# Effects of Bioactive Factors of the Pineal Gland on Thymus Function and Cell Composition of the Bone Marrow and Spleen in Mice of Different Age

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The effects of factors from the pineal gland on the titer of thymic serum factor in the supernatant of 3-h thymus stroma cultures, number of stromal precursor fibroblasts and $CD4^+$ cells in the bone marrow, and $CD8^+$ cells in the spleens of adult and old CBA mice were studied *in vitro*. Epithalamin, Epithalon, and melatonin appreciably increased the titer of thymic serum factor in the supernatant of thymus stroma cultures from mice of different age and increased the percentage of $CD4^+$ cells in the bone marrow suspension from old animals *in vitro*. The percentage of $CD8^+$ lymphocytes decreased after incubation of splenic cells from old mice with melatonin. The percentage of bone marrow fibroblast precursor cells from adult and old mice did not appreciably change after incubation with the preparations.

**Key Words:** *Epithalamin; Epithalon; melatonin; thymus; bone marrow*

The pineal gland plays a special role in the neuroendocrine effects on the immune system during aging [7, 14]. On the other hand, the age-associated dysfunction of its peripheral component depends on the severity of disorders in the hormonal function of the thymus and in the function of the bone marrow microenvironment, which is performed, among other components, by stromal fibroblasts and T helper cells [2,4,12]. We showed that injections of indole and peptide factors of the pineal gland to adult and old CBA mice increased blood level of thymic serum factor (TSF), number of stromal fibroblast precursor cells (SPC-F) and T helper cells in the bone marrow [5,10]. This was paralleled by a decrease in splenic T suppressor count in old mice. Apart from these *in vivo* effects, we detected a direct effect of Epithalamin and melatonin on the levels of some lymphokines in the supernatant of 3-h splenocyte cultures from adult and old CBA mice [5].

Here we studied the direct effects of melatonin, Epithalamin, and synthetic tetrapeptide Epithalon on the capacity of the thymic stromal component to TSF secretion and on the number of SPC-F and T cells with the regulatory functions in the bone marrow and spleen in CBA mice of different age.

## MATERIALS AND METHODS

The study was carried out on adult (4-5 months) and old (23-24 months) male CBA mice from Breeding Center of Institute of Gerontology. The thymus, bone marrow, and spleen were collected in the morning after decapitation under ether narcosis.

TSF was measured in supernatants of short-term cultures of mouse thymus stroma [5]. To this end, the cells were isolated from the organ, and the stroma was incubated at 37°C for 3 h in 1.0 ml medium without preparations (control), and with the following preparations: 0.1 and 1.0 mg Epithalamin, 0.1 μg Epithalon, 25 pg and 100 pg melatonin. Control and experimental supernatants were filtered through CF-25 ultrafilter (Amicon) and used for TSF titration [8]. The results were expressed in the hormone titer $\log_2$.

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The number of SPC-F was evaluated by culturing $10^6$ bone marrow cells in monolayer cultures [9]. In parallel, the same number of cells was cultured with 0.1 mg Epithalamin and 25 pg melatonin. After 12 days all cultures were fixed in 96% ethanol and stained with Azur-eosin. Colonies containing at least 50 cells grown in the presence and absence of the test preparations were counted.

For detection of T cells with regulatory functions, $10^6$ bone marrow and splenic cells were incubated for 1 h at 37°C in 0.1 ml medium 199 (control) or in 0.1 ml medium containing 0.1 mg Epithalamin, 0.1 μg Epithalon, 125 pg melatonin (for bone marrow cells) and 25 pg melatonin for splenocytes. After washout the percentage of $CD4^+$ cells (bone marrow) and $CD8^+$ cells (spleen) was evaluated by the immunofluorescent method [3]. Biotinylated monoclonal antibodies to L3T4 and Lyt-2 (Sigma) were used. $CD4^+$ and $CD8^+$ cells (in per cent of 300,000 cells) were counted on a FACStar Plus cytofluorometer (Becton Dickinson).

The data were statistically processed using Student's *t* test.

## RESULTS

The titer of TSF in the thymus stroma supernatant from old mice was notably lower than in adult animals (Table 1). *In vitro* treatment with the peptide preparations appreciably increased this parameter in mice of different age. The effect of epithalamin *in vitro* depended on its dose and animal age. In adult mice the titer of TSF increased significantly only in response to high dose of epithalamin, while in old mice it increased even in response to 0.1 mg. The effect of epithalon on TSF titer in adult and old mice manifested in response to a dose which was 10,000 and 1000 times lower, respectively, than the effective epithalamin dose in mice of the same age.

**TABLE 1.** *In vitro* Effect of Factors of the Pineal Gland on TSF Titer in Supernatants of 3-Hour Cultures of Thymus Stroma from Mice of Different Age ($log_2$; $n$=6)

| Preparation, dose | Adult mice | Old mice |
|---|---|---|
| Control (medium 199) | 4.1±0.7 | 1.3±0.4$^+$ |
| Epithalamin | | |
| 0.1 mg | 4.6±0.9 | 4.4±1.1* |
| 1.0 mg | 6.0±0.4* | 6.0±0.9* |
| Epithalon, 0.1 μg | 6.4±0.2* | 6.8±0.9* |
| Melatonin | | |
| 25 pg | 8.2±0.6* | 7.0±1.1* |
| 100 pg | 7.5±0.8* | 6.4±0.5* |

**Note.** $p<0.05$ compared to *control, $^+$adult mice.

The percentage of SPC-F and $CD4^+$ cells virtually did not change after culturing of adult mouse bone marrow cell with epiphyseal factors (Table 2). In old mice the percentage of SPC-F somewhat decreased and the percentage of $CD4^+$ cells appreciably increased after incubation of the bone marrow suspension with all preparations. The differences between control values of $CD4^+$ cells in old mice can be attributed to the season when the study was carried out.

