Expression of *S100B* and *S100A6* Genes during Long-Term Posttetanic Potentiation in the Hippocampus

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The expression of *S100B* and *S100A6* mRNA in CA1 region of rat hippocampal sections was studied after tetanizing stimulation. The level of *S100B* expression increased 2-4-fold in comparison with the control after 30 min and gradually returned to the basal level 120 min after tetanization. The level of *S100A6* mRNA was very low and did not change after tetanization.

Key Words: *gene expression; hippocampus; long-term posttetanic potentiation; S100*

The physiological role of S100 protein family remains an object of great interest [2,11]. However, the facts needed for evaluating the significance of their dynamics for brain plasticity are obviously insufficient. The relationships forming in the available plasticity models between the level of synaptic efficiency and dynamics of expression of genes encoding these proteins are particularly interesting. This is primarily so for the S100B and S100A6 (calcyclin) proteins, characterized by high expression in various compartments of the brain and involved in its development and diseases [8].

We studied the dynamics of expression of *S100B* and *S100A6* genes at different stages of the formation and maintenance of long-term posttetanic potentiation (LPTP), a classical model of neuronal plasticity.

MATERIALS AND METHODS

Experiments were carried out on the hippocampal sections of male Wistar rats (180-220 g). After decapitation, the brain was plunged into carbogen-aerated (95% O_2, 5% CO_2) saline of the following composition (mM): 126 NaCl, 4 KCl, 1.24 NaH_2PO_4, 1.3 $MgSO_4$, 2.3 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose. The sections (a series of four 400-μ sections from the dorsal part of the left hippocampus) were put into a flow (2 ml/min, 32-33°C) chamber (7 ml). Extracellular recording and bipolar stimulating electrodes filled with saline were placed in the pyramidal and radial layers of CA1 field, respectively.

One section from each animal was used in experiments to verify the tetanization protocol. The intensity of the test/tetanizing stimuli was selected so that the p-spike amplitude in the response was ~50% of the maximum. Tetanization (4 series of stimuli at 100 Hz frequency, 1 sec long at 30 sec interval) was started 2.5 h after preparation of sections.

One of the sections served as the control in studies of tetanization effect on mRNA concentration; no manipulations were carried out with it throughout the entire incubation period (4.5 h). Three other sections were tetanized 120, 60, or 30 min before the end of incubation. The electrodes were mounted on the section 10 min before tetanization and the intensity of the tetanizing stimulus was selected. The electrodes were removed directly after tetanization. After the experiment, the sections were plunged in cold saline and the CA1 field was isolated and transferred into liquid nitrogen. The corresponding sections from 5 animals were pooled for isolation RNA specimens.

Summary RNA was isolated from rat hippocampal sections frozen in liquid nitrogen and treated with

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DNase I using Aurum Total RNA Fatty and Fibrous Tissue Kit (Bio-Rad Laboratories) according to the instruction. The quality of RNA was tested by electrophoresis in 1% agarose gel. The quantity of RNA was measured on a spectrophotometer by absorption at λ=260 nm. Summary RNA (0.5 μg) was used to obtain cDNA. Reverse transcription was carried out in reaction volume of 20 μl using iScript cDNA Synthesis Kit (Bio-Rad Laboratories) according to the instruction. Hexanucleotide random primers were used for reverse transcription reaction.

The expression of *S100B* and *S100A6* genes was evaluated by real-time PCR using iTaq SYBR Green Supermix with ROX (Bio-Rad Laboratories) on an IQ5 amplifier (Bio-Rad Laboratories) with *β-actin* as the reference gene. The following primers were used: *S100B* F: 5'-TTGCCCTCATTGATGTCTTCCA-3' and R: 5'-TCTGCCTTGATTCTTACAGGTGAC-3'; *S100A6* F: 5'-CTTCTCGTGGCTATCTTCC-3' and R: 5'-ACTGGACTTGACTGGGATAG-3'; *β-actin* F: 5'-ACCCACACTGTGCCCATCTA-3' and R: 5'-CGGAACCGCTCATTGCC-3'. The optimal concentration for all primer pairs in the reaction mixture was 300 nM. PCR was carried out in 25 μl volume containing 1 μl cDNA under the following conditions: pre-heating (95°C, 3 min), followed by 40 cycles: denaturing (95°C, 15 sec), annealing (58°C, 20 sec), elongation (72°C, 20 sec), measurement of fluorescence (80°C, 10 sec). PCR specificity was verified by melting curves. In each experiment, the specimens of the studied cDNA with primers for the target genes and the reference gene were put on the same plate; experiments were carried out twice. The gene expression level was evaluated using Ct threshold cycle values with consideration for reaction efficiency (E) for the studied gene and reference gene.

