GENETICS

Time Course of Expression of "Early" Genes during Long-Term Posttetanic Potentiation in Rat Hippocampal CA1 Field

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The expression of *egr-1*, *junB*, *c-jun*, and *c-fos* genes in rat hippocampal CA1 field was studied by the real-time PCR 30, 60, and 120 min after induction of long-term posttetanic potentiation. The content of *egr-1*, *junB*, and *c-jun* mRNA gradually increased and doubled 120 min after tetanization. The increase in *c-jun* mRNA level lagged behind the increment of *egr-1* and *junB*. The level of *c-fos* mRNA increased 30 min after tetanization, returned to the initial level by min 60, and again increased 120 min after induction of long-term posttetanic potentiation.

Key Words: *expression of "early" genes; hippocampus; long-term potentiation; egr-1; junB; c-jun; c-fos*

Long-term posttetanic potentiation (LPTP) is a classical model for studies of the mechanisms of neuronal plasticity. The critical event leading to LPTP induction is an increase of calcium concentration in a postsynaptic cell. This increase triggers a series of biochemical cascades leading to unfolding of genetic programs underlying stable long-term changes in the neuronal phenotype. Time-dependent changes in the expression of "early" genes play an important role in this process. Many of these genes encode transcription factors, which activate the "late response" genes encoding proteins significant for LPTP maintenance.

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Until recently, the methods of immunohistochemical [6] and *in situ* hybridization [8] were the main technologies used for studies of the time course of the expression of "early" genes in LPTP. In addition, the results obtained by different authors were often contradictory not just because they studied different brain compartments, but also because of the particulars of the experimental protocols [5]. We studied the dynamics of *egr-1*, *junB*, *c-jun*, and *c-fos* transcription factors mRNA in rat hippocampal CA1 field sections by real-time PCR.

MATERIALS AND METHODS

The procedure of the experiment was described in detail [1]. Experiments were carried out on hippocampal sections from Wistar rats. Four sections were put into a flow chamber. One section served as the control, 3 were subjected to tetanization 120, 60, or 30 min be-

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fore the end of incubation. Extracellular recording and bipolar stimulating electrodes were placed in the pyramidal and radial layers of the CA1 field, respectively. After the experiment, the sections were removed from the chamber, the CA1 field was cut out and frozen in liquid nitrogen. Specimens from 5 animals were pooled for isolation of mRNA.

The levels of *egr-1*, *junB*, *c-jun*, and *c-fos* mRNA were evaluated by real time PCR. The β-actin gene served as the reference gene. The following primers were used: *c-fos* F: 5'-AGCTGAAGGCTGAACCCTTTGATG-3', R: 5'-TCCCAGTCTGCTGCATAGAAGGA-3'; *egr-1* F: 5'-AGCACCTGACCACAGAGTCC-3', R: 5'-CCACTGACTAGGCTGAAAAGG-3'; *c-jun* F: 5'-ATGGAGTCTCAGGAGCGGATCAA-3', R: 5'-ACTTTCTGCTTAAGCTGTGCCACC-3'; *junB* F: 5'-AGACCAAGAGCGCATCAAAGTGGA-3', R: 5'-GGGTCATGACCTTCTGTTTGAGCT-3'; *β-actin* F: 5'-ACCCACACTGTGCCCATCTA-3', R: 5'-CGGAACCGCTCATTGCC-3'.

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

RESULTS

The content of the reference (β-actin) gene mRNA in aliquots of the summary mRNA 60 and 120 min after tetanization virtually did not differ from the control (99±2 and 98±3% of control level, respectively). The level of β-actin mRNA was slightly lower 30 min after tetanization (93±2% of control level; $p<0.03$; $n=6$), which could be due to increased level of mRNA of other genes (for example, *S100B*) [1].

The content of *egr-1*, *junB*, *c-jun*, and *c-fos* mRNA in the control hippocampal CA1 field after 4.5-h incubation in a flow chamber was 14±7, 1.4±0.3, 2.0±0.3, and 2.4±0.4% of β-actin mRNA level, respectively.

