MODULATING EFFECT OF OPIOID PEPTIDES ON HEMATOPOIESIS DURING STRESS

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The writers previously showed that, in principle, medullary hematopoiesis can be modulated with the aid of enkephalins during immobilization stress [1]. The aim of the present investigation was to study the effect of opioid peptides and, in particular, of Leu-enkephalin and its synthetic analog, dalargin, on the formation of responses of the blood system at different periods of immobilization stress, and also to discover the individual mechanisms of their action.

EXPERIMENTAL METHOD

Experiments were carried out on 500 noninbred male mice weighing 18-20 g. The animals were immobilized for 6 h in the supine position, and at the end of that time they were given a single intraperitoneal injection of Leu-enkephalin or dalargin in a dose of 100 μg/kg body weight (these substances were obtained in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Director — Dr. Chem. Sci. M. I. Titov). Animals of the control group received physiological saline under analogous conditions. Material for investigation was taken at different times after the beginning of exposure (up to 10 days). Peripheral blood parameters (total and differential leukocyte count, erythrocyte and reticulocyte counts) and the total number of myelokaryocytes (per femur) were determined in the experimental animals. The myelogram was determined by examination of bone marrow films. Mitotic activity of the medullary cells was estimated by a stathmokinetic method. For this purpose, 2 h before sacrifice, the mice were given a single intraperitoneal injection of colchicine in a dose of 4 mg/kg. The stathmokinetic index was calculated per 1000 cells of the erythroid and granulocytic series of hematopoiesis, potentially capable of dividing. The concentration of 11-hydroxycorticosterone (11-OHCS) in the plasma of the mice was determined by a fluorometric method [6] on a "Hitachi" spectrofluorometer (Japan) with an outlet slit width of 5 nm, excitation of fluorescence by light with a wavelength of 475 nm, and identification of the peak of fluorescence at a wavelength of 525 nm.

EXPERIMENTAL RESULTS

In the early stages (1st day) after immobilization the animals developed a combination of characteristic redistributive reactions involving the blood system [2]. For instance, 3 h after the beginning of immobilization a neutrophilic leukocytosis developed in the peripheral blood, with an increase in the number of polymorphs up to 191% of the initial level. The eosinophil count fell progressively until total disappearance (after 12 h). A lymphoid peak developed in the bone marrow (after 3 h), with an increase in the content of lymphoid cells up to 233% of the initial value, whereas the peripheral blood showed lymphocytopenia, which lasted until 12 h after the beginning of immobilization. Values of all these parameters returned to their initial levels 24 h after the beginning of immobilization.

A single injection of Leu-enkephalin or of its synthetic analog changed the character of development of the early peripheral blood responses. For instance, in animals receiving the preparations mentioned, lymphocytopenia did not develop 12 h after the beginning of immobilization and the eosinopenia was less marked.

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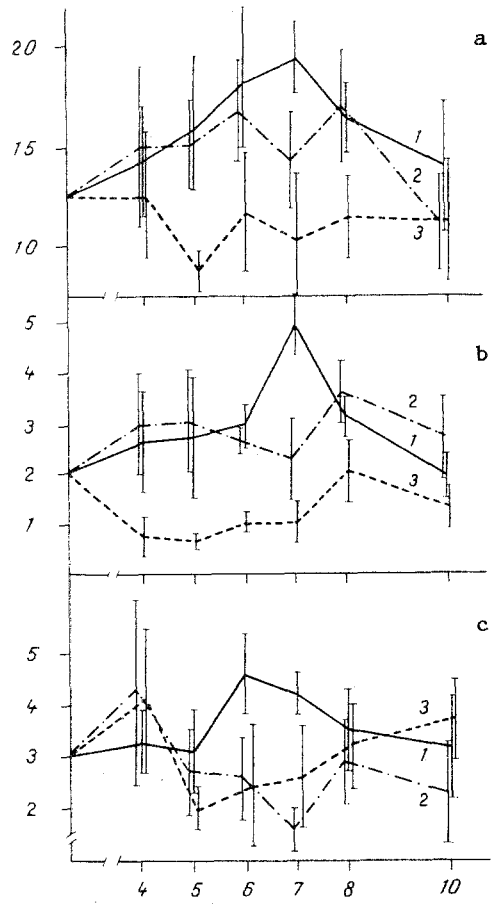

