ISSN 0012-4966, Doklady Biological Sciences, 2008, Vol. 421, pp. 241–243. © Pleiades Publishing, Ltd., 2008.
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PHYSIOLOGY

Intranasal Administration of the Peptide Selank Regulates BDNF Expression in the Rat Hippocampus in vivo

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Presented by Academician N.F. Myasoedov December 25, 2007

Received January 11, 2008

DOI: 10.1134/S0012496608040066

INTRODUCTION

There is strong evidence confirming the correlation between stress factors and mammalian central nervous system (CNS) pathologies, such as depressive and anxiety states [1]. At the same time, the pathophysiological effects of stress are associated with the influence on hippocampal functions [2]. In turn, the hippocampal functions, mainly the memory formation, are regulated by brain derived neurotrophic factor (BDNF), whose expression is inhibited by stress and glucocorticoids [3]. The participating of neurotrophins (mainly BDNF) in the learning processes and engram forming in mammalian brain has been shown [4]. BDNF regulates short-term synaptic functions, as well as long-term synaptic potentiation via binding with specific TrkB receptors on postsynaptic neurons [5, 6]. The increase in BDNF mRNA expression in the hippocampus after learning has also been shown [7, 8]. A decrease in endogenous BDNF levels in the rat brain using BDNF antibodies or antisense oligonucleotides impairs learning and memory [4, 8, 9].

On the basis of molecular structure modification of the endogenous regulatory tetrapeptide tuftsin (Thr–Lys–Pro–Arg), which possess a wide spectrum of pharmacological activity, including psychotropic activity, the prolonged-action heptapeptide Selank (Thr–Lys–Pro–Arg–Pro–Gly–Pro) was synthesized in the Department of Regulatory Peptides of the Institute of Molecular Genetics of the Russian Academy of Sciences. Large-scale pharmacological study of Selank performed in the Research Institute of Pharmacology of the Russian Academy of Medical Sciences revealed a unique spectrum of psychotropic activity of this compound, combining the anxiolytic, antidepressant and antiamnestic effects [10–12]. On the basis of these data, as well as on the Selank ability to influence the cognitive brain functions (nootropic action) and anxiety state, we tested the hypothesis that Selank-induced stimulation of BDNF expression on the mRNA and protein levels in the hippocampus is a stage of the effect of this peptide on the CNS.

MATERIALS AND METHODS

Animal experiments. We used male Wistar rats with a body weight of 200–250 g (Central Nursery of Laboratory Animals of the Russian Academy of Medical Sciences). The animals were kept under standard conditions, a 12-h light–dark cycle (light on at 9:00, off at 21:00), and received water and food ad libitum. All conditions were created to minimize stress. The animals of the control and experimental groups were caged together. Selank was administered intranasally at doses of 250 and 500 μg/kg body weight in a volume of 100 μl/kg body weight once between 08:45 and 10:15. Control animals received an equal volume of distilled water. Each group consisted of four animals. Rats were decapitated 1, 3 or 24 h after the Selank administration, and immediately after that, the studied brain regions were isolated on ice. Tissue was frozen on dry ice and stored at –80°C.

RNA extraction and reverse transcriptase polymerase chain reaction. Total RNA was extracted from samples by the phenol–chloroform method using a PeqGold TriFast kit (PeqLab, Germany) as recommended by the manufacturer. The extracted RNA was washed three times in 80% ethanol (4°C), dissolved in 100 μl of water free of DNases, and stored at –80°C. The RNA level was measured by spectrophotometry, and only the samples where the ratio between the optical densities at 260 and 280 nm exceeded 1.6 were used.

Reverse transcription was performed using a Sileks kit (Russia) as recommended by the manufacturer. A

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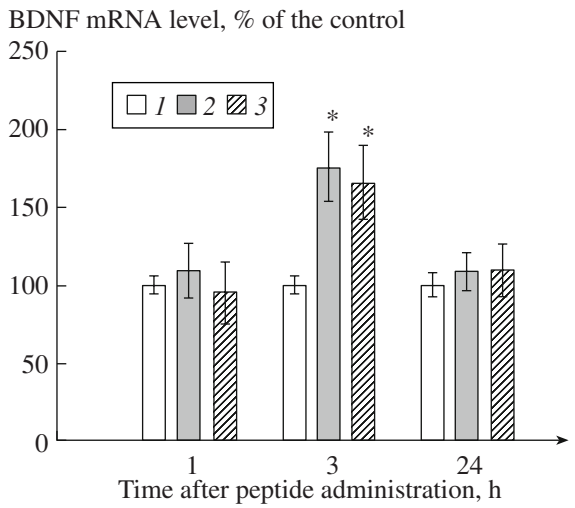

Fig. 1. Effect of Selank on BDNF mRNA expression in the rat hippocampus 1, 3 and 24 h after intranasal administration of the peptide at doses of 250 and 500 μg/kg body weight: *1*, control; *2*, Selank at a dose of 250 μg/kg; *3*, Selank at a dose of 500 μg/kg. * – $p < 0.05$ (one-way ANOVA).

sample of 0.25 μg of total RNA was taken, and the reaction was performed as follows: 1 h at 37°C in 50 μl of a mixture containing 0.1 μl of random hexaprimers, 200 units of Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase, 150 μM deoxytriphosphate nucleotide (dNTP) mixture, 70 mM Tris–HCl (pH 8.3 at 25°C), 16 mM $(NH_4)_2SO_4$, and 7.5 mM $MgCl_2$. After incubation at 70°C for 10 min, the samples were stored at –20°C.

