DALARGIN STIMULATES CELL DIVISION THROUGH OPIATE RECEPTORS

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The writers showed previously that endorphins and enkephalins, and also the synthetic Leu-enkephalin analog, dalargin, stimulate processes of DNA synthesis and mitotic activity in various epithelial tissues [1, 3, 4]. There is evidence in the literature that some neuropeptides (substance P, bombesin, vasopressin), which are not ligands of opiate receptors, also stimulate proliferative processes [6, 9]. It was decided to investigate the role of opiate receptors in the realization of the mitogenic effect of dalargin.

To study this problem the character of the effect of dalargin on cell division was investigated in the corneal epithelium of albino rats during blockade of the opiate receptors by naloxone, and also to analyze the effect of peptides similar to dalargin in their chemical structure, but possessing opiate properties, in some cases, or having lost them, in others.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 150-180 g. Dalargin was injected intraperitoneally at 2 p.m. in a dose of 10 μg/kg, and naloxone ("Endo Laboratories," USA) was given in a dose of 200 μg/kg. When combined administration of naloxone and dalargin was used, the naloxone was given 20 min before the dalargin. Animals receiving an equal volume of isotonic sodium chloride solution by intraperitoneal injection served as the control. The experimental and control animals were killed 24 h after injection of the preparations.

To analyze the role of opiate receptors in the stimulation of cell division after injection of dalargin, two synthetic Leu-enkephalin analogs which, like dalargin, are agonists of opiate receptors, with structural formulas: Tyr-Leu-Gly-Phe-Leu-Arg (a) and Try-DAla-Gly-Phe-Leu-Arg-NH-C_2H_5 (b), and two analogs which are not ligands of opiate receptors, and with the structural formulas: Phe-DAla-Gly-Phe-Leu-Arg (c) and Tyr-Ala-Gly-Phe-Leu-Arg (d), also were used. The substances were synthesized in the Laboratory of peptide Chemistry, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. All these peptides were injected intraperitoneally at 2 p.m. in a dose of 10 μg/kg. Control animals were given an equal volume of isotonic sodium chloride solution intraperitoneally. In some experiments, to rule out any change in the rate of mitosis itself, 2 h before sacrifice the animals were given an intraperitoneal injection of colchicine (0.2 mg/100 g). Experiments were carried out 24 h after injection of the peptides. The mitotic index of the colchicine-blocked metaphases (MI_{col}) was determined.

In each group of experiments, there were 8-10 control and experimental animals. Altogether, 132 rats were used. To obtain autoradiographs, one cornea was incubated at 37°C in an ultrathermostat in medium 199 with ^{3}H-thymidine (3 μCi/ml), and a total preparation was obtained from the second retina, in which the mitotic index (MI, in $^0/_{00}$) was determined. Autoradiographs were prepared, the index of labeled nuclei (ILN, in %) and intensity of labeling (IL) were determined, total preparations were obtained and MI determined by methods described previously [2]. The numerical results were subjected to statistical analysis by Student's test.

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TABLE 1. Effect of Dalargin and Naloxone on Cell Division in Corneal Epithelium of Albino Rats (24 h after injection)

Experimental conditions	MI, ‰	ILN, %	IL
Control	2,3	6,2	14,6
Injection of dalargin	5,9*	11,2*	224,4*
Control	9,5	5,7	17,3
Injection of naloxone	4,6*	4,1*	14,1
Control	12,0	5,7	14,7
Combined injection of naloxone and dalargin	12,1	6,0	16,7

Note. Here and in Table 2, asterisk indicates significant differences compared with control.

TABLE 2. Effect of Peptide Agonists of Opiate Receptors (a) and (b) and Peptides which are not Ligands of Opiate Receptors (c) and (d) on Proliferation of Albino Rat Corneal Epithelium (24 h after injection)

Experimental conditions	MI, ‰	MI_{col}, ‰	ILN, %	[illegible]
Control	2,6	8,1	5,7	17,3
Injection of peptide (a)	5,9*	12,5*	8,9*	31,4*
(b)	5,9*	17,3*	8,9*	30,3*
(c)	2,9	8,7	5,8	16,4
(d)	2,0	9,9	6,1	17,2

EXPERIMENTAL RESULTS

Injection of naloxone in a dose of 200 μg/kg caused significant inhibition of the number of dividing cells in the corneal epithelium (Table 1). MI in the experimental animals was reduced by half. Inhibition of cell division was due to a decrease in the number of cells embarking on the S-period — this was shown by a reduction of 1.4 times in the value of ILN. The rate of DNA synthesis, judging by the intensity of labeling, showed no significant change (Table 1).

