

# Effect Of Epitalon On Interleukin-1 $\beta$ Signal Transduction And The Reaction Of Thymocyte Blast Transformation Under Stress

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## Abstract

**OBJECTIVES:** The aim of this research consisted in studying the effects of tetrapeptide Epitalon on both thymocyte proliferation and interleukin-1 $\beta$  (IL-1 $\beta$ ) signal transduction via sphingomyelin pathway in the cerebral cortex membranes of mice exposed to stresses exerting diverse effects upon humoral immune response. **DESIGN AND SETTING:** The experiments were performed on male (CBA $\times$ C<sub>57</sub>BL<sub>6</sub>) F<sub>1</sub> mice aged 8-10 weeks. Two models of experimental stress were used: immune-stimulatory rotation stress and immune-suppressive combined stress (cooling followed by immobilization). The concomitant effect of Epitalon was determined according to its influence on thymocyte proliferation stimulated by concanavalin A at a sub-optimal dose and recombinant IL-1 $\beta$ . The activity of membrane neutral sphingomyelinase (nSMase), the key enzyme of the sphingomyelin signal transduction pathway, was assayed according to modified Rao and Spence's method (1976). **RESULTS:** The investigation demonstrated that Epitalon increased thymocyte proliferative activity, both enhanced under rotation stress and suppressed under combined one. It also increased IL-1 $\beta$  concomitant effect. These findings corresponded to Epitalon effect on diverse stress-induced changes in nSMase activity in cerebral cortex fraction P2. Epitalon activated nSMase in the cerebral cortex membranes of intact mice and increased IL-1 $\beta$  stimulatory effect on the enzyme activity. **CONCLUSIONS:** The obtained results provided a conclusive evidence of Epitalon stress-protective effect at the level of IL-1 $\beta$  signal transduction via sphingomyelin pathway in the nerve tissue, as well as at the level of target thymocyte proliferation.

#### List of abbreviations

AB	– antibodies
PFC	– plaque forming cells
IL-1	– interleukin-1
Con A	– concanavalin A
nSMase	– neutral sphingomyelinase
RTBT	– reaction of thymocyte blast transformation
rIL-1 $\beta$	– recombinant interleukin-1 $\beta$
SM	– sphingomyelin
c.p.m.	– counts per minute

## INTRODUCTION

The maintenance of the organism resistance to damaging factors and diseases of various etiology, as well as the prospects of active life prolongation and inhibition of aging much depend upon the preservation of both innate and acquired (immunological) defense functions and the mechanisms of their regulation. Hence, experimental investigations and subsequent clinical application of substances modulating the activity of these mechanisms and promoting their functional safety are of high priority today.

Tetrapeptide Epitalon (Ala-Glu-Asp-Gly) belongs to the most promising medications. It has been synthesized at the St. Petersburg Institute of Bioregulation and Gerontology on the basis of the amino acid analysis of Epithalamin, a complex peptide substance extracted from the pineal gland. High biological activity of these bioregulators has been confirmed experimentally [4, 6, 7].

As it has been shown before, small peptides administered intramuscularly or intravenously do not only modulate immunological reactions but also influence brain functions and, thus, participate in the interactions of neuroendocrine and immune systems. Epitalon is found capable to restore some disturbed neuroendocrine processes and, namely, to stimulate melatonin synthesis and normalize cortisol secretion circadian rhythm in senescent monkeys [6].

Especially interesting are the effects of Epitalon on the indices of defense functions changed (increased or lowered), for instance, under stress of various origin, intensity and duration, and the modulating effect of this peptide on the activity of natural endogenous bioregulators.

Cytokine interleukine-1 (IL-1) is one of the key endogenous regulators of defense functions. It is the most important mediator of neuroimmune interactions indispensable in the development of stress reaction [1, 2, 9]. Over the recent years, the sphingomyelin (SM) pathway has been found to play the principal role in intracellular IL-1 $\beta$  signal transduction. This pathway is initiated by the hydrolysis of membrane SM to the secondary cellular messenger ceramide under the effect of membrane enzyme nSMase, the key enzyme of the sphingomyelin cascade [3, 8, 11, 12, 13].

This research has been designed to study Epitalon effects on IL-1 $\beta$  signal transduction via SM pathway in the membranes of cerebral cortex nerve cells and on the blast transformation reaction of murine thymocytes under stress impacts diversely affecting humoral immune response.

