3. T. Ch. Vinogradova, Instrumental Methods of Investigation of the Cardiovascular System [in Russian], Moscow (1986).
4. L. G. Moiseeva and Yu. A. Vlasov, Hypothermic Protection in Heart Surgery [in Russian], Novosibirsk (1980), pp. 302-303.
5. V. M. Pokrovskii, The Circulation and Surrounding Medium [in Russian], Simferopol' (1983), pp. 143-149.
6. S. A. Seleznev, S. M. Vashetina, and G. S. Mazurkevich, Comprehensive Evaluation of the Circulation in Experimental Pathology [in Russian], Leningrad (1976).
7. V. A. Frolov, O. I. Kiselev, and E. A. Demurov, Byull. Éksp. Biol. Med., **62**, No. 3, 19 (1964).
8. D. E. Donald and J. T. Shepherd, Am. J. Physiol., **207**, No. 6, 1325 (1964).
9. L. J. Greenfield, P. A. Ebert, W. G. Austek, and A. C. Morrow, Surgery, **51**, No. 3, 356 (1962).
10. K. M. Kent and T. Cooper, New Engl. J. Med., No. 19, 1017 (1974).

EFFECT OF DALARGIN ON PROLIFERATION OF THE GASTRIC EPITHLIUM DURING REPEATED EXPOSURE TO STRESS

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UDC 612.841.014.3:612.6.014.43

KEY WORDS: opioid peptides; dalargin; histamine; DNA synthesis; stress.

Dalargin, a synthetic analog of Leu-enkephalin, has a broad spectrum of biological activity [8]: it accelerates physiological and reparative regeneration [9, 10], has an immunomodulating action [13], and possesses antistress activity [8]. Dalargin depresses poststress ulcer formation [6]. According to the results of our previous investigations, this property of dalargin is realized through preservation of noradrenalin reserves in the gastric tissue, reduced accumulation of lipid peroxidation products in the tissues, weakening of poststress depression and activation of compensatory proliferative cell division in the epithelium of the pyloric part of the stomach, in response to a single exposure to fixation stress [12]. In our previous investigations we found that dalargin can increase the histamine content in the gastric tissues, and this is accompanied by lowering of the blood histamine concentration [1]. According to one view [15], this trend of histamine metabolism promotes activation of reparative regeneration in the gastric mucosa.

The aim of this investigation was to determine the effect of dalargin on DNA synthesis in the epithelial cells of the mucosa of the pyloric part of the stomach during repeated exposure to various kinds of stress, and also to evaluate the role of histamine in the regulation of cell division of the gastric epithelium under these conditions.

EXPERIMENTAL CONDITIONS

Experiments were carried out on female albino rats weighing 160-190 g. Fixation stress was produced by immobilizing the animals in the supine position by the method described previously [11]. Hypoxic stress was created by elevation

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Central Research Laboratory, Khabarovsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman*.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 8, pp. 130-132, August, 1991. Original article submitted February 5, 1991.

 0007-4888/91/0008-1076$12.50

TABLE 1. Action of Dalargin on DNA Synthesis in Epitheliocytes of the Pyloric Part of the Stomach of Albino Rats Subjected to Five Exposures to Stress (M ± m)

Group of animals	Time after end of exposure to stress, h			
	1		24	
	ILN	LI	ILN	LI
Intact control	5,5±0,7	20,6±1,1	3,4±0,6	16,2±2,5
Hypoxia	3,5±0,1	17,6±1,1	9,7±1,1*,**	28,0±1,9*
Hypoxia + dalargin	4,6±1,3	22,3±3,3	6,3±0,6*,**	17,5±1,8
Intact control	5,5±0,6	20,6±1,1	3,4±0,6	16,3±2,5
Hyperthermia	3,3±0,4*	16,3±1,0*	1,5±0,2*	13,5±2,2
Hyperthermia + delargin	9,5±1,6*	22,0±1,6	3,8±0,6	11,5±1,8
Hypoxia	6,3±0,5	25,1±1,5	6,3±0,5	25,1±1,5
Immobilization	3,3±0,2*,**	20,4±1,7	9,8±0,3*	33,7±1,6*
Immobilization + dalargin	4,5±0,3*,**	24,5±3,0	7,0±0,8	24,0±1,1