Polymerase chain reaction (PCR) was performed using a Sileks kit (Russia) in a Tertsik DNA amplifier (DNK-Tekhnologiya, Russia) in 25 μl of a reaction mixtur, contained *Taq* buffer, 1.5 mM dNTP mixture, 1.25 units of *Taq* polymerase, 1 μl of complementary DNA, and 10 pmol of each primer. The following primers (Sintol, Russia) were used: for the *gapdh* gene [13], 5'-TCCATGACAACTTTGGCATTGTGG-3' (forward), 5'-GTTGCTGTTGAAGTCGCAGGAGAC-3' (reverse); supposed amplicon length, 376 bp; for the *bdnf* gene [14], 5'-CACAGCGGCAGATAAAAAGA-3' (forward), 5'-CGGCAACAAACCACAAGATT-3' (reverse); supposed amplicon length, 527 bp.

The PCR conditions for the *gapdh* gene were the following: start, 5 min at 95°C; 28 cycles of 45 s at 95°C, 45 s at 66°C, and 45 s at 72°C; and final incubation for 10 min at 72°C. The PCR conditions for the *bdnf* gene were the following: start, 5 min at 95°C; 40 cycles of 45 s at 95°C, 45 s at 62°C, and 45 s at 72°C; and final incubation for 10 min at 72°C.

The conditions and number of PCR cycles were selected in preliminary experiments and provided the proportionality of amplification products to the amount of complementary DNA added into the reaction mixture.

The products were separated by gel electropgoresis in 2% agarose gel, with ethidium bromide UV visualization. The intensity of fluorescence was measured using a BioDocAnalyze system (Biometra, Germany), and the relative values of complementary DNA initial concentrations were obtained using the manufacturer's software. Normalization of the *bdnf* gene expression level was carried out relative to the *gapdh* gene expression level.

Immunoenzyme assay. To obtain protein extracts, the tissue was weighed, placed into the reaction buffer solution (pH 7.7) containing 100 mM Tris–HCl, 400 mM NaCl, 0.4% Triton X-100, 1 mM PMSF (phenylmethylsulfonyl fluoride), 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM benzamidine, and dissociated by pipetting. Then, the tissue was homogenized with a polytron (Tissue Tearor Biospec Products, United States) at 4°C. The homogenated samples were then centrifuged at 15 000 *g* and 4°C for 30 min, the supernatant was removed, and the obtained extracts were stored at –80°C. The total protein concentration was measured by the Lowry method. The BDNF concentration in the samples was measured by sandwich-modified ELISA using a BDNF E_{max} Immumoassay System kit (Promega, United States) as recommended by the manufacturer. Subsequent normalization was performed with respect to the total protein concentration in the sample.

The significance of differences was estimated using one-way ANOVA. Differences were regarded as significant at $p < 0.05$. The data on the figures is shown as the group mean ± standard error of the mean ($n = 4$).

RESULTS AND DISCUSSION

Effect of Selank on BDNF mRNA expression in the rat hippocampus. We investigated the influence of Selank on BDNF mRNA expression in the rat hippocampus after intranasal introduction of the peptide at doses of 250 and 500 μg/kg body weight after 1, 3 or 24 h after administration (Fig. 1). As shown in the figure, Selank significantly increased the concentration of BDNF mRNA in the hippocampus by a factor of 1.5–2 compared to the control 3 h after administration at a dose of 250 or 500 μg/kg body weight. There was no significant change in the mRNA level of this neurotrophic factor 1 or 24 h after peptide administration.