## MATERIAL AND METHODS

The investigation was carried out on 8–10-week old male mice, the 1<sup>st</sup> generation hybrids of (CBA x C<sub>57</sub>BL<sub>6</sub>)F<sub>1</sub>, weighing 18–20 g. The animals were kept under the conditions of a 12-hours' light/darkness interchange with an unrestricted access to water and food (standard rations according to the norms for laboratory animals). The animals had been adapted to the experimental conditions for 7 days.

Two models of experimental stress were used in this work:

- rotation stress, i.e. revolving of the animals in a container at 78 rev/min for an hour, 10 minute rotations interchanged with 5 minute intervals;
- combined stress, i.e. cooling of the animals in individual containers for 2 hours at 4–5°C with their subsequent 18 hours' immobilization at room temperature.

Epitalon ability to activate lymphocytes and/or modulate IL-1 $\beta$  concomitant effect was defined according to their effects upon the proliferation of murine thymocytes stimulated by a sub-optimal dose of Con A and rIL-1 $\beta$  (Sigma, USA) [15].

Membrane fraction P2 was obtained from the cerebral cortex of mice according to E.G. Lapetina et al. (modified method, 1967).

The specific activity of nSMase in the cerebral cortex membrane fractions was assayed according to the modified method of B. Rao and M. Spence (1976).

The activity of nSMase was assessed according to the level of marked [<sup>14</sup>C]-phosphoryl choline transferred in the process of [<sup>14</sup>C]-SM hydrolysis to the water phase, which was formed after precipitating the membranes with 100% trichloroacetic acid.

To bind Epitalon and rIL-1 $\beta$  with the membranes of cerebral cortex nerve cells, Epitalon at various concentrations (0.1; 1; 10; 50; 100 ng/ml) and rIL-1 $\beta$  with the specific activity of 10<sup>8</sup> units/mg of protein at 10<sup>-9</sup> M (State Scientific Center "Research Institute of Extra-Pure Biopreparations", St. Petersburg) had been prior incubated with the aliquots of cerebral cortex fractions P2 in a water bath for 30 min at 37°C. Instead of the peptide preparations, equal volumes of PBS-buffer were applied to control samples. The specific activity of nSMase was calculated in nM of [<sup>14</sup>C]-SM/mg of protein/min and expressed percent of its baseline level.

The experimental results were statistically processed with the application of t-Student criterion. The data were presented as arithmetical mean  $\pm$  standard errors. The difference was considered significant at  $p < 0.05$ .

## RESULTS

### 1. Cellular molecular mechanisms of stress reaction in mice under rotation and combined stress impacts

We studied the effect of rotation stress upon the intensity of humoral immune response to ram erythrocytes. In this experiment, rotation stress was proven not to inhibit humoral immune response, which was assessed by total AB titers in blood serum and by the amount of PFC in the spleen. On the opposite, the response tended to increase. No signs of destruction in the secretion segment of the stomach mucous membrane were observed. The weight of the thymus and the spleen remained almost unchanged.

Contrariwise, the combined impact exerted an expressed decrease in the number of PFC in the spleen and total AB titers in murine blood, i.e. it provoked humoral immune response suppression and significant changes in the thymic-lymphatic system: the weight of the thymus decreased 1.3–2.1 times and that of the spleen – 1.2–1.4 times. The stomach mucous membrane developed numerous erosions (10–13 per mouse).

### 2. Epitalon effect on the proliferative activity of murine thymocytes stimulated with Con A and IL-1 $\beta$ under rotation and combined stress impacts

We studied the effect of rotation immune-stimulating stress on the proliferative activity of murine thymocytes induced by lectine Con A at the sub-optimal dose of 0.75  $\mu$ g/ml and intensified by the concomitant effect of rIL-1 $\beta$  at 250 ng/ml (without Epitalon). In this exper-

iment, rotation stress was found to increase RTBT by 64% (incubation of cells with Con A) and by 73% (incubation of cells with Con A and rIL-1 $\beta$ ) as compared to RTBT level in intact animals, whose thymocytes were stimulated with the same agents (Figure 1).

At the concentration of  $2.5 \times 10^{-5}$  Epitalon intensified RTBT in stress-exposed mice by 70% (incubation of thymocytes with Con A). In case of concomitant stimulation of the cells with Con A and rIL-1 $\beta$ , the peptide intensified RTBT on average by 90–120% in the range of concentrations from  $2.5 \times 10^{-3}$  to  $2.5 \times 10^{-7}$  (Figure 1).