The content of *egr-1*, *junB*, and *c-jun* mRNA gradually increased after tetanization, increasing approximately 2-fold after 120 min (Fig. 1). However, the increase in *c-jun* mRNA level at the early stage was negligible. The concentrations of *egr-1* and *junB* mRNA increased significantly as early as 30 min after tetanization (Fig. 1). For *junB* the level of significance in this case was underrated because the sample did not correspond to normal distribution; after excluding the

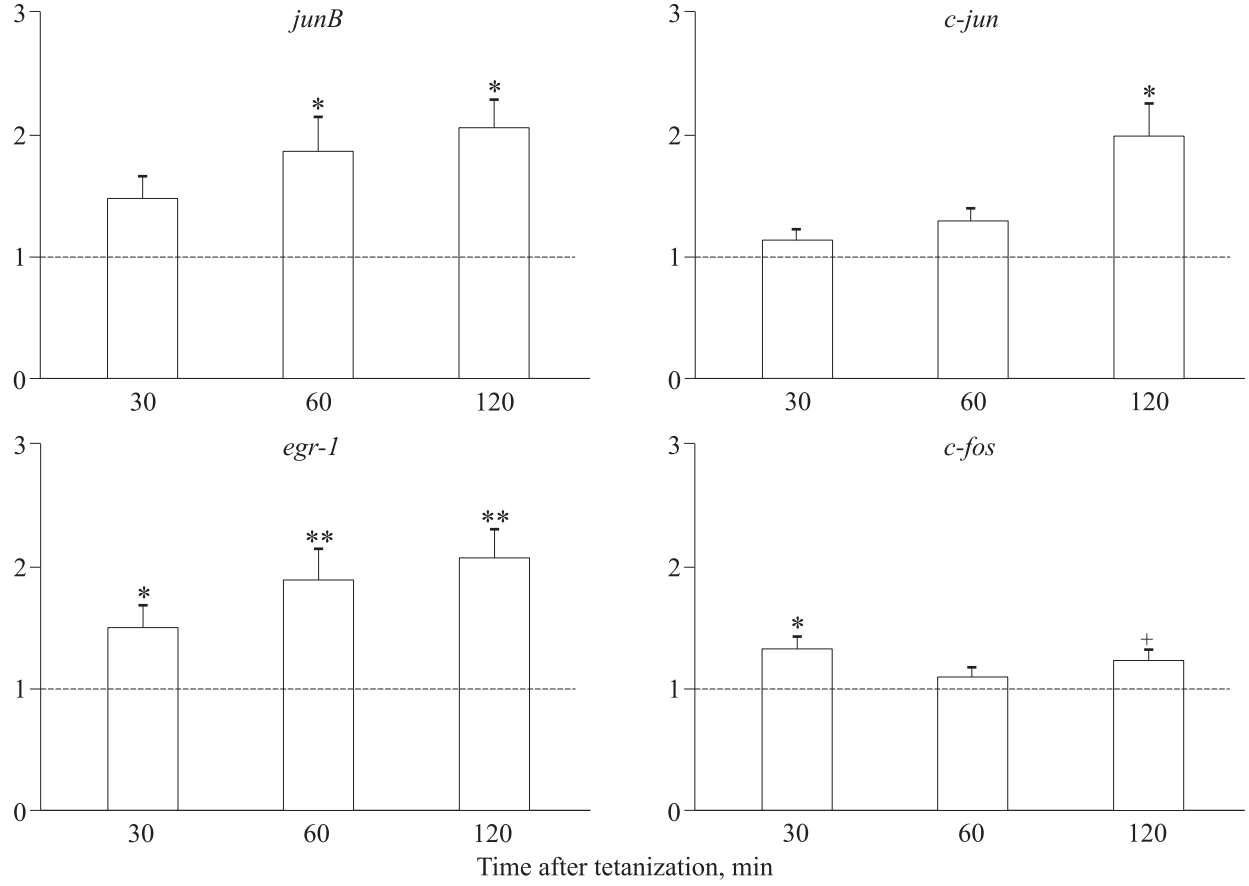

Fig. 1. Dynamics of the content of *egr-1* ($n=4$), *junB* ($n=4$), *c-jun* ($n=4$), and *c-fos* ($n=6$) mRNA in rat hippocampal CA1 field. Ordinate: multiplicity of increase in the expression in comparison with control (non-tetanized sections; dotted line). $^{*}p<0.05$, $^{**}p<0.01$, $^{+}p<0.05$ (sign test) compared to the control.

