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ORIGINAL ARTICLE

# Epitalon influences pineal secretion in stress-exposed rats in the daytime

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**Abstract**

**OBJECTIVES**. The content of C-Fos protein was tested in rat pinealocytes in the norm and stress and in case of intranasal administration of Epitalon (Ala-Glu-Asp-Gly), which regulated pineal secretion processes, presumably, via protooncogenes.

**SETTING**. Intact and osmotic-stress-exposed rats were used for the immunohistochemical detection of C-Fos protein. All animals were intranasally administered with Epitalon, the last infusion made in two hours before the biopsy. Simultaneously, light microscopy of the pineal parenchyma was performed in all groups of animals.

**RESULTS**. A slight but significant C-Fos increase was observed only in stress-exposed pinealocytes of rats after intranasal Epitalon infusions. C-Fos was irregularly distributed throughout pineal cells. In stress, the clusters of 5–10 cells containing C-Fos in their cytoplasm were detected. The dilation of capillaries and pericapillary space induced by an osmotic stress was partially reduced by the intranasal infusions of Epitalon.

**CONCLUSIONS**. Tetrapeptide Epitalon is synthesised on the basis of the amino acid composition of pineal peptide extract Epithalamin. Epitalon modulates pineal secretion only under a stress impact but never in the norm. It prevents osmotic-stress-induced pathologic changes in the pineal parenchyma structure. Besides, the physiological activity of Epitalon seems to be mediated by the activation of protooncogenes in pinealocytes.

## Introduction

Both the pineal gland and the hypothalamus-hypophysis complex take part in the formation of general adaptation syndrome [1, 2]. Stress causes the pineal gland to increase the secretion of melatonin and peptide substances [2, 11], which are known to reduce the damaging effect of oxygenic, osmotic, psychic and other stresses. The peak of pineal melatonin secretion occurs only at night, while in the daytime pinealocytes secrete other substances, peptides in particular. Yet, the works dedicated to pineal peptides are disproportionally few as compared to available publications on melatonin.

The pineal gland belongs to circumventricular organs having no blood-brain barrier. Consequently, this organ is highly sensitive to macromolecular biologically active substances circulating with the cerebral blood flow and, especially, to peptides. The unique location of the olfactory system, its chemical links both to the environment and the central nervous system turn it to a convenient pathway for the non-invasive delivery of substances to the cerebral blood flow and circumventricular organs. This pathway bases on the anatomic connection of the nasal submucosa to the subarachnoid space surrounding olfactory nerves as they penetrate the cribriform plate of the skull and enter the brain [6]. The cribriform region has no significant barrier to cerebrospinal fluid drainage [3, 8]. This is the possible way for metals, dyes, viruses, peptides [14, 15], proteins and narcotics to enter the brain via nasal cavity avoiding the blood-brain barrier [4]. Previously we have shown that intranasally infused epiphyseal peptides reach the pineal gland and specifically regulate its electric activity and pinealocytic ultrastructure. Furthermore, these effects have been observed only in stress, but not in the norm [20]. An important role in intracellular pineal synthesis activation, for example, in stress, belongs to heterodimer AP-1 formed of C-Fos and C-Jun transcription factors [10, 13]. C-Fos protein exists longer than its mRNA. The peak of its content in the cytoplasm is usually observed in two hours after, for instance, a stress impact. Some authors [12] have suggested cytomedins to influence the cells through C-Fos synthesis activation. To prove the hypothesis on the participation of oncogenes in the pineal humoral self-regulation, we have performed an immunohistochemical detection of C-Fos protein in the pinealocytes of stress-exposed rats subjected to the intranasal infusions of Epitalon.

## Material and Methods

To detect C-Fos in pinealocytes, we used male Wistar rats – intact ones and those exposed to a 24-hours' osmotic stress (n = 24). Twelve rats had an unlimited access to water and food: six of them served as the control and the other six were administered with four intranasal Epitalon infusions at 12-hours' intervals at the dose of 50$\mu$g (0.5 $\mu$g per animal). Twelve rats were deprived of water and food for 48 hours: six of them received Epitalon infusions by the same scheme and at the same dose. In two hours after the last infusion, the animals were decapitated (according to FELASA guidelines), their pineal glands were subjected to a standard histological procession and embedded in paraffin. Microtome was used to make 5-μm sections for the indirect immunohistochemical detection of C-Fos: the sections were incubated with rabbit C-Fos antibodies (Santa-Cruz, USA) and subsequently with secondary

