EFFECT OF DALARGIN ON PROLIFERATION AND VERTICAL MIGRATION OF CORNEAL EPITHELIAL CELLS DURING STRESS

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Previous research by the writers showed that an increase in the frequency of pathological mitoses and acceleration of vertical migration of epithelial cells are important components of the structural trace of tissue disadaptation during stress [5, 10]. Among the many different properties of opioid peptides, an important role is played by their antistressor activity [4, 6, 7]. At the same time they play an active role in the regulation of cell division, and induce stimulation of proliferative processes [1, 2]. The aims of the present investigation included determination of the effect of the synthetic Leu-enkephalin analog, dalargin, on cell division during stress and to assess the possibility of using it to prevent disturbances induced by stress in the tissues.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-200 g. In the first group of experiments the animals were subjected to fixation in the supine position once for 4 h in a frame, from 6 to 10 a.m. by means of a metal clamp. Previous experiments showed that exposure to stress in the morning most frequently induces pathological mitoses in rats, whereas at other times of day this is not always observed [8, 9]. Some animals were given an intraperitoneal injection of dalargin in a dose of 10 μg/kg 1 h before fixation. Animals were killed and material collected 1 h after the end of fixation.

In the second group of experiments the rats were subjected to fixation for 1 h daily for 5 days. It is under such conditions that compensatory stimulation of DNA synthesis is observed in the epithelium [1], a characteristic feature of chronic stress situations [4], and that vertical migration of the epithelial cells [10], an important component of the disturbance of cell differentiation, are observed. Immediately before immobilization the animals of one group were given dalargin by intraperitoneal injection in a dose of 10 μg/kg, whereas rats of the other group received isotonic sodium chloride solution. Immediately after final immobilization of the animals they were given an injection of ^{3}H-thymidine (0.6 μCi/g, specific activity 81 Ci/mmole), and in addition, ^{3}H-thymidine was applied to the cornea in a dose of 5 μCi. Some of the animals were killed 1 h after injection of thymidine. To assess the velocity of vertical migration of thymidine-labeled cells, animals of another group were killed 24 h after injection of thymidine. The number of labeled nuclei in each layer of the cornea was counted by the method described previously [10].

Determination of the mitotic index (MI, promille), the number of pathological mitoses (PM, % of the total number of mitoses), preparation of autoradiographs, and determination of the index of ^{3}H-thymidine-labeled nuclei (ILN, %), the intensity of labeling (IL, mean number of grains of silver above the nucleus), were carried out by methods adopted in the laboratory [3]. The total number of rats used in the experiments was 86. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

A single exposure to stress in the morning led to disturbances of DNA synthesis in the cornea: ILN fell to 2.4% compared with 7.6% in the control group (Fig. 1). The fall of ILN was accompanied by a significant decrease in IL, evidence of the slowing

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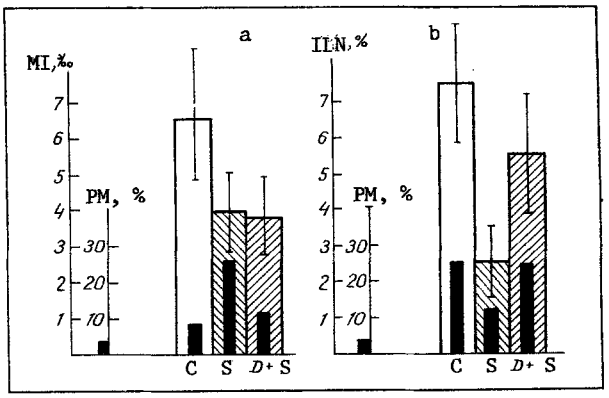

Fig. 1. Effect of dalargin on cell division in corneal epithelium following a single exposure to stress. a) Mitotic index (MI, promille) and number of pathological mitoses (PM, %); b) index of labeled nuclei (ILN, %) and intensity of labeling (IL, %). C) Control, S) stress, D + S) dalargin + stress.

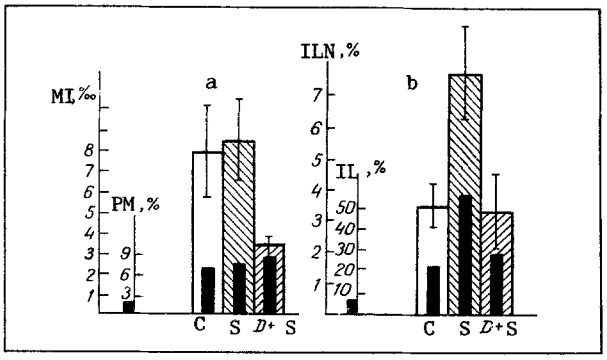

Fig. 2. Effect of dalargin on cell division in corneal epithelium during repeated exposure to stress. Legend as to Fig. 1.

of DNA synthesis. Besides a disturbance of DNA synthesis, exposure to stress also induced a more than threefold increase in the level of PM. In animals receiving dalargin before exposure to stress, no significant changes took place in the parameters of DNA synthesis. The level of PM likewise was unchanged compared with the intact control: 8.2 (compared with 10.1 in the control, animals receiving dalargin before stress).