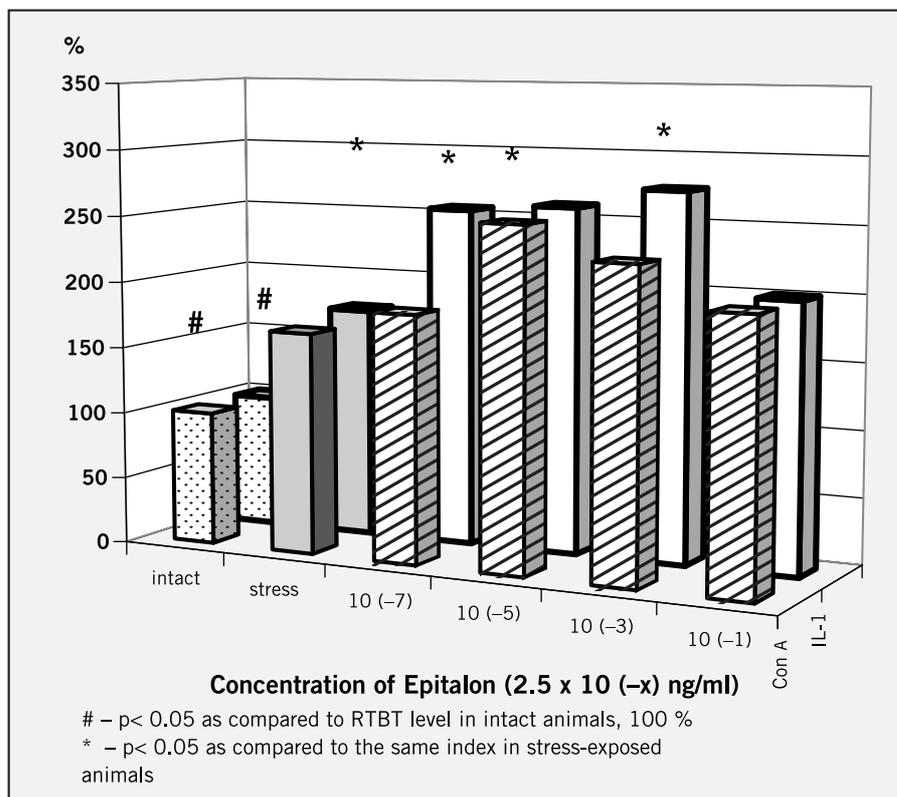
The combined stress was entailed by cooling and immobilization of the animals and induced a pronounced immune suppression decreasing RTBT intensity by 44% (incubation with Con A) and by 68% (Con A + rIL-1 $\beta$  stimulation of thymocytes) (Figure 2).

Epitalon was effective as an RTBT stimulant in stress-exposed animals at the concentration of  $2.5 \times 10^{-1}$  ng/ml (incubation with Con A) and at all the tested concentrations from  $2.5 \times 10^{-1}$  to  $2.5 \times 10^{-7}$  ng/ml (incubation with Con A + rIL-1 $\beta$ ) (Figure 2).