Blockade of opiate receptors by naloxone evidently makes the cell insensitive to the stimulating action of endogenous ligands of opiate receptors, which leads to depression of proliferation in the corneal epithelium. In previous investigations naloxone in a dose of 10 μg/kg, and also dalargin, caused stimulation of cell division. When explaining the stimulating effect of naloxone, an opiate receptor blocker, in several experimental situations, Bruzzone suggests that in cases when the effect of opiates is realized through the δ-receptor, naloxone, by blocking the μ-receptor, unmasks the δ-receptor and makes it more accessible for opiates [7]. This may evidently explain why naloxone, in our previous experiments, stimulated cell division whereas in a dose of 200 μg/kg, naloxone blocked the δ-receptors also, causing inhibition of cell division.

Injection of dalargin, just as in the previous investigations, stimulated DNA synthesis and raised the mitotic index. Blockade of the opiate receptors by naloxone prevented stimulation of cell division by dalargin: MI, ILN, and LI showed no significant changes. These data explain the experimental results [5] showing that naloxone abolishes the stimulating action of dalargin on regeneration of the head end of the body of planarian worms. The results are evidence that dalargin exerts its stimulating effect on cell division through specific opiate receptors.

Further proof that the mitotic effect of dalargin is exerted through opiate receptors was given by the results of experiments with a group of peptides similar to dalargin in their chemical structure, and possessing affinity for opiate receptors, namely the peptides (a) and (b), Table 2. Both preparations, like dalargin itself, led to an increase in the number of dividing cells and activated DNA synthesis. MI and MI_{col} were increased under the influence of these peptides by 2.2 times, and ILN by 1.5 times.

The intensity of labeling, reflecting the rate of DNA synthesis, was increased by 1.7 times. At the same time, peptides (c) and (d), which are not ligands of opiate receptors, did not cause any significant changes in the number of dividing cells or in the level of DNA synthesis.

The results of the investigations into involvement of opiate receptors in cell division confirm the view that endogenous opiate peptides are an important component of the system maintaining structural homeostasis.

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ROLE OF THE STROMAL MICROENVIRONMENT IN REGULATION OF BONE-MARROW HEMATOPOIESIS AFTER ADMINISTRATION OF DIPYRIDAMOLE

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The construction of hematopoietic territories following heterotopic bone marrow transplantation is accompanied by transfer of the hematopoiesis-inducing microenvironment, which determines the type and volume of the developing hematopoietic tissue, and the cells transferring it are stromal cells of the hematopoietic organs [4-6]. A general feature of cells of connective-tissue nature is the presence of a receptor for platelet-secreted growth factor (PSGF), by means of which the stimulating action of PSGF on proliferative is realized [1, 10]. Blocking of platelet aggregation, leading to a decrease in the secretion of PSGF, causes inhibition of proliferative activity of connective-tissue cells.

The aim of the investigation was to study the role of the stromal microenvironment in the regulation of bone-marrow hematopoiesis following administration of dipyridamole, used as a platelet disaggregant, by the method of experimental heterotopic bone marrow transplantation.

EXPERIMENTAL METHOD

Experiments were carried out on 540 male (CBA × C57BL)F_1 mice weighing 18-20 g. A fragment of bone marrow isolated from the femoral medullary cavity was transplanted beneath the connective-tissue capsule of the kidney of syngeneic recipients under hexobarbital anesthesia [11]. Some animals received an intraperitoneal injection of dipyridamole (D) in a dose of 30 mg/kg body weight daily for 50 days after bone marrow transplantation. Control animals received physiological saline. From the rest of the experimental and control animals, the foci of heterotopic hematopoiesis (FHH), formed 7 days after transplantation, was retransplanted into a fresh group of intact recipients. The animals were killed by cervical dislocation on the 7th, 19th, 30th, and 50th days of administration of D. Animals with retransplanted foci were killed on the 30th day of the experiment. The dimensions of FHH were estimated from the weight of the bony capsule and of the cellular bone marrow in it, in both experiment and control. Part of the material was analyzed histologically. In preparations stained with hematoxylin-eosin the mitotic

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