Effect of Selank on the BDNF protein level in the rat hippocampus. The influence of Selank intranasal administration on the *bdnf* gene protein product level was estimated at the next stage of the study. A statistically significant 30% increase relative to the control value was found in the rat hippocampus BDNF level 24 h after the peptide administration at a dose of 250 or 500 μg/kg body weight (Fig. 2). On the other hand, a statistically significant 40% decrease in the hippocampal BDNF level was observed 3 h after the Selank administration at a dose of 500 μg/kg body weight,

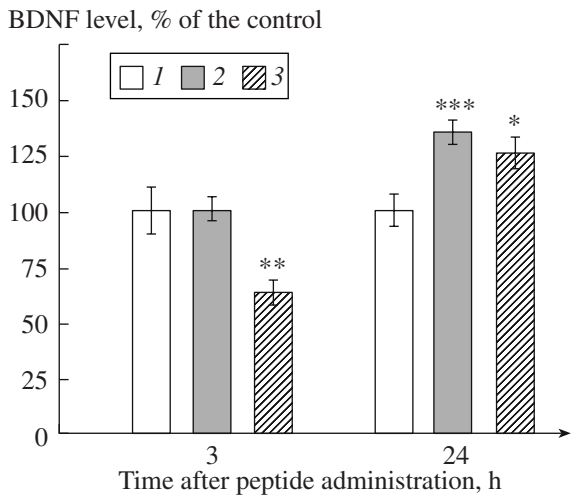

Fig. 2. Effect of Selank on BDNF level in the rat hippocampus 3 and 24 h after intranasal administration of the peptide at doses of 250 and 500 μg/kg body weight: *1*, control; *2*, Selank at a dose of 250 μg/kg; *3*, Selank at a dose of 500 μg/kg. * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$ (one-way ANOVA).

while there was no change in the hippocampal BDNF level in rats injected with Selank at a dose of 250 μg/kg body weight. The increase in both BDNF mRNA and protein levels suggests that Selank stimulates the expression of this neurotrophic factor exactly in the hippocampal cells, rather than its axonal transport from distant brain areas. The results indicate that Selank, possessing anxiolytic and nootropic properties, can regulate the hippocampal BDNF level in rats. To date, no other anxiolytic compounds are known to regulate BDNF expression in the mammalian brain. However, there are some examples of such compounds among substances with nootropic activity. For example, we demonstrated earlier that the peptide Semaks (Met–Glu–His–Phe–Pro–Gly–Pro), the effective agent of the nootropic drug Semaks, 0.1% solution, stimulated the expression of several neurotrophic factors, including BDNF, in the rat hippocampus and basal forebrain [15]. Thus, the Selank effects on processes in the CNS may be connected with changes in the expression of some genes, especially the BDNF gene.

ACKNOWLEDGMENTS

The study is supported by the Russian Foundation for Basic Research (project no. 05-04-49187).

REFERENCES

1. Esch, T., Stefano, G.B., Fricchione, G.L., et al., *Neuroendocrinol. Lett*, 2002, vol. 23, no. 3, pp. 199–208.
2. Phillips, L.J., McGorry, P.D., Garner, B., et al., *Aust. N. Z. J. Psychiatry,* 2006, vol. 40, no. 9, pp. 722–724.
3. Smith, M.A., Makino, S., Kvetnansky, R., et al., *J. Neurosci.*, 1995, vol. 15, no. 3, p. 1768.
4. Alonso, M., Vianna, M.R., Depino, A.M., et al., *Hippocampus*, 2002, vol. 12, no. 4, pp. 551–560.
5. Minichiello, L., Korte, M., Wolfer, D., et al., *Neuron*, 1999, vol. 24, no. 2, pp. 401–414.
6. Kovalchuk, Y., Hanse, E., Kafitz, K.W., et al., *Science*, 2002, vol. 295, no. 5560, pp. 1729–1734.
7. Hall, J., Thomas, K.L., and Everitt, B.J., *Nat. Neurosci.*, 2000, vol. 3, no. 6, pp. 533–535.
8. Mizuno, M., Yamada, K., Olariu, A., et al., *J. Neurosci.*, 2000, vol. 20, no. 18, pp. 7116–7121.
9. Mu, J.S., Li, W.P., Yao, Z.B., et al., *Brain Res.*, 1999, vol. 835, no. 2, pp. 259–265.
10. Kozlovskaya, M.M., Sarkisova, K.Yu., and Kozlovskii, I.I., *Psikhofarmakol. Biol. Narkol.*, 2005, vol. 5, no. 2, pp. 939–945.
11. Seredenin, S.B., Kozlovskaya, M.M., Blednov, Yu.A., et al., *Zh. Vyssh. Nervn. Deyat.*, 1998, vol. 48, no. 1, pp. 153–160.
12. Semenova, T.P., Kozlovskaya, M.M., and Val'dman, A.V., *Zh. Vyssh. Nervn. Deyat.*, 1988, vol. 38, no. 6, pp. 1033–1040.
13. Tabuchi, A., Sakaya, H., Kisukeda, T., et al., *J. Biol. Chem.*, 2002, vol. 277, no. 39, pp. 35920–35931.
14. Miller, R., King, M.A., Heaton, M.B., et al., *Brain Res.*, 2002, vol. 950, nos. 1/2, pp. 137–147.
15. Dolotov, O.V., Seredenina, T.S., Levitskaya, N.G., et al., *Dokl. Biol. Sci.*, 2003, vol. 391, no. 1, pp. 292–295 [*Dokl. Akad. Nauk*, 2003, vol. 391, no. 1, pp. 131–134].