Legend. Asterisk indicates significant differences between intact control and experimental group; two asterisks indicate significant differences between experimental groups subjected to a particular type of stress.

in a pressure chamber to an altitude of 9000 m, and staying at that altitude for 1 h. The animals were raised and lowered by the method described previously [2]. Exposure of the rats to sublethal hyperthermia for 2 h, up to a temperature of 41.5°C, was carried out in a ventilated hot chamber with a volume of 1.35 m^3 by the method in [7]. Dalargin was injected intraperitoneally in a dose of 10 μg/kg into the animals of one group 40 min before the beginning of exposure. The control group consisted of intact animals. Cell division was studied during the first hour and 24 h after the final exposure to stress. The animals were given an intraperitoneal injection of ^{3}H-thymidine (specific activity 87 Ci/mmole) in a dose of 0.06 μCi/g, 45 min before sacrifice. Autoradiographs were prepared by the usual laboratory method. The index of labeled nuclei (ILN) was expressed as a percentage and the labeling intensity (LI) as the average number of grains of silver above a cell nucleus. The histamine concentration was determined by the method described previously [1] and expressed in micromoles/g. Altogether 86 rats were used in the experiments. The results were analyzed by Student's test.

EXPERIMENTAL RESULTS

The results show that during hypoxic, hyperthermic, and immobilization stress significant inhibition of DNA synthesis took place during the first hour of exposure in the epithelium of the pyloric part of the stomach. This was manifested as a decrease in ILN by 1.57, 1.6, and 1.9 times respectively compared with the intact control (Table 1). In the experiments with hyperthermia the decrease in ILN was combined with a significant fall of LI; this was interpreted as evidence of disturbance of the rate of DNA synthesis under these conditions. During immobilization and hypoxia, LI did not differ significantly from values in the intact control. Preliminary injection of dalargin into the animals restored normal DNA synthesis during hypoxic stress, and during hyperthermia, ILN was significantly higher than in the intact control. In immobilization stress, injection of dalargin did not restore the normal value of ILN, but it significantly weakened the poststress decrease in its value.

Significant activation of DNA synthesis was noted 24 h after exposure to hypoxic and immobilization stress This was shown by an increase in ILN in hypoxia by 2.8 times and in immobilization by 1.6 times compared with values for the intact control. The increase in ILN during immobilization and hypoxia was accompanied by a significant increase in LI, evidence of an increase in the rate of DNA synthesis. According to our preliminary data, activation of DNA synthesis at this time of the investigation is compensatory in character and is aimed at maintaining tissue homeostasis [5]. During five exposures to sublethal hyperthermia, normalization of ILN 24 h after exposure was not observed. This was confirmed by the results of our previous investigations, showing that sublethal hyperthermia causes the most persistent disturbances of cell division compared with other stressors [7].

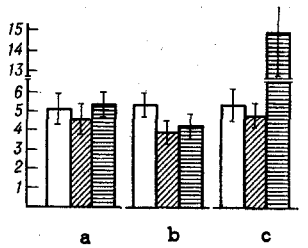

Fig. 1. Histamine concentration in gastric tissue 24 h after exposure to hypoxia (a), hyperthermia (b), and immobilization (c). Unshaded columns — control, obliquely shaded — exposure to stress, horizontally shaded — exposure to stress preceded by injection of dalargin. Ordinate, histamine concentration (in μg/g).