A significant decrease in MI in the animals receiving dalargin before stress was evidently due to premitotic delay, which under these conditions prevents the PM level from rising [8].

During five repetitions of immobilization stress compensatory stimulation of proliferative processes, characteristic of repeated and chronic exposures, took place [1, 5]. ILN and IL in the corneal epithlium increased by 2.2 and 2.6 times respectively (Fig. 2). MI and the level of PM showed no significant changes. The stability of MI in these experiments can be explained by the acceleration of mitosis itself. In rats receiving dalargin before stress no changes were observed in the value of ILN, although IL was significantly higher than the control value. The PM level was unchanged in these experiments. The significant decrease in MI was perhaps due to premitotic delay, although changes in the velocity of mitosis itself cannot be ruled out.

Thus dalargin had different effects on DNA synthesis in the epithelium in the case of single and repeated exposures to stress: in acute stress it activated DNA synthesis, but during repeated exposures it prevented activation. It may be recalled that when dalargin was given to intact animals marked stimulation of DNA synthesis took place [1, 12] in the corneal and lingual epithelium. In our view, this can be explained, not by the different character of the effect of dalargin depending on the initial background, but by the antistressor properties of the preparation. By alleviating the course of stress dalargin prevents inhibition of DNA synthesis in the case of acute exposure to stress. This prevents the loss of the cell population which determines compensa-

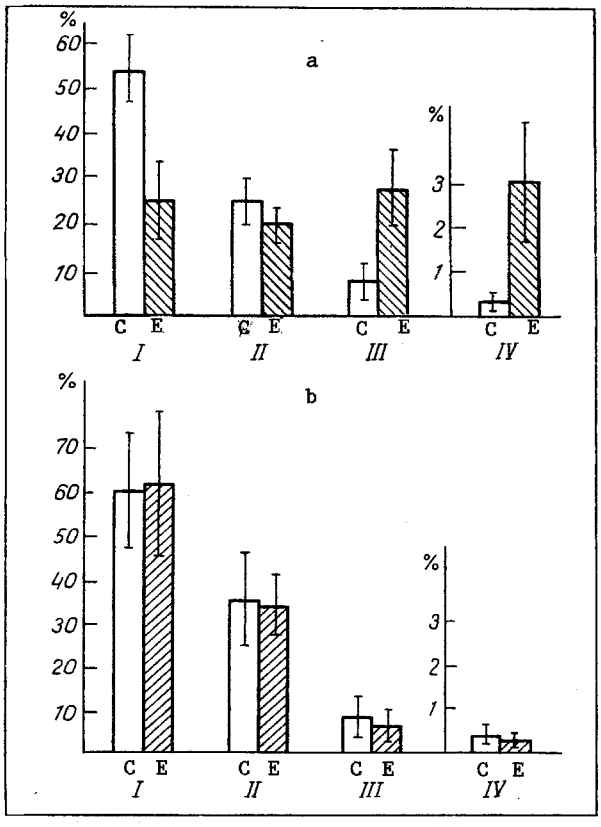

Fig. 3. Distribution (in %) of labeled nuclei among layers of corneal epithelium during repeated exposures to stress, 24 h after injection of ^{3}H-thymidine. a) Intact animals (C) and animals exposed to stress (E). b) Intact animals (C) and those exposed to stress, treated with dalargin before stress (E). I-IV) Layers of corneal epithelium: I) basal, II) spinous, III) intermediate, and IV) inner squamous layer.

tory stimulation under the conditions of repeated stress. We have shown that preparations alleviating the course of stress prevent compensatory stimulation of DNA synthesis during repeated exposures to stress [2, 5]. Previous investigations showed that maximal acceleration of migration of labeled cells from the basal layer into the higher layers takes placed 24 h after the final exposure to stress [10]. Injection of dalargin into animals before stress led to normalization of the velocity of migration of labeled cells from the basal layer into the higher layers. Parameters of rats subjected to stress and receiving dalargin beforehand, when measured 24 h after injection of thymidine, were indistinguishable from values of the intact control (Fig. 3). Induction of PM is one indicator of cytopathic action.