Fig. 1

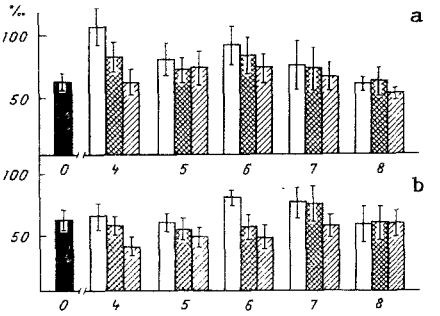

Fig. 2

Fig. 1. Time course of total number of myelokaryocytes (a), erythroid cells (b), and immature neutrophilic granulocytes (c) in bone marrow of noninbred mice immobilized for 6 h, and then given an injection of physiological saline (1), Leu-enkephalin (2), or dalargin (3). Abscissa, time of investigation (in days); ordinate, number of bone marrow cells ($\times 10^6$ per femur).

Fig. 2. Dynamics of mitotic activity of cells of erythroid (a) and granulocytic (b) series of hematopoiesis in noninbred mice immobilized for 6 h and subsequently receiving injection of: physiological saline (empty columns), Leu-enkephalin (cross-hatched columns), and dalargin (obliquely shaded columns). Abscissa, time of investigation (days); ordinate, stathmokinetic index (in ‰).

TABLE 1. 11-OHCS Concentration (μg/100 ml) in Plasma of Noninbred Mice Immobilized for 6 h and Subsequently Given an Injection of Physiological Saline or Dalargin (M±m)

Time of investigation before immobilization	Physiological saline	Dalargin
	22,4±1,8	22,4±1,8
6 h	38,3±3,3*	—
12 h	19,0±2,8	17,6±3,4
1 days	30,4±3,1*	13,4±1,3*
3 days	31,5±1,5*	10,6±1,1*
4 days	24,0±3,8	20,2±1,2
5 days	17,6±1,2	10,0±0,7*
6 days	21,2±2,9	19,3±2,7
7 days	10,7±1,0*	14,8±2,1*
8 days	20,6±3,6	9,8±0,7*

Legend. *$p<0.05$ compared with corresponding control.

On the 6th-8th day the control, immobilized animals developed marked hyperplasia of their bone marrow. The total number of myelokaryocytes in this group reached peak values after 7 days of investigation (up to 140% of the initial value). Analysis of the myelograms showed that the increase in the number of bone marrow cells under these circumstances took place on account of stimulation of both erythro- and granulocytopoiesis up to 245 and 140% respectively of the initial value (Fig. 1). One result of the medullary hyperplasia was an increased production of mature blood cells, as shown by the presence of neutrophilic leukocytosis (up to 150% of the initial values), monocytosis (up to 240%), and erythrocytosis (up to 110%) on the 6th-7th day after the beginning of immobilization. Activation of erythropoiesis also was confirmed by the development of marked reticulocytosis (up to 180% of the initial value).

Injection of the enkephalins caused a significant change in the character of response of the hematopoietic tissue to stress. For instance, Leu-enkephalin reduced the severity of development of medullary hyperplasia in animals exposed to immobilization, whereas dalargin completely abolished the changes in the blood system characteristic of the general adaptation syndrome, or even led to the development of hypoplasia of medullary hematopoiesis on the 6th-8th days of the experiment (Fig. 1). The suppressive effect of opioids on hematopoiesis led to a change in the dynamics of the peripheral blood parameters. In both cases (injection of Leu-enkephalin or of dalargin), for instance, neutrophilic leukocytosis, monocytosis, and erythrocytosis did not develop. The effect of dalargin, moreover, was stronger. After injection of dalargin the number of reticulocytes in the peripheral blood likewise did not increase significantly.