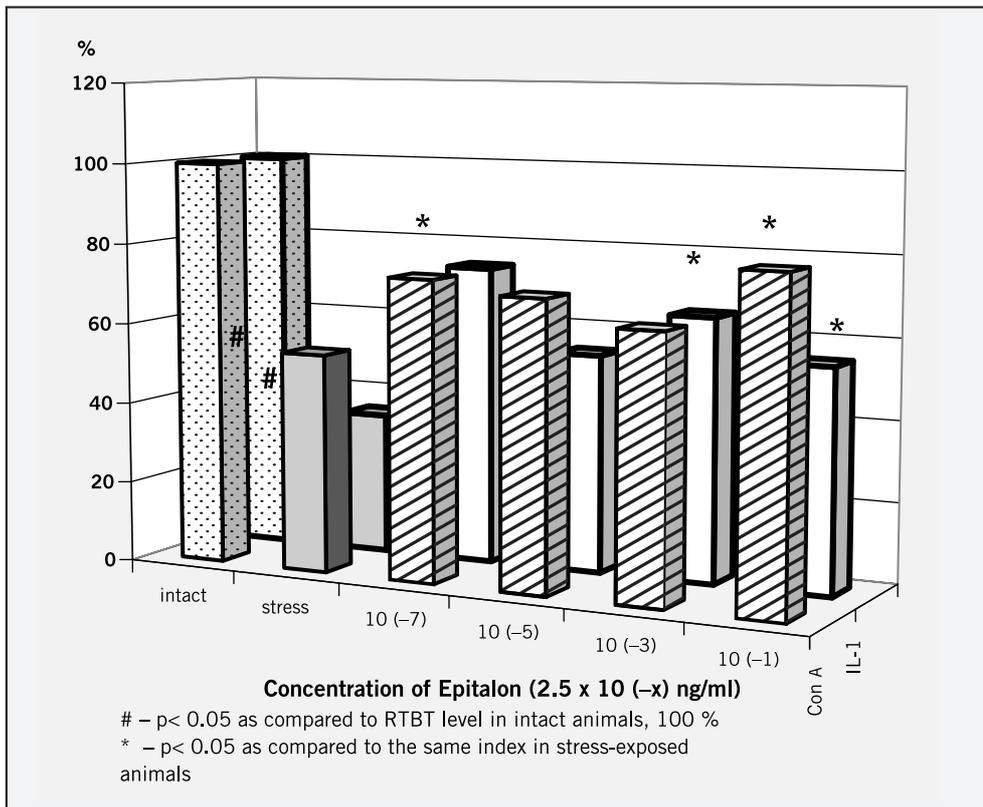
### 3. Concomitant effect of IL-1 $\beta$ and Epitalon on the activity of nSMase in the membranes of murine nerve cells

Since nSMase is an enzyme initiating the SM pathway of signal transduction, any change in its activity reflects the intensity of SM cascade.

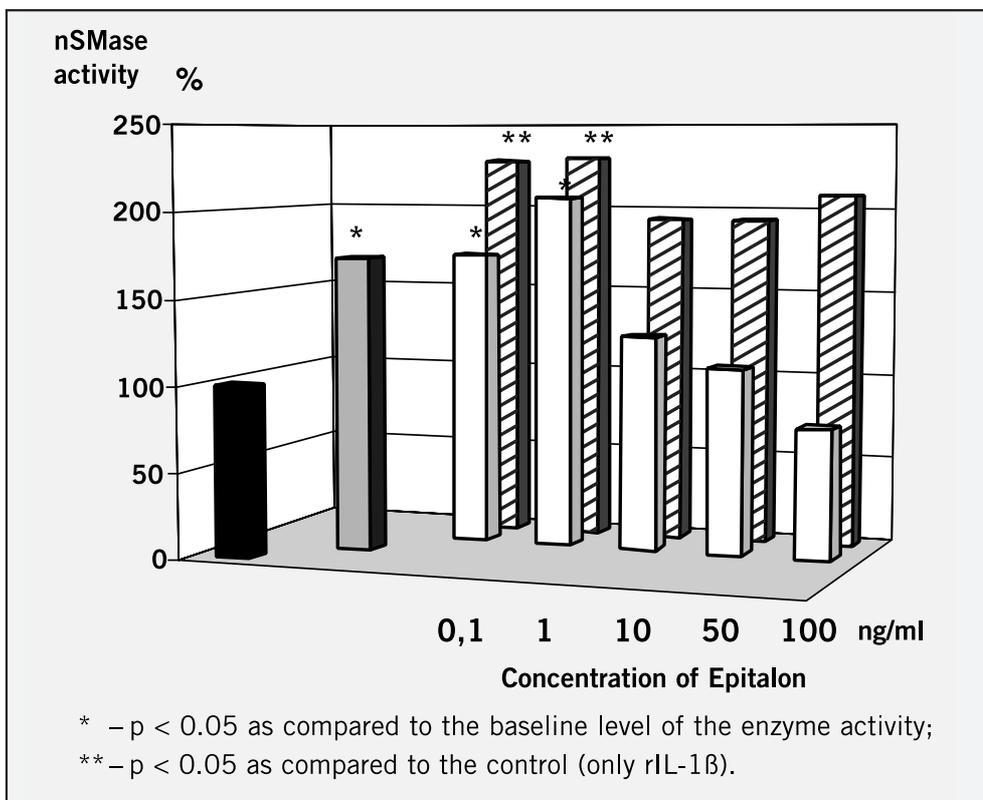
We studied the concomitant modulating effect of rIL-1 $\beta$  and Epitalon on the activity of nSMase – the key enzyme of the SM pathway of IL-1 $\beta$  signal transduction. Membrane fraction P2 obtained from the mice'