The ability of dalargin to prevent an increase in the frequency of aberrant mitoses during stress is further confirmation of its cytoprotective properties [13]. Acceleration of vertical migration of cells during stress may lead to a disturbance of cell differentiation. Combinations of acceleration of cell migration into higher layers with elevation of the PM level may be a vital component of the structural trace of disadaptation. The property of the opioid peptide, dalargin, of preventing the appearance of a structural trace of disadaptation, is evidence that dalargin exhibits its antistressor properties at the cellular and tissue level.

LITERATURE CITED

1. N. I. Berezhnova and S. S. Timoshin, Byull. Éksp. Biol. Med., No. 12, 727 (1984).
2. S. V. Vdovenko and S. S. Timoshin, Byull. Éksp. Biol. Med., No. 1, 29 (1985).
3. G. M. Kalivetskaya and S. S. Timoshin, Byull. Éksp. Biol. Med., No. 4, 92 (1982).
4. Yu. B. Lishmanov, M. N. Maslov, and M. I. Titov, Byull. Éksp. Biol. Med., No. 9, 268 (1985).

5. E. I. Mel'nik, "Effect of chronic stress on cell division in different types of epithelium in the albino rat," Author's Abstract of Candidate's Dissertation, Medical Sciences, Vladivostok (1987).
6. M. G. Pshennikova, Patol. Fiziol., No. 3, 85 (1987).
7. V. D. Slepushkin, Yu. B. Lishmanov, G. K. Zoloev, et al., Usp. Fiziol. Nauk, 65, No. 4, 106 (1985).
8. S. S. Timoshin, "Effect of stressors on cell division in the corneal epithelium of albino rats," Author's Abstract of Doctoral Dissertation, Medical Sciences, Moscow (1982).
9. S. S. Timoshin, N. B. Simon, and A. P. Baranov, Scientific and Technical Progress and Circumpolar Medicine [in Russian], Novosibirsk (1978), pp. 146-147.
10. S. S. Timoshin and N. I. Berezhnova, Byull. Éksp. Biol. Med., No. 8, 167 (1985).
11. S. S. Timoshin, T. D. Pan'kova, and M. I. Titov, Byull. Éksp. Biol. Med., No. 8, 229 (1987).
12. S. S. Timoshin and T. F. Zhdanova, Byull. Éksp. Biol. Med., No. 9, 354 (1987).
13. M. I. Titov, V. A. Vinogradov, and Zh. D. Bespalova, Byull. Vses. Kardiol. Nauch. Tsent., No. 2, 72 (1985).

RESPONSE OF CIRCADIAN RHYTHMS OF THE LYMPHOID SYSTEM TO DEEP SCREENING FROM THE EARTH'S GEOMAGNETIC FIELD

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KEY WORDS: biorhythms; lymphoid system; hypomagnetic state

Circadian rhythms of the lymphoid system form the spatiotemporal organization for the distribution of functional steps in lymphoid tissue during the 24-h cycle. This applies in particular to the power of migration and recirculation flows of lymphoid cells, maintaining the morphological composition of the lymphoid organs: bone marrow, thymus, spleen, lymph nodes. During the daytime, in the period of rest, proliferative processes predominate in the lymphoid tissue of inbred mice and recirculation processes of cells, which left the circulation during the previous period of activity, come to an end. The period of dark motor activity itself is characterized primarily by more intensive migration of young lymphoid cells and the more rapid migrations of mature lymphoid cell forms [3]. Data have also been obtained on the effect of changes in the intensity of the earth's magnetic field on the level of leukocytosis in peripheral blood [1, 6], and also on the effect of deep screening on young rabbits: the number of large, undifferentiated lymphoid cells in the structure of their spleens was increased [5].

We have studied the state of the set of circadian biorhythms of the lymphoid system of laboratory mice, kept under conditions of deep screening.

EXPERIMENTAL METHOD

Experiments were carried out from May 11 through May 26, 1988, in a reinforced concrete bunker, at a depth of 3-4 m; a cylindrical screening chamber was made in it, from four layers of permalloy steel, reducing by 10^4 times the intensity of the earth's magnetic field. The experimental series of seven inbred male C57BL/6 mice aged 13-14 weeks and weighing 23-26 g was kept under conditions of constant dim lighting and with free access to water and food. A similar control series of animals was kept in the same bunker in the immediate vicinity of the hypomagnetic chamber. On the 1st and 7th days (throughout the 24-h period

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