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Hydra Peptide Morphogen Weakens Poststress Disturbances in Albino Rats

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The effect of hydra peptide morphogen on poststress disturbances in albino rats is evaluated. A 4-h immobilization leads to a rise of corticosterone, activates lipid peroxidation, impairs antioxidant defense system, and induces a marked decrease in the content of thyrotropic hormone and thyroxine. The relative weight of the thymus significantly decreases 24 h after immobilization. Moreover, stress inhibits proliferative processes in corneal and pyloric epithelium immediately and 24 h after immobilization. Hydra peptide morphogen prevents the endocrine shift, normalizes the content of lipoperoxides and α-tocopherol immediately after stress, weakens poststress proliferation disturbances, induces compensatory stimulation of proliferative processes in the corneal epithelium 24 h after stress, and normalizes DNA synthesis in the pyloric epithelium, the level of malonic dialdehyde being elevated.

Key Words: *stress; peptide hydra morphogen; cell division*

Hydra peptide morphogen (HPM) stimulates proliferation [11,12], participates in the regulation of endocrine status of the organism [2], and exerts an immunomodulating effect [9].

It has been demonstrated that HPM corrects DNA synthesis in the tracheal epithelium of newborn rat pups subjected to prenatal hypoxia [5].

The aim of the present study was to evaluate the effect of HPM on poststress disturbances in albino rats.

MATERIALS AND METHODS

Experiments were carried out on random-bred male rats weighing 150-160 g. The animals were immobilized in supine position for 4 h as described previously. Some animals were injected with 100 μg/kg HPM undecapeptide (synthesized at the Laboratory of Peptide Synthesis, Cardiology Research Center, Moscow) 1 h before immobilization. Preliminary experiments showed that this is an optimal dose for studying the stimulating effect of HPM on proliferation and proliferation-specific hormone rearrangement [11]. Control animals received an equivalent volume of sterile isotonic NaCl solution. The animals were decapitated immediately and 24 h after immobilization. The development of stress reaction was judged from the serum corticosterone level immediately after a 4-h immobilization (Amersham kits) and thymus weight 0 and 24 h after immobilization. No gastric ulceration was seen after 4-h immobilization, therefore, stress-induced disturbances in the stomach were assessed by the intensity of DNA synthesis in the pyloric mucosa epithelium. Additionally, plasma concentrations of thyrotropic hormone (TSH), triiodothyronine (T_3), and thyroxine (T_4) (kits manufactured by Institute of Bioorganc Chemistry, Academy of

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Science of Byelarus Republic) and the intensity of lipid peroxidation (LPO) were measured as stress-related parameters. To this end the concentrations of malonic dialdehyde [3] and α-tocopherol [13] were fluorometrically determined, and lipoperoxides were measured spectrophotometrically [4]. The data were standardized per gram lipids. Total lipids were measured using Lachema kits. Suppression of proliferation in epithelial tissues is an indirect indication of the anxiety stage of the general adaptation syndrome and a marker of poststress disturbances [10]. The corneal and pyloric epithelium were used as test-objects. DNA synthesis was assessed by ^{3}H-thymidine incorporation. The isotope was injected in a dose of 0.6 μCi/g (145 GBq/liter volume activity, 1530 GBq/liter molar activity) 45 min before sacrifice. Radioautographs were routinely processed. Index of labeled nuclei was expressed in percents and label intensity was counted as the number of silver grains above the nucleus. Mitotic index was counted in total corneal preparation and expressed in promille. The data were processed statistically using the Student's *t* test, the differences were significant at $p<0.05$.

RESULTS

Immobilization stress induced pronounced changes characteristic of the anxiety stage of the general adaptation syndrome. Serum corticosterone increased 1.6-fold immediately after a 4-h immobilization. At the end of immobilization, the relative weight of the thymus remained unchanged, but 24 h later it was significantly decreased (185±10.40 mg/g *vs.* 247.39±17.45 mg/g in the control). The decreased contents of TSH (more than 2-fold) and a dramatic drop of T_4 are indirect indications of the general adaptation syndrome. Moreover, we observed a pronounced activation of LPO: the contents of lipoperoxides and malonic dialdehyde increased more than 3-fold. A significant decrease (more than 3-fold) in serum α-tocopherol indicates suppression of the antioxidant system (Fig. 1). Stress also suppressed proliferative processes, which is characteristic of the anxiety stage [10]. In the corneal epithelium, mitotic index at the end of immobilization decreased 9.3-fold, while the index of labeled nuclei decreased 1.3-fold. In the pyloric epithelium these parameters were decreased 1.8- and 1.2-fold, respectively. Twenty-four hour later, the index of labeled nuclei remained significantly decreased in both corneal and pyloric epithelium, while label the intensity did not differ from the control values (Table 1).

Injection of HPM prevented the stress-induced shift of the endocrine balance. The concentrations of corticosterone, TSH, T_3, and T_4 at the end of 4-h immobilization did not differ from the control values. The LPO parameters were also normalized: content of lipoperoxides and α-tocopherol were close to the control level. However, the content of malonic dialdehyde remained increased (Fig. 1). Our previous experiments have demonstrated that, apart from the nonenzymatic antioxidant defense system, activation of the antioxidant enzymes can participate in normalization of the content of lipoperoxides [6].