Preliminary injection of dalargin into the experimental animals led to normalization of the parameters of DNA synthesis during hyperthermia and immobilization In the experiments with hypoxia, at this stage of the investigations dalargin weakened the poststress increase in ILN by 1.8 times but did not restore it to normal, as was the case in experiments with immobilization and hyperthermia. In all three groups injection of dalargin led to restoration of the normal LI — this index did not differ from its value in the intact control. Our results, indicating normalization of proliferative processes in the stomach during stress under the influence of dalargin, agree with those obtained by the authors of [3], who noted a similar effect of Leu-enkephalin on hematopoiesis during stress. The ability of dalargin to stimulate cell division was manifested differently in our experiments. Immediately after the final exposure to stress, it took the form of weakening of poststress depression or its correction. As a result, compensatory stimulation was weakened or abolished after 24 h. Besides its ability to have a direct stimulating effect on cell division, dalargin also maintained tissue homeostasis during stress through many other mechanisms. This applies to its modulating effect on the endocrine balance during stress, its ability to prevent the fall of the noradrenalin concentration, to reduce accumulation of lipid peroxidation products in the stomach during stress [12], and to slow the rate of vertical migration of the cells [11]. The role of each of these factors evidently changes depending on the character of the stressor. This conclusion is confirmed by the results of investigations of the histamine content in the stomach in the present experiments.

When the histamine concentration in the stomach was determined 24 h after extremal exposure to stress no significant changes were found in its content in all three experimental groups (Fig. 1). Preliminary injection of dalargin in the experiments with hyperthermia and hypoxia likewise caused no significant changes in the histamine content. In experiments with fixation stress dalargin induced an almost threefold increase in the histamine concentration in the gastric tissues. This fact confirms the view that histamine is involved in realization of the effects of opiate receptor ligands [4]. It will be recalled that, according to some views [15], an increase in the histamine concentration in the tissues of the stomach improves its blood supply and stimulates repair processes. The fact that during immobilization stress injection of dalargin led to an increase in the histamine concentration in the gastric tissues during the period of compensatory activation of proliferative processes, and also our previous observations [1] are evidence in support of the involvement of histamine in realization of the effects of dalargin in immobilization stress, However, the stability of the histamine concentration in the stomach in experiments with hyperthermia and hypoxia suggests that this mechanism is not universal.

LITERATURE CITED

1. A. G Aleksandrovich, T. F. Zhdanova, S. S. Timoshin, et al., Byull. Éksp. Biol. Med., No. 8, 209 (1989).
2. S. V. Vdovenko and S. S. Timoshin, Byull. Éksp. Biol. Med., No. 8, 86 (1983).
3. E. D. Gol'dberg, A. M. Dygai, O. Yu. Zakharova, and V. P. Shakhov, Byull. Éksp. Biol. Med., No. 8, 216 (1989).
4. O. I. Karpov. Farmakol. Toksikol., No. 1, 36 (1990).
5. E. I. Mel'nik, "Effect of chronic stress on cell division in different types of epithelium of albino rats," Author's Abstract of Dissertation for the Degree of Candidate of Medical Sciences, Vladivostok (1987).
6. S. B. Pashutin, Byull. Éksp. Biol. Med., No. 7, 24 (1990).

7. M. I. Radivoz, "Effect of hyperthermia on cell division processes in the corneal and lingual epithelium of albino rats," Author's Abstract of Dissertation for the Degree of Candidate of Medical Sciences, Vladivostok (1987).
8. V. D. Slepushkin, Yu. B. Lishmanov, G. K. Zoloev, et al., Usp. Fiziol. Nauk, No. 4, 3 (1985).
9. S. E. Spevak, A. I. Solov'eva, and A. V. Shekhter, Byull. Vses. Kardiol. Nauch. Tsent., No. 2, 78 (1986).
10. S. S. Timoshin, T. D. Pan'kova, and M. I. Titov, Byull. Éksp. Biol. Med., No. 7, 97 (1988).
11. S. S. Timoshin, N. I. Berezhnova, and S. I. Shvets, Byull. Éksp. Biol. Med., No. 2, 101 (1990).
12. S. S. Timoshin, S. I. Shvets, N. B. Murzina, et al., Byull. Éksp. Biol. Med., No. 10, 399 (1990).
13. D. D. Kharkevich and Z. G. Kadagidze, Byull. Éksp. Biol. Med., No. 9, 315 (1989).
14. I. M. Hermens, I. M. Eberty, and I. M. Hanifin, Anesthesiology, **61**, 351 (1984).
15. S. Parson, Gut, **26**, No. 11, 1159 (1985).