The development of hyperplasia of medullary hematopoiesis in mice subjected to immobilization stress was linked with stimulation of proliferation of hematopoietic cells (Fig. 2). For instance, an increase in the number of dividing erythroid cells was observed in the control animals as early as on the 4th day of the experiment (up to 160% of the initial value) and it continued through the 6th day. Stimulation of mitotic activity of cells of the granulocytic series of hematopoiesis was observed on the 6th-7th days of the experiment (up to 133% of the initial value). Injection of Leu-enkephalin reduced the number of proliferating bone marrow cells, and injection of dalargin arrested the increase in mitotic activity of the hematopoietic cells.

No significant differences were found between values of the stathmokinetic index in the experiment with dalargin and the initial state (before immobilization; Fig. 2).

In recent years sufficient evidence has been obtained in support of an important role of adrenocortical hormones (glucocorticoids) in stimulating proliferation and differentiation of hematopoietic cells, leading to the development of medullary hyperplasia during stress [4]. Meanwhile we know that opioid peptides can substantially modify activity of the pituitary–adrenal system during exposure to extremal influences [3, 5]. To confirm the possibility of an indirect effect of enkephalins on medullary hematopoiesis in stress the 11-OHCS concentration was determined in the blood plasma of the experimental animals (Table 1). In mice subjected to immobilization a marked increase was observed in the corticosteroid concentration in the plasma 6 h after the beginning of immobilization (up to 171% of the initial value). Later the 11-OHCS concentration fluctuated in a wavelike manner and remained elevated through the 3rd day of the experiment. Thus the rise of the hormone level preceded the development of medullary hyperplasia in the experimental animals with immobilization stress. Meanwhile, in mice receiving dalargin, no increase in the plasma corticosteroid concentration was observed. Conversely, the concentration of the hormones in some cases was significantly lower than the initial values and than the corresponding values in the group of control animals.

Opioid peptides, which have a universal antistressor action [7], thus exert a suppressive effect on hematopoiesis during stress and modify the course of the early redistributive reactions of the blood system. The effect of dalargin, a stable analog of Leu-enkephalin, is stronger. An important role in the formation of the early responses of the blood system is known to be played by activation of the sympathico-adrenal system [2]. Inhibition of activity of that system by exogenous enkephalins may perhaps lead to a decrease in the intensity of the quantitative changes in the peripheral blood cell composition in the early stage of the general adaptation syndrome. The effect of opioids on medullary hematopoiesis during stress is evidently mediated through their inhibition of activity of the pituitary–adrenal system, which leads to reduction of the proliferative capacity of the medullary cells and to abolition of reactions of hematopoietic tissue characteristic of the general adaptation syndrome.

LITERATURE CITED

1. E. D. Gol'dberg, O. Yu. Zakharova, A. M. Dygai, et al., Byull. Éksp. Biol. Med., No. 5, 589 (1987).
2. P. D. Gorizontov, O. I. Belousova, and M. I. Fedotova, Stress and the Blood System [in Russian], Moscow (1983).
3. V. N. El'skii, V. D. Slepushkin, V. A. Samsonenko, et al., Patol. Fiziol., No. 6, 15 (1985).
4. E. V. Kirienkova, Mechanisms of Pathological Reactions [in Russian], Vol. 4, Tomsk (1986), pp. 8-11.
5. Yu. B. Lishmanov, L. N. Maslov, and M. I. Titov, Byull. Éksp. Biol. Med., No. 9, 268 (1985).
6. É. Z. Naugol'nykh, Lab. Delo, No. 9, 532 (1969).
7. V. D. Slepushkin, Yu. B. Lishmanov, G. K. Zoloev, and I. A. Prum, Usp. Fiziol. Nauk, 16, No. 4, 106 (1985).