production of active oxygen species. In 1997 UCP2 was discovered [6]. The level of its expression in different tissues varies greatly and depends on the physiological status of a cell. It was shown that it plays a signal role in pancreatic β-cells, reducing insulin secretion [3]. The function of UCP2 in the brain is not identified; according to some data, it can play a neuroprotective role [11].

We studied the expression of this protein in the hippocampus and prefrontal cortex after hippocampal injury.

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MATERIALS AND METHODS

The hippocampus was damaged by microinjection of kainic acid (KA), a cainate receptor agonist widely used in experimental biology as a neurotoxin and epileptogenic agent [13]. Previously we selected a subconvulsive dose of KA (0.2 μg), causing no epileptization of the brain after injection into the dorsal hippocampus. It has been shown that this hippocampal injury leads to specific disorders in the animal long-term memory (mainly in information reproduction processes and in the capacity to inhibit the reactions which lost biological sense) [4]. With time the defects in animal behavior, caused by injection of this KA dose, are compensated for. In addition, microinjection of KA (0.2 μg) into the hippocampus stimulates mitochondrial functions in the hippocampus and prefrontal cortex [1] and modulates the expression of metabotropic glutamate mGluR5 receptor and synaptophysin [2], which also attests to compensation for the disorders caused by neurotoxin. If UCP2 is a neuroprotector, the expression of its gene can be changed in hippocampal injury of this kind.

Experiments were carried out on male Wistar rats (180-200 g). The animals were kept under normal con-

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ditions with a special feeding protocol: they received fodder during behavioral tests intended for monitoring of functional disorders in the hippocampus caused by its injury by the neurotoxin. The rats were trained (5 days, 10 attempts daily) to food-procuring behavior in a box (60×80×60 cm) with a starting platform and several target shelves located at different height with food revard on one of them. This methods for studies of rat behavior was described in detail previously [4]. Stereotaxic bilateral injection of KA (Sigma) was carried out under pentobarbital narcosis (30 mg/kg). Kainic acid (0.2 μg) was injected directly after training with a Hamilton syringe, 1 μl solution into the left and right dorsal hippocampus (AP coordinates: -3.0; ML: ±3.0, V: -3.0). Control animals were injected with isotonic NaCl in the same volume during the same period as KA to experimental rats. As mentioned above, this dose of the neurotoxin provides minimum but lasting effect on behavior, which can be detected by behavioral tests used in the study. The rats developed transitory limbic convulsions within 2-4 h after KA injection; no convulsions were observed later, which suggests that convulsive activity is located within the limbic system.

The animals were decapitated 3, 7, or 20 days after injection of KA. Brain structures (prefrontal cortex, hippocampus) were isolated, plunged in denaturing buffer, and homogenized. The homogenate was stored at -20°C. Total RNA was isolated from brain structure homogenates by phenol-chloroform extraction using guanidine isothiocyanate (Sigma). This method leads to isolation of pure intact RNA suitable for further 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 GeneBank database. Primers for *UCP2 F* – 5'-TCCTCTCGTGCAATGGTCTT-3'; *UCP2 R* – 5'-CCTGGCAGGTAGCACCAC-3'. For cytochrome C-oxidase subunit III: *COX III F* – 5'-GGTATGTTCCTTCACGGATGA-3'; *COX III R* – 5'-ACCAAACCCATGCATACCATA-3'.

