

and has a direct excitatory influence on neuron membranes [12]. Our studies (in press) have shown that glutapyrone, although a derivative of 1,4-DHP, does not block voltage-dependent Ca channels. It seems likely that this compound realizes its anticonvulsive activity predominantly via the GABA-ergic mechanisms.

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# Elimination of Stress-Induced Changes in the Thymus within Laser Irradiation of the Endocrine Glands

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Adaptation medicine, a new scientific discipline, studying the mechanisms of adaptation of the organism to the environment or to the behavior of the organism itself and elaborating adaptive methods for the prevention and treatment of diseases has been formed in recent years [8]. One of the main aspects of this trend is the use of the adaptation for the prevention and treatment of stress-induced disorders.

The possibility of using low-energy laser radiation (LLR) in the infrared (IR) range as a stress-

limiting factor needs to be explored. IR LLR (0.89 m) is a physical agent widely used at present and, unlike laser radiation of the visible part of the spectrum, it offers greater depth of penetration in tissues (of the order of 5-6 cm) and thereby the possibility of direct contact not only with the skin but with the deeper-lying tissues as well [5,6]. The feasibility of using of IR laser radiation to correct cardiovascular [2] and immune [9,12,13] disorders has been shown in a series of experimental and clinical investigations. A positive effect is noted in inflammatory processes of different localization [3,7]. Beneficial shifts in the biological and immunological blood indexes ensue from the use of

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TABLE 1. Indexes of Activity of Thymus Cells in Stress and after Laser Irradiation of Thymus Region ( $M \pm m$ )

Index	Group ( $n=5$ )				
	1st	2nd	3rd	4th	5th
Weight of thymus, mg	225 $\pm$ 16.9	118 $\pm$ 25.7*	235 $\pm$ 28.1	112.5 $\pm$ 16.9	200 $\pm$ 10.7*
Number of TM, mill. cells/ml	92.4 $\pm$ 7.8	26.1 $\pm$ 2.4*	99.0 $\pm$ 5.6**	43.6 $\pm$ 8.8*	70.9 $\pm$ 10.7**
Binding of AO with TM nucleus, rel. units	0.29 $\pm$ 0.06	0.40 $\pm$ 0.07	0.53 $\pm$ 0.05*	0.22 $\pm$ 0.05	0.20 $\pm$ 0.05**
Protein content in TM, mg/ml	21.6 $\pm$ 3.0	19.4 $\pm$ 3.0	23.0 $\pm$ 1.8	26.0 $\pm$ 0.4	17.0 $\pm$ 3.0
Level of LPO in TM, mM/mg protein	0.142 $\pm$ 0.006	0.248 $\pm$ 0.057	0.125 $\pm$ 0.001**	—	0.183 $\pm$ 0.01*

Note. Here and in Table 2 asterisks signify reliability of differences ( $p < 0.05 - 0.01$ ); one asterisk relates to comparison with control, two asterisks to comparison with second group.

the low-energy pulse IR radiation in patients with autoimmune diseases (psoriasis, rheumatoid arthritis). IR radiation exhibits an antioxidant effect when used in doses of up to 3.0 J/cm [10].

The aim of the present study was to assess the functional state of the thymus cells of stressed animals according to the activity of the genetic apparatus of the thymocytes (TM) and the level of LPO of the membranes of these cells depending on the localization of laser action, and to choose the regime optimal for the elimination of stress-induced changes in the thymus or for the prevention of their development.

## MATERIALS AND METHODS

Fifty male albino rats weighing 150-200 g were used. The experimental animals were subjected to immobilization stress [1]. The source of laser radiation was the serial laser physiotherapeutic Uzor ALT apparatus (radiation wavelength 0.89  $\mu$ m, mean pulse power 2 W, pulse frequency 300 Hz, pulse duration  $7 \times 10^{-8}$  sec). Regions of rat thymus and thyroid gland were irradiated. Depending on the localization, the animals were divided into two

series, each series consisting of 5 groups with 5 animals in each group: the 1st group was the control, in which the animals received sham procedures; the animals of the 2nd group were stressed; the 3rd group consisted of the animals subjected to laser procedures; animals exposed to preliminary laser radiation made up the 4th group; the 5th group included stressed animals exposed afterwards to laser radiation. The state of the organism was assessed by indexes characterizing the functional activity of the thymus of experimental animals, namely, weight, number of cells per unit weight, physicochemical state of TM nuclei, level of LPO processes, and protein content in TM. TM were isolated by homogenization of the thymus at +4°C. The isolation medium consisted of 145 mM NaCl, 5.6 mM KCl, 2 mM  $\text{KH}_2\text{PO}_4$ , and 10 mM glucose (pH 7.5). The thymus homogenate was centrifuged at 1000 g for 10 min at +4°C. Precipitated and washed TM were dissolved in isolation medium in a ratio of 1:10. Cells were counted with a Picoscale photoelectric particle counter (Hungary). The structural and functional state of the nuclear chromatin of TM was studied with acridine orange (AO) staining photometrically

