EFFECT OF TRANSCRANIAL LASER IRRADIATION IN THE NEAR INFRARED BAND ON ANTINOCICEPTIVE RESPONSES OF MICE RECEIVING DIAZEPAM, CLONIDINE, AND MORPHINE

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Low-energy lasers are increasingly being used in clinical medicine, including in the treatment of neurological diseases [1, 6]. In recent years laser radiation has begun to be used in the treatment of acute and chronic pain syndromes. The principal method in this case is laser-puncture with the use of traditional indications of acupuncture points [5] and irradiation of painful zones [2, 8]. The effect of laser irradiation on mechanisms of pain perception may be connected with changes in the properties of the biological membranes and enzyme systems acting on the endogenous opioid system [4, 7, 10].

Meanwhile problems concerned with direct irradiation of the brain with low-energy lasers and a combination of irradiation with pharmacotherapy, have not yet been studied from the standpoint of nociceptive reactions. The aim of this investigation was to study the effect of transcranial laser irradiation on nociceptive responses in mice and also the effect of diazepam, clonidine, and morphine under these conditions.

EXPERIMENTAL METHOD

Experiments were carried out on 110 noninbred albino mice weighing 20-25 g. The animals' hair was removed from the region of the vault of the skull 24 h before the experiment began. During laser irradiation, in order to ensure constant conditions of exposure, all the mice were fixed by special forceps in the neck region without disturbing breathing or the cerebral circulation. Irradiation was given with a "Uzor" semiconductor laser apparatus acting from the vault of the skull and with the source of laser radiation in contact with the skin. The characteristics of laser irradiation were: wavelength 0.89 μ, pulse repetition frequency 1500 Hz, average pulse power $7 \cdot 10^{-8}$ sec, duration of irradiation 20 min.

Nociceptive responses were assessed by the "tail flick" tests on an "analgesimeter" ("Hugo Sachs Electronic," Germany) and a hot plate (55°C), with recording of the original latent periods (LP) of response (background), followed by their values 30, 45, and 60 min and 24 h after the beginning of laser irradiation or its combination with the drugs chosen for study. The control animals were fixed for 20 min after intraperitoneal injection of physiological saline. The drugs were injected intraperitoneally: morphine 3 mg/kg, clonidine 0.5 mg/kg, and diazepam 1 mg/kg. The doses used were chosen on the grounds that morphine and clonidine, in these doses, exhibit clear antinociceptive effects of opioid and nonopioid types respectively, although their level permits detection of their enhancement or weakening under the conditions of a particular experiment. Diazepam in the above dose has a marked anxiolytic action in animals, without exhibiting antinociceptive activity in the analgesimetric tests used.

For the morphological investigations 4-5 h after laser irradiation the animals were decapitated, and this was quickly followed by fixation of the brain in 10% formalin. Sections were embedded in paraffin wax and stained with hematoxylin and eosin.

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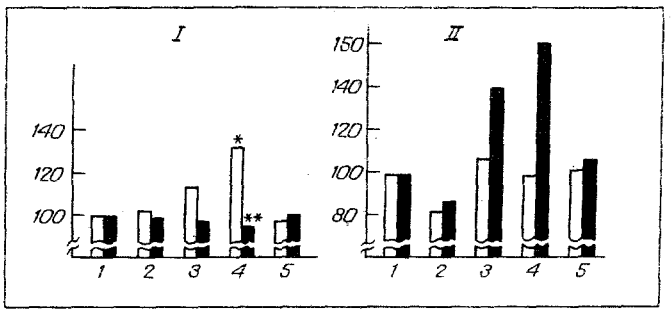

Fig. 1. Effect of transcranial laser irradiation in the infrared band on nociceptive responses of mice in "tail flick" (I) and "hot plate" (II) tests. Abscissa, 1) background, 2) 30 min, 3) 45 min, 4) 60 min, 5) 24 h. Ordinate, LP (in % relative to background). Unshaded columns – control group, black columns – group with laser irradiation. *) Statistical significance of differences not under $p < 0.05$ compared with background values of corresponding group; **) between groups at the same stage.