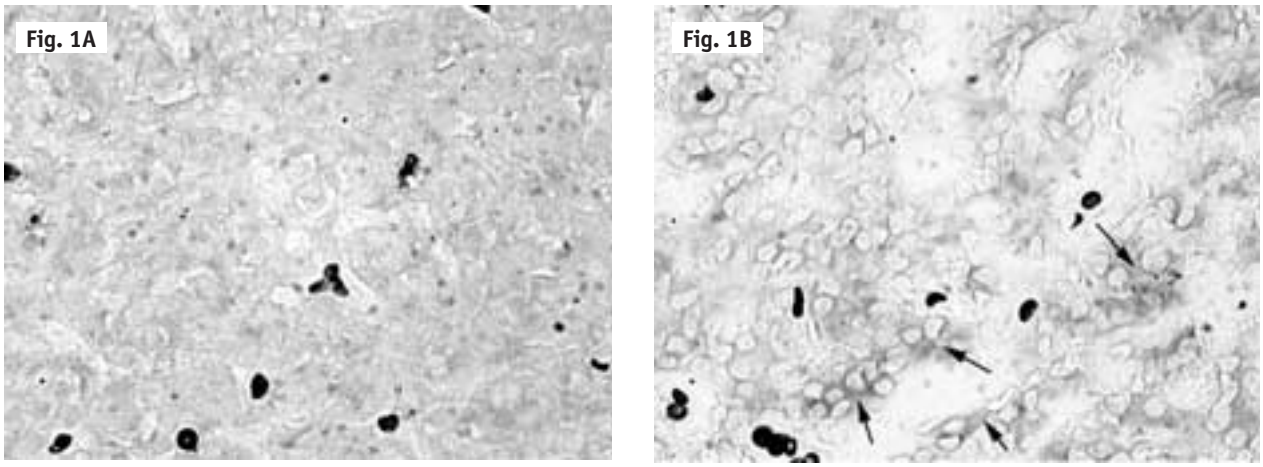


**Figure 1.** Immunohistochemical detection of C-Fos protein. A – without Epitalon infusion. B – with intranasal Epitalon infusion (cell clusters producing C-Fos protein)

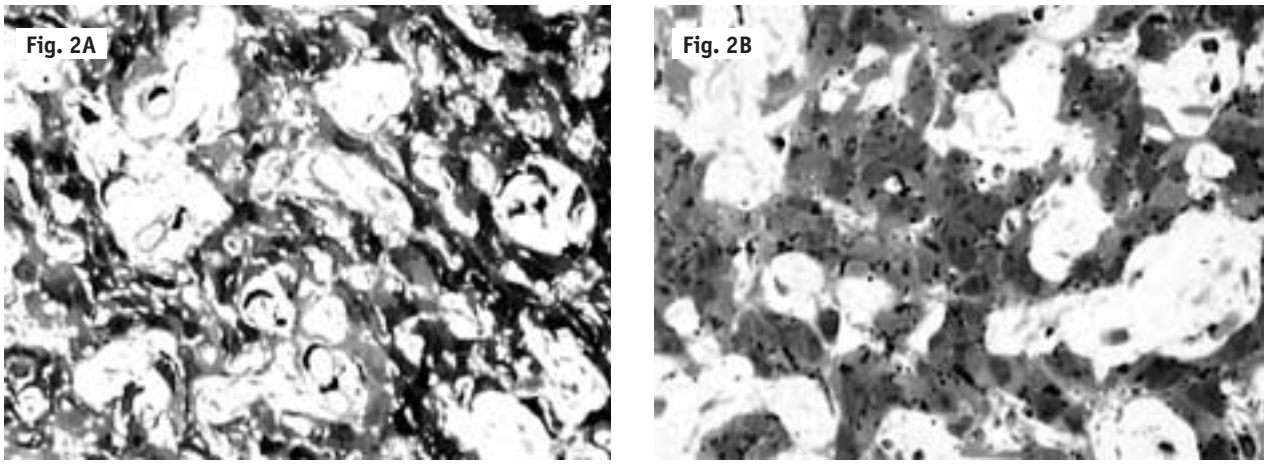


**Figure 2.** Pineal parenchyma after a 48-hours' water and food deprivation. A – without Epitalon infusion. B – after intranasal Epitalon infusion

anti-rabbit antibodies conjugated with horseradish peroxidase (Sigma). The peroxidase was revealed with a diaminobenzidine-$H_2O_2$ mixture. The stained sections were digitised in a microscope with a 48-bit CCD camera. The presence of C-Fos protein was confirmed by the appearance of the characteristic brown colour.

Simultaneously, the sections were dyed in the system of methylene blue – azure – fuxine. Digitising was done the same way as above. Morphometry was performed with "Ista Video-Test" software (Russia) and in "SigmaScan Professional 5" (SPSS Inc., USA).

## Results and Discussion

C-Fos protein belongs to the triggers activating pineal synthetic processes in response to extreme factors, in particular, to stress [10]. In our experiments, the nearly complete absence of C-Fos in the rats' pineal glands was probably associated with the chronic type of the stress, since according to the published data, the maximum C-Fos content was usually observed in 1–2 hours after a short stress impact [16, 17]. Chronic stress suppressed C-Fos gene activity in the paraventricular hypothalamic nucleus [5] due to the high blood level of glucocorticoids [9, 22]. Besides, the nuclear glucocorticoid receptor and AP-1 transcription factor revealed a regulatory antagonism [21].

A slight but statistically significant C-Fos increase was observed only in stress-exposed pinealocytes of rats after intranasal Epitalon infusions (Figure 1). The obtained results complied with the hypothesis [12] on the possible mechanism of action of cytomedins and their components via C-Fos activation. The fact that Epitalon (a derivative of pineal regulatory peptides) exerted this effect only in case of stress confirmed its participation in the specific mechanisms of pineal self-regulation under extreme conditions.

Only some pinealocytes were found to produce C-Fos. In stress, the clusters of 5–10 C-Fos synthesising cells were registered. Presumably, these clusters represented the very groups of interacting pinealocytes, which we had discovered before in our electrophysiological investigations [18, 19]. Certainly, this assumption would require a more valuable experimental confirmation in the future.

C-Fos detection was accompanied by pineal parenchyma morphometry. The dilation of capillaries and pericapillary space induced by an osmotic stress (Figure 2) was partially suppressed with intranasal Epitalon infusions. This activity of Epitalon resembled its effect upon the gamma-irradiated pineal gland of rats described elsewhere [7].

Thus, synthetic peptide Epitalon (a derivative of pineal regulatory peptides) modulates pineal oncogenes only in stress but not in the norm. It also prevents osmotic-stress-induced pathologic changes in the pineal parenchyma structure. To add, the effect of Epitalon on the pineal gland is probably mediated by the activation of protooncogenes.

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