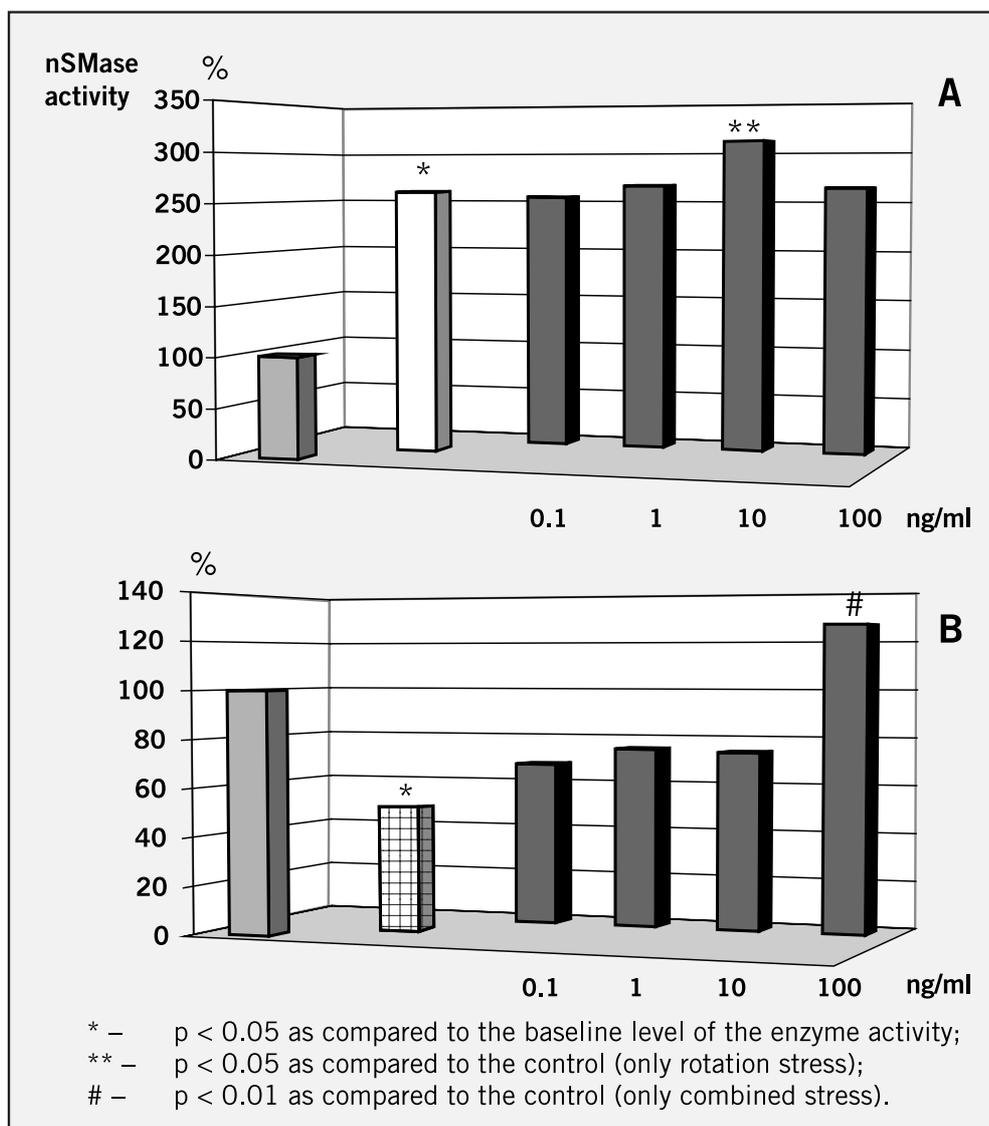
**Figure 1.** Epitalon effect on the proliferation of murine thymocytes stimulated with a sub-optimal dose of Con A and IL-1 $\beta$  after rotation stress, % of RTBT in intact animals.



**Figure 2.** Epitalon effect on the proliferation of murine thymocytes stimulated with a sub-optimal dose of Con A and IL-1 $\beta$  after combined stress, % of RTBT in intact animals.



**Figure 3.** Combined effect of rIL-1 $\beta$  ( $10^{-9}$  M) and Epitalon on the activity of neutral sphingomyelinase in membrane fraction P2 of murine cerebral cortex:  
 Black column – intact animals (baseline level of nSMase activity);  
 Grey column – control (rIL-1 $\beta$ ,  $10^{-9}$  M);  
 White columns – only Epitalon;  
 Striped columns – Epitalon + rIL-1 $\beta$ ,  $10^{-9}$  M.