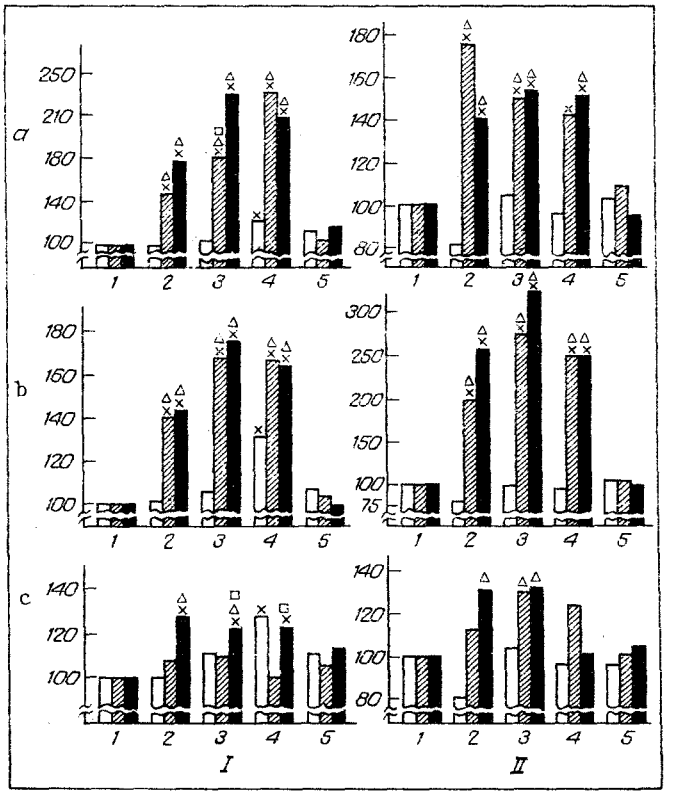

Fig. 2. Effect of transcranial laser irradiation in the infrared band on nociceptive responses of mice in "tail flick" and "hot plate" tests. a (I, II) Effects of combination of laser irradiation with morphine, b (I, II) with clonidine, c (I, II) with diazepam. I (a, b, c) Testing by "tail flick" method, II (a, b, c) by "hot plate" method. Unshaded columns – control group of animals, shaded columns – group receiving drug, black columns – combination of laser irradiation with administration of drug. *) Statistical significance of differences not under $p < 0.05$ compared with background values of corresponding group; triangles – relative to control group at the same stage of testing; squares – between groups with administration of drug and combination of drug with laser irradiation at the same stage of testing. Remainder of legend as to Fig. 1.

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The results indicate that (Fig. 1) transcranial laser irradiation of mice by the method described has no effect on the character of nociceptive response in "tail flick" and "hot plate" tests. A relatively small (by 29%, $p < 0.5$) increase was observed in LP in the "tail flick" test after 60 min in the control group. In the experimental group of mice, the value of LP after laser irradiation returned to normal in 60 min.

It will be clear from Fig. 2 that under the influence of morphine, LP of the nociceptive response in the "tail flick" test increased after 30, 45, and 60 min by 50, 83, and 137% respectively ($p < 0.01$), whereas in the "hot plate" test it increased by 77, 51, and 42% ($p < 0.05$) compared with background values. During laser irradiation this antinociceptive effect of morphine was found to be increased by 39% ($p < 0.05$) 45 min after the beginning of irradiation, in the "tail flick" test only.

Under the influence of clonidine LP of the nociceptive response in the "tail flick" test was increased after 30, 45, and 60 min by 42, 68, and 67% respectively ($p < 0.05$), whereas in the "hot plate" test it was increased by 101, 178, and 140% respectively ($p < 0.05$) compared with background values. Laser irradiation had no significant effect on the antinociceptive activity of clonidine.

