Article

Transcriptomic reaction of the rat hippocampus and spleen to singular and course injection of selank peptide

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Abstract: The *aim* of the study was to evaluate the effect of the regulatory peptide selank on the action and expression of the genome. *Methods*. In order to do this, we needed to locate the genes responsible for the change in expression in the hippocampus and spleen of a rat. Male Wistar rats were used in the experiment. The animals were split into 3 groups (of 8 rats with the average weight of 260 grams): control group (C), singular injected group (SI) and injected over a course (CI). 2 groups- C and SI were intranasally injected with water over the course of 5 days once a day, group CI was injected with a watered solution of selank (200 mcg/kg). *Results*. During the first stage, hybridization was carried out on a micromatrix for RNA from hippocampal tissues. The data obtained showed that both single and course administration of Selank changed the expression of 5 genes by more than 2 times. Since Selank is also an antiviral drug, of particular interest is the study of the mechanism of action of this peptide on the expression of these five genes in the rat spleen. A quantitative assessment carried out during the study showed that the effect of Selank on the expression of the five selected genes in the rat spleen was much more pronounced than in the hippocampus. In the spleen, there is an increase in the expression of all selected genes. The strongest increase (more than 4.5 times) was noted after a single injection of the peptide. With a course introduction, the effect of the peptide is less pronounced, the expression of selected genes increases by no more than 2 timesThe data obtained showed that both single and course administration of selank changed the expression of 5 genes by more than 2 times. The change of expression in the CX3CR1 gene is of particular interest because it is involved in the regulation of inflammatory processes. *Conclusions*. Our data indicate that selank may be involved in the regulation of inflammatory processes in the body. The complex biological effects of selank on the body may at least partially be due to the systemic effect of the peptide on genome expression. This mechanism of action of peptides opens up new opportunities for directed changes in the transcriptional profile under the action of oligopeptides, homologues of natural bioactive peptides. However, this will require further study of the mechanisms of action of peptides, including selank, on different systems of the body and the processes occurring in them.

Keywords: Transcriptomic Response, CX3CR1 Expression, Selank peptide.

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1. Introduction

An innovation in the field of pharmacology, is creating new drugs that are affectively able to lower the level of anxiety without side effects, using endogenous regulative peptides. "Selank"- is a new drug that has been synthesized at the laboratories of the Institute of Molecular Genetics of the Russian academy of sciences and the Research Institute of Pharmacology (im. Zakusova) of the Russian academy of medical sciences, the active substance of which is a synthetic peptide - an analogue of a short fragment of the heavy chain of human immunoglobulin G Thr–Lys–Pro–Arg, elongated from the C end with the tripeptide Pro–Gly–Pro. Selank has been proven to be an effective stable nootropic and anxiolytic drug, while promoting the survival of brain cells during hypoxia, and also having an antiviral effect. Research in the recent years has shown that many peptides like semax, can alter the expression of the genome. Therefore, given the selank is also a regulative peptide, it will be interesting to research its influence on the expression of the genome. In

order to do that, we had to localise the genes responsible for altering the expression of the genes in hippocampus and spleen of a rat under the influence of said peptide.

2. Methods

Male Wistar rats were used in the experiment. The animals were split into 3 groups (of 8 rats with the average weight of 260 grams): control group (C), singular injected group (SI) and injected over a course (CI).

2 groups- C and SI were intranasally injected with water over the course of 5 days once a day, group CI was injected with a watered solution of selank (200 mcg/kg). on the sixth day group SI was injected with the watered solution of Selank (200 mcg/kg). After one hour the animals were decapitated. Using the RNAgentsTM Total RNA Isolation System ("Promega", USA) a total strand of RNA was isolated out of the hippocampus and spleen, then using the "RevertAid™H Minus First Strand with DNA Synthesis Kit" ("Fermentas", Lithuania) a strand of coking DNA was synthesized. The influence of selank on the expression of genes was analyzed via a micromatrix SBC–R–RC–100–13, containing 12000 genes ("Shanghai Biochip™", China). A quantitative assessment of the level of expression of individual genes was carried out using real-time PCR on the device "Mx3000P™ RealTime QPCR System" ("Stratagene Equipment", USA) using the SYBR Green I ("Syntol", Russia) and primers RT2 qPCR Rrimer Assay SYBR® Green ("Super Array", USA). The obtained values of reaction cycle thresholds (Ct) were normalized to Ct of the gene of the ribosomal protein L3 and statistically processed using the software "Relative Expression Software Tool384", version 2 [8].