TABLE 2. Indexes of Activity of Thymus Cells in Stress and after Laser Irradiation of Thyroid Region ( $M \pm m$ )

Index	Group ( $n=5$ )				
	1st	2nd	3rd	4th	5th
Weight of thymus, mg	348 $\pm$ 21.4	97 $\pm$ 13.9*	304 $\pm$ 36.4	118 $\pm$ 19.3*	254 $\pm$ 72.9
Number of TM, mill. cells/ml	111.22 $\pm$ 2.6	30.54 $\pm$ 4.9*	$\pm$ 84.8 $\pm$ 8.3*	42.52 $\pm$ 9.96*	$\pm$ 60.5 $\pm$ 18.6*
Binding of AO with TM nucleus, rel. units	0.40 $\pm$ 0.015	0.28 $\pm$ 0.04*	0.23 $\pm$ 0.006*	0.33 $\pm$ 0.047	0.44 $\pm$ 0.06**
Protein content in TM, mg/ml	20.0 $\pm$ 1.0	14.2 $\pm$ 1.4*	18.6 $\pm$ 0.8	17.2 $\pm$ 1.8	19.6 $\pm$ 3.6
Level of LPO in TM, mM/mg protein	0.075 $\pm$ 0.001	0.143 $\pm$ 0.003*	0.088 $\pm$ 0.001**	0.145 $\pm$ 0.001*,**	0.082 $\pm$ 0.002

at 492 nm. The level of LPO in TM was determined by the reaction of peroxide fission, namely, the reaction between malonic dialdehyde (MDA) and 2-thiobarbituric acid, spectrophotometrically at 535 nm [11]; the protein content in TM was measured by the biuretic method.

## RESULTS

It was found in the first experimental series that the stressed animals had a twofold decreased thymus weight ( $p < 0.02$ ), a 3.5-fold decrease of the number of TM ( $p < 0.001$ ), and a tendency toward an increase of LPO by 75% (Table 1). Laser irradiation of the thymus activated the genetic apparatus of TM and reduced LPO. Preliminary laser treatment did not prevent the development of the stress reaction to immobilization, and such parameters as thymus weight and number of TM remained low. The use of laser after the development of stress proved to be more effective: none of the indexes studied except the LPO level, which increased by 25%, differed significantly from the control values.

In the second experimental series (Table 2) the stressed animals were characterized by a more than threefold decrease of the thymus weight and of the number of TM ( $p < 0.001$ ), the activity of the TM genome dropped by 30% ( $p < 0.05$ ), and the protein content in them was also diminished ( $p < 0.01$ ), while the LPO level rose 1.9 times ( $p < 0.02$ ). Laser irradiation of the thyroid resulted in a 34% decrease of TM ( $p < 0.002$ ) and a 45% reduction of the activity of their genetic apparatus ( $p < 0.001$ ); there was also a tendency for the thymus weight to decrease. Preliminary laser irradiation did not prevent the development of the stress reaction to immobilization, and the thymus weight and number of TM remained lowered more than 2.5 times ( $p < 0.001$ ) while LPO was increased 1.9 times ( $p < 0.001$ ). The use of laser radiation after the development of stress caused no significant changes of any of the studied parameters compared to the control.

On the basis of an analysis of the data obtained, it may be assumed that the effect of laser radiation depends to a marked degree not on the localization of action but on the sequence of its use in stressed animals. Its stress-limiting properties appear only in already stressed animals and it is not a preventive agent. The same fact was noted for the use of He-Ne laser as a reparative agent for genetic disturbances after the action of ionizing radiation [4]. As there were no significant differences between the test and control indexes for irradiation of the region of the thyroid gland in the 5th group, despite the more pronounced changes in the stressed animals (compared to the 1st series of experiments), it may be concluded that the stress-limiting effect of IR laser radiation is more pronounced precisely in this localization.

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