**Figure 4.** Epitalon effect on the activity of neutral sphingomyelinase in membrane fraction P2 of murine cerebral cortex changed after rotation (a) and combined (b) stress:  
 Grey columns – intact animals (baseline level of nSMase activity);  
 White column – control (rotation stress);  
 Striped column – control (combined stress);  
 Black columns – Epitalon under stress.

cerebral cortex hemispheres was incubated until the enzyme reaction was initiated with rIL-1 $\beta$  at  $10^{-9}$  M, the concentration, at which the cytokine produced its highest stimulating effect on nSMase specific activity [13], and with Epitalon at 0.1; 1; 10; 50; 100 ng/ml.

The introduction of Epitalon into the incubation medium at 0.1 ng/ml and 1 ng/ml without rIL-1 $\beta$  was found to stimulate nSMase activity in the fraction P2 of murine cerebral cortex. The combined application of Epitalon and rIL-1 $\beta$  resulted in a more expressed stimulation of nSMase activity in the fraction as compared to the activity of this enzyme in the control samples containing only rIL-1 $\beta$  at  $10^{-9}$  M (Figure 3).

#### 4. Epitalon effect on nSMase activity in the membranes of nerve and immune-competent cells of mice subjected to rotation and combined stress impacts

The last experimental session was designed to investigate Epitalon effect on nSMase activity in the membranes of murine nerve cells against the background of stress-modified defense functions. Epitalon was added to cerebral cortex fractions P2 of the stress-exposed mice at 0.1; 1; 10; and 100 ng/ml.

Under rotation stress intensifying nSMase activity in fraction P2, Epitalon at 10 ng/ml stimulated the enzyme activity already increased after rotation stress, as compared to the control (Figure 4A).

In case of combined stress suppressing nSMase specific activity in fraction P2, Epitalon at 100 ng/ml stimulated the enzyme activity restoring it up to its baseline level (Figure 4B).

## DISCUSSION

In this investigation, the biological activity of synthetic tetrapeptide Epitalon in the model of stress-modified reaction of murine thymocyte blast transformation was analyzed in combination with its effect on the SM pathway of rIL-1 $\beta$  signal transduction in nerve membranes modified by immune-stimulating and immune-suppressing stress impacts.

Over the recent years, the stress-protective effect of some peptide and protein immune-regulators, such as defensins, IL-1 [9, 16], has been demonstrated in a number of investigations, which makes this work more significant.

The obtained experimental results enable a conclusion on Epitalon ability to increase RTBT in mice exposed both to rotation and combined stress impacts. Consequently, Epitalon intensifies the proliferative activity of murine thymocytes raised by rotation stress and suppressed by combined stress.

These findings also confirm Epitalon modulating effect on IL-1 $\beta$  signal transduction via SM pathway in cerebral cortex nerve membranes assessed by nSMase activity alterations.

The data on Epitalon ability to enhance IL-1 $\beta$  effect on nSMase activity have been obtained for the first time and are definitely very important. This ability implies the modulating effect of Epitalon on IL-1 $\beta$  signal transduction via SM pathway in murine nerve membranes, which is manifested as a more pronounced increase in the enzyme activity in case of the combined application of the peptide and IL-1 $\beta$ .

The obtained results on Epitalon influence on stress-modified nSMase activity in murine cerebral cortex membranes deserve a special notice. Epitalon-promoted intensification of nSMase activity in the nerve membranes of mice subjected to immune-stimulating rotation stress can be apparently explained by the peptide ability to increase the enzyme-stimulating effect of endogenous IL-1 $\beta$ , which increases in the blood of stress-exposed animals. Under immune-suppressing combined stress Epitalon at high doses restores nSMase activity in nerve cell membranes up to its baseline level.

Consequently, the investigation using the model of immune-suppressing experimental stress for the first time confirms Epitalon stress-protective effect in two experimental models – the mitogen- and IL-1 $\beta$ -induced reaction of thymocyte blast transformation and IL-1 $\beta$  signal transduction via SM pathway in murine cerebral cortex membranes.

These results conform to the earlier reported modulating effects of Epitalon both on immunological and neuroendocrine processes and can serve as a fundamental prerequisite for devising a method of its application in the correction of immune dysfunction and disturbed immune-neuroendocrine interactions [5].

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