Effect of Pulsed Infrared Laser Radiation on DNA Synthesis in Tissues of Intact Rats and during Strenuous Physical Exercise

S. M. Zubkova and L. V. Mikhailik

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 6, pp. 625-627, June, 1995
Original article submitted February 17, 1995

The motor zone of the rat brain cortex is subjected to pulsed infrared (0.89 μ) laser radiation, which is found to stimulate DNA synthesis both in intact animals and after strenuous physical exercise (swimming). Preliminary laser irradiation exerts a stress-limiting effect on cells of the brain cortex and thymus but does not prevent swimming-induced reduction of ^{3}H-thymidine incorporation in nuclear DNA of muscles.

Key Words: *laser; brain cortex; physical exercise; stress; adaptation*

In sum, the experimental data gathered over many years suggests that adaptation to short-term stress raises the organism's resistance not only to stress, but also to cold, physical strain, ischemic necrosis of the heart, chemical necrosis of the gastric mucosa, and ionizing radiation [8]. Studies with the use of physical interventions have revealed that microwave and laser radiation exerts antioxidant and radioprotective effects [4,5]. Exposure of endocrine glands to infrared (IR) laser radiation prevents the development of stress-induced changes in the organism [1]. It has also been demonstrated that pulses of a direct current (10 and 1000 Hz) passed through the brain before and after immobilization have a stress-limiting effect on animals [3]. This opens up broad prospects for the use of physical factors as active adaptogens.

The present study explores the effect of pulsed IR-laser radiation on the level of DNA synthesis in the motor zone of the cortex in rats under normal conditions and under a physical load. To this end we chose a frequency modulation of 10 Hz, since it coincides with the a-rhythm of the central nervous system and, as was previously found, most actively stimulates DNA synthesis in the cortex and skeletal muscles [6].

Russian Research Center of Rehabilitation and Physical Therapy, Ministry of Health of the Russian Federation, Moscow. (Presented by V. M. Bogolyubov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Twenty male nonpedigree rats weighing 180-200 g were used. Swimming with a ballast attached to the tail (6% of body weight) served as the physical load. The rats swam in pairs in a plastic bath (30×30×50 cm) in water at room temperature during 5-7 min until they started to sink. Thereafter they were removed from the bath and placed in dry cages for 3-3.5 hours (until sacrifice). The generator of IR-laser radiation was an Orion apparatus (Zhiva, Moscow), radiation mode: 0.89 μ wavelength, 4.8 W pulse power, 3000 Hz carrier frequency, 8-10 sec pulse duration, and 10 Hz frequency modulation, and the duration of exposure was 10 min. The motor zone of the cortex was subjected to radiation. The animals were divided into 5 groups: 1) control animals, 2) sham-radiated controls, 3) animals subjected to a single laser exposure, 4) animals irradiated 2-3 min prior to the physical load, 5) animals receiving the load 2-3 min prior to irradiation. The general state of the organism was evaluated using a fluorometric method by measuring the plasma level of 11-hydroxycorticosteroids (11-HOCS) [9]. The rate of

0007-4888/95/0006-0602$12.50

TABLE 1. Levels of DNA Synthesis in Cells of the Brain Cortex, Skeletal Muscle, and Thymus and Plasma Content of 11-HOCS during Physical Exercise, Laser Irradiation, and Their Combination ($M \pm m$, $n=4$)

Group	Specific activity of DNA, counts/sec			Level of 11-HOCS, nmol/liter	Weight of thymus, mg
	in thymus per 10^8 nuclei	in brain cortex (per 100 μg)	in skeletal muscle (per 100 μg)		
Group 1	1.6±0.5	8.3±0.6	14.4±.3	622.7±122.5	310.5±35.0
Group 2	2.0±0.7	10.6±0.5* $p_{2,3}<0.001$	13.2±0.3 $p_{2,3}<0.001$	795.8±122.2 $p_{2,3}<0.05$	237.0±36.0
Group 3	4.1±0.9*	31.3±1.1** $p_{3,4}<0.001$	25.3±0.7** $p_{3,4}<0.001$	301.7±66.9	397.5±82.6
Group 4	6.3±2.2 $p_{1,4}<0.05$	15.8±1.1** $p_{2,4}<0.02$	11.7±1.0 $p_{1,4}<0.05$	334.3±11.6 $p_{2,4}<0.02$	318.0±59.0
Group 5	4.0±1.2 $p_{1,5}=0.1$	15.4±0.2** $p_{2,5}<0.001$	22.1±0.6 $p_{4,5}<0.002$	264.0±70.8 $p_{2,5}<0.02$ $p_{2,5}<0.001$	295.7±58.0

Note. *$p<0.05$, **$p<0.01-0.001$ in comparison with the control. p with the indexes (group numbers) indicates reliability of differences between these groups.

DNA synthesis in the cortex, skeletal muscle, and thymus was determined from the incorporation of ^{3}H-thymidine in nuclear DNA. To this end the rats were injected intraperitoneally with ^{3}H-thymidine (specific activity of 590 GBq/mol, St. Petersburg) in a dose of 0.25 mCi per rat in 0.3 ml physiological saline 3 hours before irradiation or the physical exercise. The animals were decapitated 3-3.5 hours after irradiation or swimming and nuclear DNA was isolated from the cortex, sartorius muscle, and thymus as described earlier [2]. After purification from lipids, proteins, and RNA, the radioactivity of DNA was radiometrically measured. The specific activity of DNA from the cortex and myocytes was estimated in terms of the optical density of DNA solutions in the UV range. Thymocytes were counted on a Picoscale photoelectron counter and the specific radioactivity of DNA was determined in terms of 10^8 nuclei.