Pretreatment with HPM partially weakened the stress-induced proliferative disturbances in corneal epithelium: at the end of immobilization, mitotic index and label intensity increased significantly 4.8- and 1.3-fold, respectively, in comparison with the corresponding control (stress without HPM pretreat-

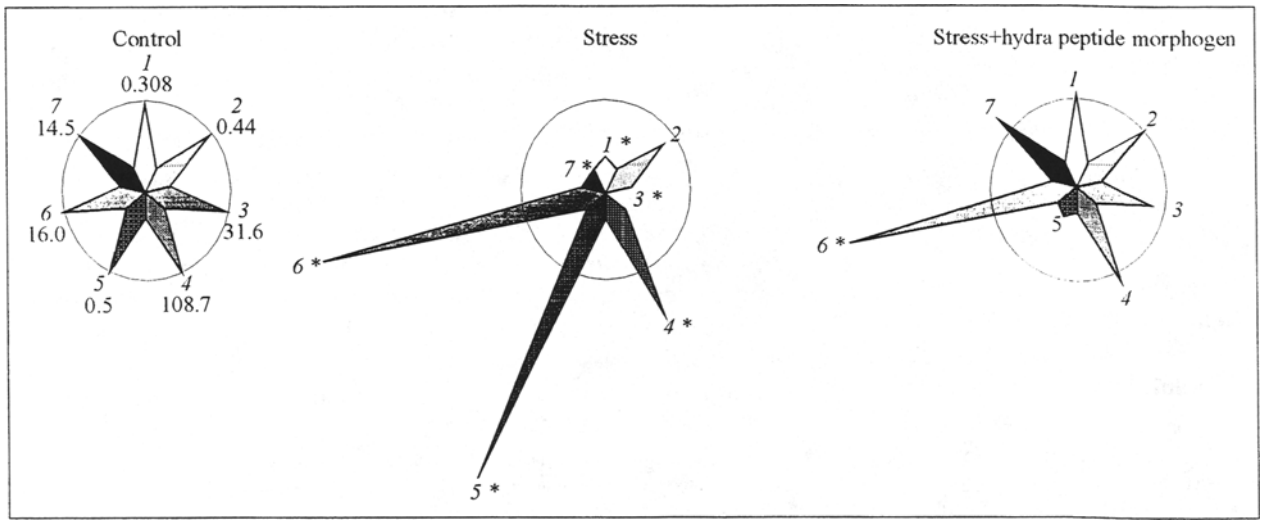

Fig. 1. Effect of a single injection of hydra peptide morphogen on stress parameters in albino rats (% of control). Significant differences are indicated with asterisks. *1*) thyrotropic hormone, μU/ml; *2*) triiodothyronine, nmol/liter; *3*) thyroxine, nmol/liter; *4*) corticosterone, nmol/liter, *5*) lipoperoxides, optical density units/mg lipids; *6*) malonic dialdehyde, fluorescence units/mg lipids; *7*) α-tocopherol, μmol/liter.

TABLE 1. Effect of Single Injection of HPM on Proliferation Processes in Corneal and Pyloric Epithelium in Stressed Animals ($M \pm m$)

Group	Cornea			Stomach	
	mitotic index, ‰	index of labeled nuclei, %	label intensity	index of labeled nuclei, %	label intensity
At the end of immobilization					
Control	4.31±0.32	6.93±0.30	13.45±0.35	5.35±0.21	13.76±0.35
Stress	0.46±0.04*	1.65±0.32*	10.16±0.31*	2.97±0.10*	12.32±0.15
HPM+stress	2.22±0.11*+	2.10±0.18*	12.82±0.27+	3.47±0.16*	13.47±0.19
24 h after immobilization					
Control	3.30±0.24	6.62±0.20	12.44±0.19	5.13±0.28	12.71±0.41
Stress	2.77±0.35	4.48±0.43*	11.83±0.26	3.94±0.16*	12.31±0.33
HPM+stress	4.28±0.37+	7.76±0.47*+	12.29±0.17	4.62±0.23	12.65±0.30

Note. $p<0.05$: *compared with the control, +compared with stress.

ment). Although complete recovery of cell division was not attained, the parameters of cell division in the pyloric mucosa were the same in HPM-treated and untreated animals. Twenty-four hours postimmobilization, we observed a compensatory stimulation of proliferative processes in the corneal epithelium of HPM-treated rats: mitotic index and index of labeled nuclei increased 1.5- and 1.7-fold. In the pyloric mucosa we observed normalization of DNA synthesis (Table 1).

When analyzing the mechanisms underlying the protective effect of HPM in stress, it should be remembered that administration of HPM markedly elevates the concentration of β-endorphin [8], which acts as a stress-limiting factor [7]. Together with other opioid peptides β-endorphin is the component of the central stress-limiting system.

Normalization of blood TSH content indirectly indicates activation of the central stress-limiting system after injection of HPM. The ability of HPM to improve the antioxidant defense system due to strengthening of both the nonenzymatic (our data) and enzymatic [6] components weakens the stress reaction through local mechanisms of stress-limiting system. HPM has been identified in the hypothalamus of mammals and human [1]. The ability of HPM to weaken stress-related disturbances cannot be extrapolated to the physiological function of this peptide and requires further investigation. Our findings suggest that HPM weakens stress-related disturbances.

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