tion of the plane of the porphyrin ring, as a result of which a change in interaction between porphyrin and the amino-acid skeleton of the molecule will take place [5].

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THE ANTISTRESSOR EFFECT OF D-Ala^2-Leu^5-Arg^6-ENKEPHALIN

Yu. B. Lishmanov, E. N. Amosova, V. D. Slepushkin, and K. V. Yaremenko

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KEY WORDS: stress, enkephalins, antistressor action.

Besides known biologically active substances, the opioid neuropeptides, whose level in the body rises regularly during exposure to extremal factors, may behave as endogenous modulators of stress [6, 7]. Accordingly the aim of this investigation was to study the effect of enkephalins on a combination of nonspecific morphological changes (Selye's triad) arising in animals and the glucocorticoid level in stress.

EXPERIMENTAL METHOD

Experiments were carried out on 50 noninbred male albino rats weighing 100-120 g and on 40 noninbred albino mice wieghing 18-20 g. Stress was induced by suspending the animals for 18 h by the neck fold. All the animals were divided into groups (10 in each group): 1) intact animals; 2) stress control; 3-5) before the beginning of exposure to stress, the synthetic arginine-containing hexapeptide analog of Leu-enkephalin (D-Ala^2-Leu^5-Arg^6-enkephalin) in various doses or a preparation of extract of eleutherococcus (1 ml/kg, perorally) was administered to the animals; the latter is known to be one of the most effective adaptogens [2, 3]. The animals were killed in accordance with the "Rules for the conduct of research using experimental animals" approved by the Ministry of Health of the USSR. The weight of the adrenals, spleen, and thymus was determined in milligrams, and the number of ulcers formed on the gastric mucosa was counted. Antistressor activity of the preparations was determined using the six-point scale for evaluation of stress suggested by Lobryakov [4]. It was considered that the preparation possessed antistressor activity if the difference in the total number of points between the stress control and the experimental groups exceeded or was equal to 2. To count the points for integral assessment of the degree of stress in an animal, values of the mean weight of the seperate organs in the groups were calculated per 100 g body weight of rat or per 20 g body weight of mouse,and changes in the parameters were expressed as percentages of the normal [4]. The 11-hydroxycorticosteroid (11-HCS) concentration in samples of blood plasma from the rats was determined by a fluorometric method [5] on a Hitachi (Japan) spectrofluorometer, and the immunoreactive cortisol level was determined by radioimmunoassay with the kit from CEA-IRE-Sorin (France) on a Tracor gamma-spectrometer (USA). The

Laboratory of Pathophysiology, Siberian Branch, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. Laboratory of Experimental Chemotherapy of Tumors, Siberian Branch, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Tomsk.

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TABLE 1. Effect of D-Ala^2-Leu^5-Arg^6-Enkephalin on Changes in Internal Organs and Plasma Cortisol Concentration in Rats with Stress (M ± m; n = 10)

Group of animals	Weight of internal organs, in mg			Number of ulcers in gastric mucosa	Total number of points	Cortisol, pg/ml
	spleen	thymus	adrenals			
1	923,7±134,5	353,2±98,2	44,8±3,4	—	—	1,67±0,48 P_t<0,01
2	440,2±43,9 P_{U_1}<0,05	297,9±27,4 P_{U_1}<0,05	56,3±2,4 P_{U_1}<0,05	4,7±3,0	14	9,54±0,83
3	332,5±20,4 P_{U_2}>0,05	314,7±16,8 P_{U_2}>0,05	47,7±3,4 P_{U_2}>0,05	6,6±1,4	10	1,35±0,57 P_t<0,01
4	457,3±42,1 P_{U_2}>0,05	306,5±24,0 P_{U_2}>0,05	50,0±3,2 P_{U_2}>0,05	5,4±1,7	10	2,15±0,84 P_t<0,01
5	420,6±51,3 P_{U_2}>0,05	296,7±29,6 P_{U_2}>0,05	51,9±2,6 P_{U_2}>0,05	7,2±3,0	11	2,22±1,04 P_t<0,01

Legend. Group 1) intact control; 2) stress control; 3) stress + enkephalin (10 µg/100 g); 4) stress + enkephalin (1 µg/100 g); 5) stress + eleutherococcus. P_t) Significance of changes compared with stress control; P_{U_1}) nonparametric criterion relative to intact animals; P_{U_2}) nonparametric criterion relative to stress control.

results were subjected to statistical analysis using nonparametric tests (the P_U test of Wilcoxon, Mann, and Whitney) and by Student's test (P_t).