Statistical processing of the results consisted of a comparison of the mean values of the studied parameters in animals of the 2nd, 3rd, 4th, and 5th groups with the same parameters in the control group (group 1). The reliability of the differences was determined using the Student t coefficient by comparing it with tabulated t values for the corresponding number of measurements.

RESULTS

The data in Table 1 show that physical strain activates the synthesis of DNA in the cortex 1.3-fold ($p<0.05$). A trend toward elevation of plasma 11-HOCS was also observed. Laser irradiation led to an activation of DNA synthesis in all studied tissues. This activation was maximal (3.8-fold, $p<0.001$) in the irradiated zone (motor zone of the cortex) and less expressed in the thymus (2.6 fold, $p<0.05$) and sartorius muscle (2.75-fold, $p<0.001$). Plasma 11-HOCS declined while the mass of the thymus tended to increase. IR-laser irradiation before the physical load resulted in a 1.9-fold activation of DNA synthesis in the cortex ($p<0.01$). A tendency toward an increase in DNA synthesis was noted in the thymus and toward a decrease in *m. sartorius*. Plasma 11-HOCS was 2.4-fold lower than in the 2nd group ($p<0.02$).

Exposure to laser radiation after the physical load led to an activation of DNA synthesis in all studied types of tissues, but whereas the degree of activation was the same in the muscle and thymus, in the cortex it was only half as high as that observed in the 3rd group ($p<0.001$). The content of 11-HOCS in the 2nd and 5th groups differed reliably, being 3 times as high in the 2nd group as in the 5th ($p<0.02$). The obtained data suggest that IR-laser radiation in the mode used is not a strong source of stress, since it does not increase the level of 11-HOCS, whereas the mass of the thymus and the functional activity of thymocytes do increase at the moment of investigation. Nevertheless, this radiation may act as an active adaptogenic factor, which provides a plastic basis for increasing functional activity, both directly in the irradiated tissue (brain cortex) and in peripheral tissues (the muscle and the thymus). When IR-laser irradiation is applied as a prophylactic measure (before strenuous physical exercise), the central nervous and the muscular systems react in different ways. In the muscular system the level of plastic processes drops after the physical load and the preliminary irradiation does not prevent this drop.

Our data on the activation of DNA synthesis in the cortex are in conformity with our previous findings on the effect of helium-neon laser irradiation of the same zone of the cortex, where, using a microfluorometric method, we showed a stimulated incorporation of acridine orange in deoxyribonucleoprotein of pyramidal and stellate neurons [7]. It is also wellknown that any homeostatic shift in the organism induces an activation of the genetic apparatus, which stimulates the synthesis of nucleic acids and proteins, thus strengthening the adaptive systems [8]. The activation of biosynthetic processes in the brain cortex establishes temporary bonds and improves the capacity of the stress-limiting systems. In the molecular mechanism of these adaptive processes an important role is played by an increased expression of certain genes and hence an accumulation of the so-called stress proteins with a molecular weight of 71-72 kD, which prevent protein denaturation and protect cell structures from stress-induced injuries [8,10,11].

Thus, single 10-min IR-laser irradiation of the motor zone of the brain cortex induces stimulation of the biosynthetic processes in irradiated cells of the brain cortex and in cells of the thymus and muscles both in intact animals and under conditions of strenuous physical exercise.

REFERENCES

1. M. N. Alekseeva, S. M. Zubkova, A. A. Minenkov, and S. B. Pershin, *Byull. Eksp. Biol. Med.*, **116**, № 10, 360 (1993).
2. V. V. Ashapkin, G. A. Romanov, N. A. Tushmalova, and B. F. Vanyushin, *Biokhimiya*, **48**, № 3, 355 (1983).
3. V. M. Bogolyubov, S. M. Zubkova, S. A. Radzievskii, *et al.*, *Vopr. Kurortol.*, № 4, 3 (1994).
4. N. D. Devyatkov, S. M. Zubkoba, I. B. Laprun, and N. S. Makeeva, *Uspekhi Sovr. Biol.*, **103**, № 1, 31 (1987).
5. S. M. Zubkova and I. B. Laprun, in: *The Bioantioxidant* [in Russian], Moscow (1983), p. 138.
6. S. M. Zubkova, L. V. Mikhailik, V. V. Trushin, and I. S. Parfenova, *The Use of Low-Intensity Laser Radiation and Magnetic Fields in Biology and Medicine* [in Russian], Obninsk (1994).
7. S. M. Zubkova and Z. A. Sokolova, *Vopr. Med. Khimii*, № 3, 326 (1978).
8. F. Z. Meerson and I. Yu. Malyshev, *The Phenomenon of Adaptational Stabilization of Structures and Protection of the Heart* [in Russian], Moscow (1993).
9. Yu. A. Pankov and I. Ya. Usvatova, in: *Methods in Clinical Biochemistry of Hormones and Transmitters* [in Russian], Moscow (1973), p. 66.
10. W. J. Welch and I. P. Suhan, *J. Cell Biol.*, **103**, 2035 (1986).
11. Y. Yazaki and I. Komuro, *J. Molec. Cell Cardiol.*, **21**, Suppl. 3, 29 (1989).