The results were presented as the mean±standard error of the mean. The significance of differences was evaluated using Student's paired *t* test.

RESULTS

Our protocol of tetanization caused a significant increase in the amplitude of evoked p-spikes in the CA1 field induced by stimulation of Schaffer collaterals. This increase persisted throughout the entire observation period (3 h; Fig. 1).

In control sections, the level of *S100B* mRNA (34±7% of *β-actin* mRNA level; *n*=4) in CA1 field more than 1000-fold surpassed that of *S100A6* (0.030±0.008%; *n*=4). This can be attributed to uneven distribution of S100A6 protein in the brain, specific of the limited areas of the entorhinal cortex and amygdala and found in some astrocytes [12], while S100B is presented more extensively: in cortical and hippocampal astrocytes, some populations of oligodendrocytes and neurons [4,5].

The effect of tetanization on the expression of the studied genes in CA1 field was also different. As

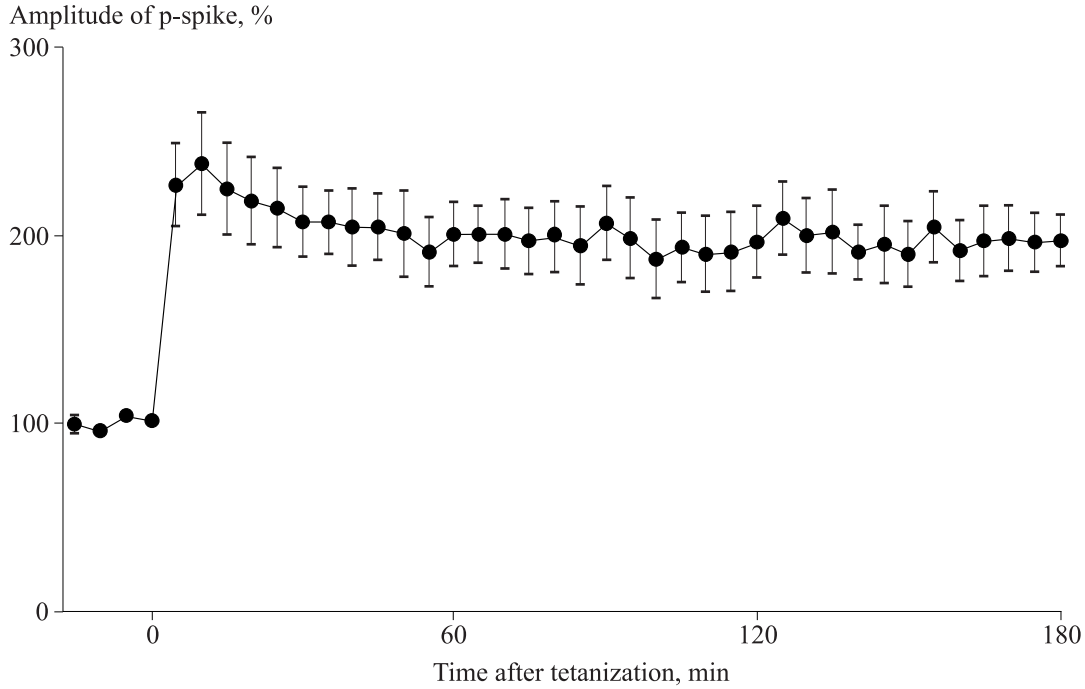

Fig. 1. Persistence of LPTP in CA1 field of the rat hippocampal sections. Ordinate: amplitude of p-spike recorded in the CA1 pyramidal layer in response to stimulation of Schaffer collaterals. Mean amplitude of p-spike in the responses to 4 stimuli, preceding tetanization, is taken for 100%. The amplitude of responses after tetanization differed significantly ($p<0.02$) from the amplitude of response to test stimulus, directly preceding tetanization ($n=5$), during the entire period of observation.