3. Results

During the first stage, hybridization was carried out on a micromatrix for RNA from hippocampal tissues. The data obtained showed that both single and course administration of Selank changed the expression of 5 genes by more than 2 times (Table 1).

Since Selank is also an antiviral drug, of particular interest is the study of the mechanism of action of this peptide on the expression of these five genes in the rat spleen. A quantitative assessment carried out during the study showed that the effect of Selank on the expression of the five selected genes in the rat spleen was much more pronounced than in the hippocampus. In the spleen, there is an increase in the expression of all selected genes. The strongest increase (more than 4.5 times) was noted after a single injection of the peptide. With a course introduction, the effect of the peptide is less pronounced, the expression of selected genes increases by no more than 2 times (Table 1).

Table 1. Relative changes in gene expression in the hippocampus and spleen of rats under the influence of a single (SI) and course (CI) administration of the selank peptide compared with the control.
*p<0.05;**p<0.01

Gene	Name of Gene	Hippocampus		Spleen	
		SI	CI	SI	CI
ACTN1	Actinin Alpha 1	0.42*	0.49*	15.96**	1.97**
CX3CR1	C-X3-C Motif Chemokine Receptor 1	0.46*	2.77*	15.93**	1.87**
FGF7	Fibroblast Growth Factor 7	2.35*	2.23*	8.15**	2.08**
PTPRN2	Protein Tyrosine Phosphatase Receptor Type N2	2.61*	3.01*	70.36**	1.09
XTRP3	Sodium- and chloride-dependent transporter XTRP3	3.98*	2.13*	4.47*	1.19

4. Discussion

The presented study is one It should be noted that for the ACTN1 and CX3CR1 genes, selank has a multidirectional effect on their expression in the rat hippocampus and spleen. The expression of the ACTN1 gene after a single and course administration and of the CX3CR1 gene after a single administration in the hippocampus is significantly reduced. In the spleen, on the contrary, there is an increase in the expression of these genes, which is especially significant after a single administration of the peptide. The greatest change in expression was observed in the spleen for three genes - PTPRN2, ACTN1 and CX3CR1 after a single injection of the drug. At the same time, the maximum increase in expression (by 70 times) was noted for the PTPRN2 gene. This gene encodes an integral glycoprotein involved in the regulation of transmembrane signaling [4]. PTPRN2 is an important autoantigen in insulin-dependent diabetes and may play a pathogenic role in the development of this disease [11]. For the ACTN1 and CX3CR1 genes, it was shown that a single administration of selank leads to a 16-fold increase in their expression level. The ACTN1 gene encodes a calcium-sensitive protein that cross-links F filaments of actin, which plays an important role in maintaining the viscosity and elasticity of the cytoplasm, which is necessary to preserve the integrity of macromolecules associated with the plasma membrane [12]. The change in the expression of the CX3CR1 gene that we found is of particular interest, since this gene is involved in the regulation of inflammatory processes. The CX3CR1 gene encodes a specific receptor for the fractalkine protein belonging to the serpentine receptor family and is involved in the maturation, transfer, and recycling of leukocytes and in the initiation of local inflammation as a result of the involvement of inflamatory cells in the chemotaxis process [5, 9].

The interaction of fractalkine with CX3CR1 can act as a regulator of communication between neurons and microglia in the brain and participate in the activation and migration of microglia [7]. Some data indicate that the CX3CR1 gene plays the role of a neuroprotector and is able to inhibit apoptosis [6]. In addition, studies show that CX3CR1 can function as a co-receptor for HIV1 entry into the cell [2].

5. Conclusions

Our data indicate that selank may be involved in the regulation of inflammatory processes in the body. The complex biological effects of selank on the body may at least partially be due to the systemic effect of the peptide on genome expression. This mechanism of action of peptides opens up new opportunities for directed changes in the transcriptional profile under the action of oligopeptides, homologues of natural bioactive peptides. However, this will require further study of the mechanisms of action of peptides, including selank, on different systems of the body and the processes occurring in them.

Conflicts of Interest: The authors declare no conflict of interest.

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