The percentage of $CD8^+$ cells in the spleens of adult ($n$=5) and old ($n$=5) CBA mice was 12.5±1.3 and 28.1±1.2% ($p<0.05$). After incubation of splenocytes with melatonin the values did not change in adult mice (18.1±2.8%) and decreased significantly in old animals (to 19.9±2.9%; $p<0.05$).

Hence, melatonin, epithalamin, and epithalon can directly modulate the secretory function of the thymus

**TABLE 2.** *In vitro* Effects of Factors of the Pineal Gland on the Percentage of SPC-F and $CD4^+$ Cells in the Bone Marrow of Adult and Old Mice

| Group | | Control (medium 199) | Epithalamin | Melatonin | Epithalon |
|---|---|---|---|---|---|
| SPC-F | adult | 23.0±3.2 | 26.7±8.0 | 23.8±7.7 | |
| | | (6) | (6) | (6) | |
| | old | 32.6±15.5 | 18.2±2.2 | 16.3±3.2 | |
| | | (6) | (6) | (6) | |
| $CD4^+$ cells | adult | 6.7±0.4 | | | 6.9±1.0 |
| | | (6) | | | (6) |
| | old | 10.9±1.3 | | | 15.6±2.4* |
| | | (6) | | | (6) |
| | old | 20.1±2.3 | 29.1±1.8* | 36.1±2.8* | |
| | | (5) | (5) | (5) | |

**Note.** *$p<0.05$ compared to the control. Number of measurements is given in parentheses.

in adult and old CBA mice and the percentage of $CD4^+$ cells in the bone marrow of old mice. Melatonin *in vitro* changed the percentage of $CD8^+$ cells in the spleen of old mice. High affinity melatonin receptors were detected in thymic epithelial cells and bone marrow T helpers [11]. It seems to explain why increase in TSF titers in supernatants of cultured thymic stroma and the percentage of bone marrow $CD4^+$ cells was observed in response even to physiological fluctuations in melatonin concentration. The hormone regulates the expression of prothymosine-$\alpha_1$ gene [13]. It is also possible that changed expression of L3T4 and Lyt-2 lymphocyte markers is also due to melatonin effect on the gene system. The modulatory effect on the markers is characteristic of melatonin. Previously the changes in the cell levels of cyclic nucleotides were considered as one of the main mechanisms of direct effects of epiphyseal peptides [6,7]. However, now the microchip technology proved the effects of Epithalon on the expression of many genes in animal cells [1]. It was hypothesized that because of low affinity of the receptors to epiphyseal peptides on thymic cells the direct effect of peptides on the thymus is realized in situations requiring rapid mobilization of hormones [6]. We showed that TSF titer increased in adult mice only after high doses of epithalamin. The relationships between the endocrine glands change during aging, and therefore the role of the direct effects of epiphyseal factors can increase not only for the epithelial component of the thymus, but for bone marrow T helpers as well. We revealed an appreciable increase in the percentage of bone marrow $CD4^+$ cells after incubation with epiphyseal peptides only in old mice. High efficiency of Epithalon for these parameters also confirms good prospects of this drug as an immunomodulator and geroprotector. The absence of *in vitro* effects of epiphyseal factors on the number of SPC-F can indicate that their effect *in vivo* is most likely realized through factors of the macroenvironment.

Thus, our results confirm the possibility of the effects of epiphyseal factors of indole and peptide nature at the cellular level in the central and peripheral organs of the immune system in adult and old CBA mice.

## REFERENCES

1. S. V. Anisimov, K. R. Bokheler, V. Kh. Khavinson, and V. N. Anisimov, *Byull. Eksp. Biol. Med.*, **133**, No. 3, 340-347 (2002).
2. G. M. Butenko, *Probl. Staren. Dolgolet.*, **7**, No. 3, 100-108 (1998).
3. *Immunocytochemistry and Monoclonal Antibodies in Oncohematology*, Eds. V. G. Pinchuk and D. F. Gluzman [in Russian], Kiev (1990).
4. I. F. Labunets, *Zh. Akad. Med. Nauk Ukrainy*, **6**, No. 4, 783-791 (2000).
5. I. F. Labunets and G. M. Butenko, *Probl. Staren. Dolgolet.*, **2**, No. 3, 280-285 (1992).
6. V. D. Slepushkin, V. N. Anisimov, V. Kh. Khavinson, *et al.*, *Pineal gland, Immunity, and Cancer (Theoretical and Clinical Aspects)* [in Russian], Tomsk (1990).
7. V. Kh. Khavinson and V. G. Morozov, *Epiphyseal and Thymic Peptides in the Regulation of Aging* [in Russian], St. Petersburg (2001).
8. J. F. Bach, M. Dardenne, and M. A. Bach, *Transplant. Proc.*, **5**, No. 1, 99-104 (1973).
9. A. J. Friedenstein, R. K. Chailakhyan, N. V. Latsinic, *et al.*, *Transplantation*, **17**, No. 4, 331-340 (1974).
10. I. Labunets, L. Magdich, T. Maksyuk, and G. Butenko, *Advances in Gerontol.*, **5**, 63-64 (2000).
11. Z. Liu, Y. Zhao, and S. Peng, *Sci. China B.*, **38**, No. 12, 1455-1461 (1995).
12. G. J. Maestroni, E. Hertens, P. Galli, *et al.*, *J. Pineal Res.*, **21**, No. 3, 131-139 (1996).
13. P. Molinero, M. Soutto, S. Benot, *et al.*, *J. Neuroimmunol.*, **103**, No. 2, 180-188 (2000).
14. R. J. Reiter, *Exp. Gerontol.*, **30**, Nos. 3-4, 199-212 (1995).