EXPERIMENTAL RESULTS

The experiments showed that suspending the rats for 18 h caused changes characteristic of stress in the weight of the internal organs and the formation of ulcers in the gastric mucosa (Table 1). For instance, the weight of the spleen was reduced in these animals by more than half compared with that in intact animals. The weight of the thymus was significantly reduced (by 15%) and the weight of the adrenals increased (by 25%). In stressed rats ulcers formed regularly in the gastric mucosa.

The level of immunoreactive cortisol in the plasma of rats after 18 h of stress was 5.7 times higher than in the intact control (Table 1).

In rats receiving D-Ala^2-Leu^5-Arg^6-enkephalin (10 µg/100 g, subcutaneously), the increase in weight of the thymus and decrease in weight of the adrenals, calculated per 100 g, differed from the stress control, but these changes taken separately were not statistically significant, but differences from the intact animals still remained. Nevertheless, comparison of the total number of points in integral evaluation of the degree of stress in the animals in the stress control group and in the group receiving the neuropeptide showed that the enkephalin analog has marked antistressor activity, for the difference in the total number of points between these groups was 4.

Ability of the enkephalin analog to inhibit the stress reaction also was indicated by the absence of any increase in the level of immunoreactive cortisol in the blood of the stressed rats receiving this peptide (Table 1). The 11-HCS level, which was due mainly to corticosterone [5], also was lower in rats receiving the enkephalin analog before exposure to stress than in the stress control group (18.4 ± 1.74 < 27.6 ± 2.52 µg%; $P < 0.05$).

The antistressor effect of the enkephalin analog described above also was preserved when the preparation was given in a dose 10 times smaller (1 µg/100 g).

Peroral administration of eleutherococcus extract (1 mg/kg) before the rats were suspended, undertaken for comparison, likewise did not prevent the change due to stress in the weight of the individual internal organs, but it led to a decrease in the cortisol level compared with the stress control. The known antistressor properties of eleutherococcus [2, 3] were confirmed in these experiments when its activity was assessed on the point scale for integral evaluation of stress.

Incidentally, D-Ala^2-Leu^5-Arg^6-enkepalin, like eleutherococcus extract, did not prevent the formation of ulcers in the gastric mucosa of the stressed rats under these conditions. The antiulcerogenic action of opioid peptides described in the literature [1] can probably by explained by their direct effect on gastric secretion in the models of ulcer formation in the gastric and duodenal mucosa used by the authors cited. At the same time, in the present experiments on mice, using enkephalin analog in a high dose (0.25 mg/100 g body weight), we

observed a significant decrease in the number of ulcers in the gastric mucosa from 4.4 ± 1.0 to 2.5 ± 1.1 ($P_U < 0.05$).

Comparison of the total number of points on the integral evaluation of stress scale in the stress control and in the group of mice receiving enkephalin analog also confirmed the antistressor action of D-Ala^2-Leu^5-Arg^6-enkephalin. In this case the difference between the total number of points was 2.

The results are thus evidence that enkephalins may have a marked antistressor action. This suggests that activation of the system of opioid neuropeptides in stress [6, 7] may be adaptive in character and aimed at natural prevention of injuries caused by excessive response to the action of extremal factors.

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EFFECT OF PENTOBARBITAL ON SODIUM PERMEABILITY OF HEART MUSCLE CELLS

N. V. Dmitrieva, E. I. Shtresgeim, N. A. Burnashev, and V. V. Chernokhvostov

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Barbituric acid derivatives are known to influence the sodium permeability of cell membranes [6, 11], and according to some workers, changes in that permeability are linked with the basic mechanism of action of general anesthetics [1, 12]. No such data are available for the myocardium, although barbiturates have been found to have a cardiodepressive effect [8].

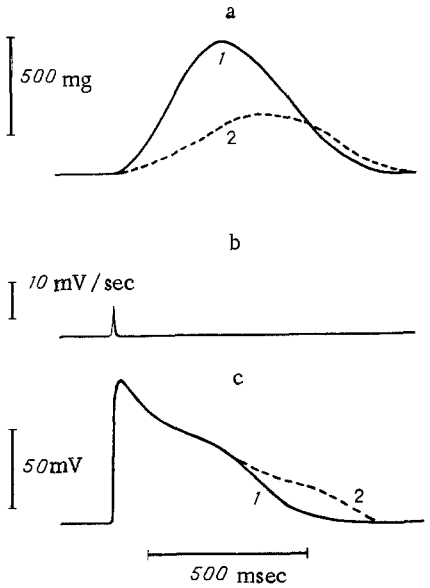

Fig. 1. Action of pentobarbital ($5 \cdot 10^{-4}$ M) on contractile (a) and electrical (b) activity of frog myocardial strip; 1) control; 2) pentobarbital.

Research Institute for Biological Testing of Chemical Compounds, Moscow.

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