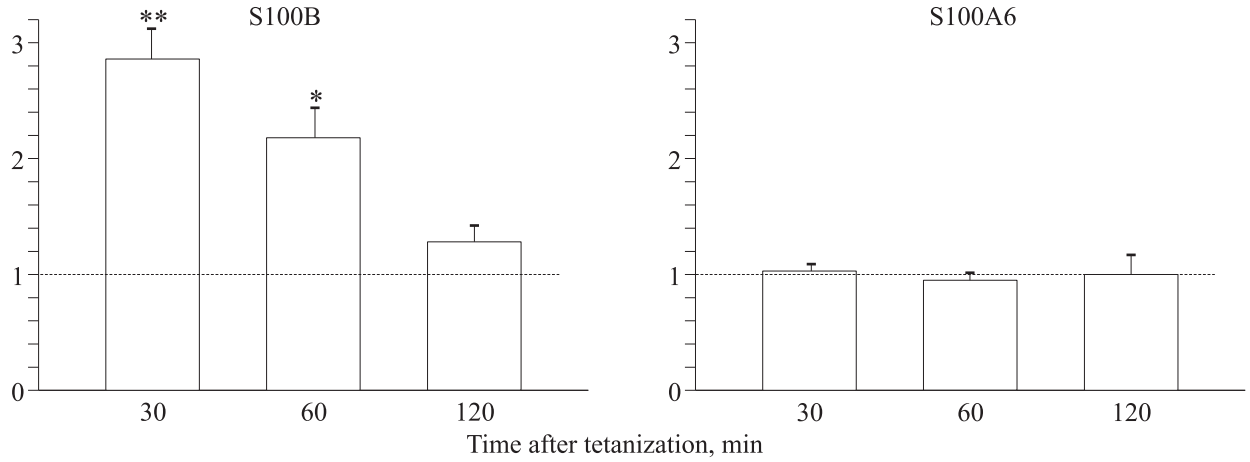

Fig. 2. Time course of *S100B* and *S100A6* mRNA in LPTP in the rat hippocampal CA1 field. Ordinate: multiplicity of increase in expression in comparison with the control (nontetanized sections; dotted line). *$p<0.05$, **$p<0.01$ compared to the control. In all cases $n=4$.

soon as 30 min after tetanization, the level of *S100B* mRNA increased significantly (2-4-fold; Fig. 2), but later this parameter gradually decreased and 120 min after tetanization virtually did not differ from the control. The content of *S100A6* mRNA in sections did not differ from the control during all the studied periods after tetanization.

The detected differences in the regulation of genes expression in tetanization can be explained by cell specificity of regulation. Presumably, the cell-specific epigenetic mechanisms (cell-specific silence of the genome resultant from methylation) are involved in case with *S100A6* [9], while with *S100B* the cell-specific interactions between the *cis*-elements and *trans*-factors can be involved [6].

The results can reflect different extracellular functions of these proteins [2]. It was recently shown that these proteins bind to various domains of the receptor for advanced glycation end-products (RAGE), which leads to activation of the anti- and proapoptotic signal systems in the neuron [8]. Significant induction of *S100B* expression presumably indicates triggering of these signal routes in the adjacent cells, which eventually leads to the "antiapoptotic" effect in them. It was found that S100B in physiological (nanomolar) concentrations is a trophic factor [2]. On the other hand, astrocyte secretion of S100B depends on neuronal activity [10]. Presumably, the dissociation between the neuronal and glial activation underlies the neuropathological phenomena with characteristic elevation of S100B and S100A6 protein levels [1,7,11].

Some other facts deserve discussion in connection with our findings. We previously described the dynamics of LPTP in the hippocampal sections plunged in solution of low-dose antibodies to protein S100B [3]. We detected a preconditioning phenomenon in that study: low-dose antibodies abolished the inhibitory effect of high-dose antibodies on LPTP. Up to the present time we attributed this effect to the desensitizing effect of low-dose antibodies to S100B through intervention in the calcium homeostasis. Interestingly, the time of the development of the preconditioning phenomenon coincides with the period of maximum expression of *S100B* gene during LPTP development. We think that this coincidence is not chance and deserves experimental analysis. Further studies of this phenomenon will extend our notions on the mechanisms of neuroglial interactions during LPTP formation.

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