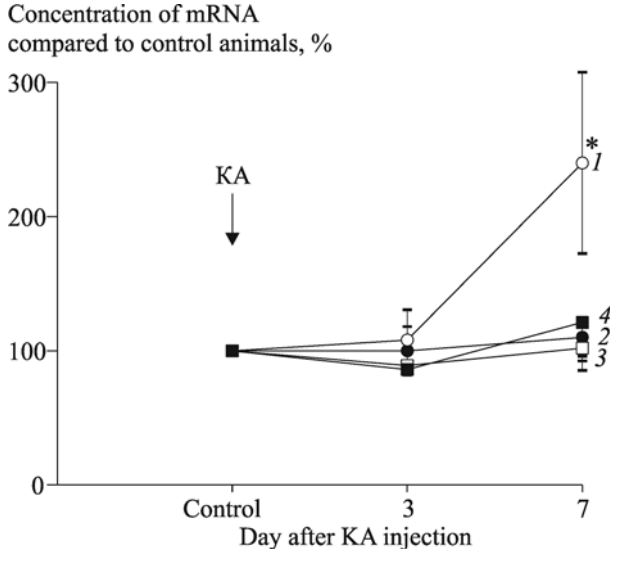

Fig. 1. Expression of *UCP2* in the hippocampus (*1*) and prefrontal cortex (*2*); expression of cytochrome C-oxidase subunit III in the hippocampus (*3*) and prefrontal cortex (*4*) of rats after hippocampal injury. At least 4 measurements per point. *$p<0.01$ compared to the control.

Reverse transcription was carried out according to the standard protocol developed by reverse transcriptase manufacturer (Fermentas). Real-time PCR was carried out in a DT-322 detecting amplifier (DNA-Technology). Results of the real time PCR were evaluated using DNA-Technology software with SYBR Green stain (Invitrogen) intercalating into double-stranded DNA. The quality and molecular weight of PCR products were evaluated by electrophoresis in 5% PAAG (230 B/110 mA). The results were statistically processed using Statistica software.

RESULTS

Behavioral tests were used for monitoring the effect of KA injected into the hippocampus. Previous study showed that this hippocampal injury disorders food-procuring behavior trained before injection of the substance and to deterioration of the inhibitory processes, which manifested in inhibition of rat capacity to suppress the reactions which lost their biological significance in the habit inhibition test [4]. These cognitive disorders were detected 3 days and later after KA injection, and hence, these periods attracted our special interest. Gene expression was carried out in rats with these disorders (Fig. 1).

Cytochrome C-oxidase plays an important role in energy metabolism. It is a transmembrane protein complex located in the mitochondria and catalyzing cytochrome C oxidation by molecular oxygen. In mammals, cytochrome C-oxidase consists of 13 subunits: 3 main catalytical (I, II, III, encoded by mitochondrial genome) and 10 minor ones, encoded by the nuclear genome. Functional activity of cytochrome C-oxidase is often regarded as a marker of functional activity of brain structures [12]. For example, the expression of its subunits in the brain can change after injury [7]. In this study we evaluated the expression of one of the major catalytical subunits of the complex, subunit III (SOX III).

The level of SOX III mRNA in the hippocampus and prefrontal cortex was similar to the control 3 and 7 days after hippocampal injury by KA microinjections. Presumably, the hippocampal damage inflicted in our study was largely reversible; the main functional po-

tentialities of the mitochondria were retained, and hence, no appreciable changes in SOX III expression were found. However, the content of mRNA for other mitochondrial protein (UCP2) changed significantly in damaged hippocampus. The expression of *UCP2* expression in the hippocampus increased after 7 days, while in the prefrontal cortex the expression was the same as in the control. This time course of the gene expression suggests an adaptive mechanism, involved at the late stages of plastic restructuring of the hippocampus. As was noted above, the physiological role of UCP2 remains not quite clear, but experimental findings indicate its involvement in the regulation of the mitochondrial membrane potential, production of active oxygen species, stabilization of calcium homeostasis, modulation of neuronal activity, and eventually reduction of cell injury [9]. It was found, for example, that higher expression of UCP2 correlated with neuronal survival after stroke and injury [10,11]. Increase of *UCP2*_expression in the brain under the effect of KA was observed by other authors [5]. These authors showed that UCP2 mRNA reached its peak level 24 h after intraperitoneal injection of this neurotoxin to mice in the convulsive dose and normalized 3 days after the injection. We also observed normal level of UCP2 expression in the hippocampus 3 days after KA injection; however, the expression increased later, on day 7 postinjection. We used a lower (subconvulsive) dose of KA, after which the probability of neuron survival was much higher. Therefore, delayed activation of UCP2 expression can be regarded as one of the mechanisms of neuroprotection in the hippocampus. Its detailed study will make it possible to evaluate the therapeutic potentialities of this class of proteins.

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