values differing much from the mean, the content of *junB* mRNA in samples 30 min after tetanization was 133±3% ($p<0.01$, $n=3$) of the control.

The increase in *c-jun* expression does not develop in all models of long-term potentiation [5], presumably because of positive feedback between the *c-jun* protein and expression of its gene [2] and, hence, the need to maintain the low basal level of the protein under normal conditions. It seems that a certain threshold concentration of *c-jun* protein is needed for stimulation of its gene expression, which then sharply increases, as we observed in our experiments.

The content of *c-fos* mRNA 30 min after tetanization was significantly higher than in the control (Fig. 1), after which it returned to the initial level (60 min after tetanization) and increased again by min 120 ($p<0.03$ *vs*. mRNA level 60 min after tetanization; $n=6$). Though the increase in *c-fos* mRNA level 120 min after tetanization was negligible in comparison with the control according to paired *t* test ($p=0.07$), it was significant by the sign test ($p<0.05$).

Transcription of *c-fos* is regulated by CREB [9], and hence, expression of *c-fos* can reflect CREB activity [7]. Biphasic activation (phosphorylation) of CREB in LPTP was experimentally demonstrated *in vivo*: peak 1 of activity was observed 30 min after tetanization, while peak 2 started after about 2 h and lasted much longer [10]. A similar phenomenon was demonstrated on hippocampal sections, though its time course was different [4]. Our data indicate that, presumably, biphasic activation of CREB also developed under conditions of our experiment and its time course was close to the previously described dynamics [10].

Study of LPTP in the rat hippocampal CA1 field revealed no increase in *c-fos* expression 45 min after τ-stimulation. It was concluded that the stimulation protocol used in the study caused no increase in *c-fos* mRNA level [8]. Our results confirm high liability to fluctuations of *c-fos* mRNA level [2] and necessitate more careful interpretation of the experimental data on the time course of its changes.

In addition to the new data on the actual dynamics of "early" genes, the main "resource" of neuronal plasticity, it will be interesting to analyze transcription factors as "products" for creation of new drugs. The choice and substantiation of such a product are partially realized [3].

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REFERENCES

1. O. O. Sokolova, M. B. Stark, P. D. Lisachev, *et al.*, *Byull. Eksp. Biol. Med.*, **148**, No. 8, 195-198 (2009).
2. K. T. Turpaev, *Mol. Biol.*, **40**, No. 6, 945-961 (2006).
3. M. B. Shtark, *Byull. Eksp. Biol. Med.*, Supplement to No. 8, 8-12 (2009).
4. T. Ahmed and J. U. Frey, *Neuropharmacology*, **49**, No. 4, 477-492 (2005).
5. C. M. Alberini, *Physiol Rev.*, **89**, No. 1, 121-145 (2009).
6. M. Dragunow, R. W. Currie, R. L. Faull, *et al.*, *Neurosci. Biobehav. Rev.*, **13**, No. 4, 301-313 (1989).
7. E. Miyamoto, *J. Pharmacol. Sci.*, **100**, No. 5, 433-442 (2006).
8. L. A. Roberts, M. J. Higgins, C. T. O'Shaughnessy, *et al.*, *Brain Res. Mol. Brain Res.*, **42**, No. 1, 123-127 (1996).
9. L. M. Robertson, T. K. Kerppolia, M. Vendrell, *et al.*, *Neuron*, **14**, No. 2, 241-252 (1995).
10. S. Schulz, H. Siemer, M. Krug, and V. Hollt, *J. Neurosci.*, **19**, No. 13, 5683-5692 (1999).