Diazepam by itself had no marked antinociceptive action, and actually promoted normalization of the increase in LP in the "tail flick" test observed in animals of the control group at the 60th minute of testing. However, during the combined action of laser irradiation and diazepam a significant ($p < 0.01$) increase of 23-27% in LP was observed, indicating the development of an antinociceptive effect. During testing of the animals by the "hot plate" method no such phenomenon could be found.

Testing the nociceptive responses after 24 h revealed restoration of LP in the tests to the original level, indicating reversibility of the changes in nociception induced by laser irradiation and by the drugs studied.

The results thus indicate that transcranial laser (within the band studied) irradiation of the mouse brain can restore to normal relatively late (after 60 min) changes in nociception, which are probably the result of immobilization stress. In some types of testing ("tail flick") irradiation examined at certain intervals (45 min after injection of the drug) can potentiate the antinociceptive action of the opioid analgesic morphine, and can also lead to the development of an antinociceptive reaction after injection of diazepam, which does not belong to the analgesic class and does not by itself exhibit an antinociceptive effect. Meanwhile laser irradiation did not lead to any significant change in the antinociceptive action of clonidine, whose effect is not connected with opioidergic processes [3], in either test. Incidentally, during testing by the "hot plate" method no change was found in the effect of the substances examined on nociceptive reactions after laser irradiation. This state of affairs suggests that the effect of transcranial irradiation of mice is evidently mediated through its interference with descending influences of the brain that control nociceptive mechanisms at the spinal level that are responsible for changes observed in nociceptive reactions and caused by diazepam and morphine in the "tail flick" test. This hypothesis may be based on indications that the nociceptive reaction in the "tail flick" test is realized predominantly at the spinal cord level [9], whereas the nociceptive response in the "hot plate" test (licking the paw) calls for a more highly organized motor response, involving supraspinal mechanisms.

Considering the appearance of an antinociceptive effect under the influence of laser irradiation and after administration of diazepam, we studied the possible connection of this effect with the opioid system. The results demonstrated the absence of effect of the specific opioid antagonist naloxone (1 mg/kg, intraperitoneally, 15 min before testing) on the effect of diazepam under conditions of laser irradiation. This fact suggests that the antinociceptive response studied is due to nonopioidergic mechanisms.

Histological investigation of the brain of the experimental animals revealed no significant morphological differences from the control. This may indicate that the effects of laser irradiation observed are unlikely to be connected with physical damage to brain structures, and it indicates the likely safety of transcranial laser irradiation within the range studied.

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CORRECTION OF HORMONAL-METABOLIC DISTURBANCES IN RATS BY NATURAL ADAPTOGENS DURING DEVELOPMENT OF AN ADAPTATION SYNDROME AND FUNCTION TESTS WITH DEXAMETHASONE AND ACTH

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Adaptogens and, in particular, those of them which belong to the group of natural origin, have been used in the combination treatment of various diseases characterized by a nonspecific component of their pathogenesis [6, 7]. The ability of these substances to control disturbances of the hormonal-metabolic status of an individual observed during the development of the adaptation syndrome is well known, but the mechanism of these effects is largely unexplained. It has been shown that the main pathogenetic stages in the formation of this group of disturbances are the successively developing (stages of anxiety and exhaustion of the stress reaction respectively) manifestations of loss of hypothalamic sensitivity to regulatory homeostatic signals and exhaustion of adrenocortical function [2, 5]. It is logical to suggest that the regulatory effects of adaptogens are largely connected with their action on this mechanism. One method of assessment of disturbances of function of the pituitary-adrenal system and, consequently, of discovering the mechanisms of the corrective effects of adaptogens, is to perform loading function tests with dexamethasone (DM) and with adrenocorticotrophic hormone (ACTH) [2, 4].

The aim of this investigation was to study the effect of adaptogens of natural origin, in the form of an extract of Baikal skullcap (*Scutellaria baicalensis*) (EBS) and its active principle – the flavonoid baikalin, on parameters of the hormonal-metabolic status of rats during the development of an adaptation syndrome, and during function tests with DM and ACTH.

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