ANTISTRESSOR EFFECT OF DALARGIN IN RATS WITH IMMOBILIZATION STRESS

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Long-term exposure to stress and, in particular, to surgical stress [7] may be accompanied by damage to target organs, namely the heart and mucosa of the gastrointestinal tract (GIT), due to the histotoxic action of hypercatecholaminemia, which develops during severe stress reactions [3]. An example of stress-induced damage to the myocardium and GIT in surgical practice is the low cardiac output syndrome in the earliest stages of various kinds of shock, and also the development of stress-induced ulcers of the gastric and duodenal mucosa [9]. Endogenous opioids and their synthetic analogs, as several research workers have shown, can considerably alleviate and even abolish pathological changes in the myocardium [6] and mucosa of GIT [2], induced by hypercatecholaminemia during exposure to different kinds of stress.

The aim of this investigation was to study the antistressor effect of the synthetic Leu-enkephalin analog D-ala^2-leu^5-arg^6-enkephalin (the preparation dalargin, synthesized in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, under the direction of Professor M. I. Titov, and generously provided by him for research) in rats during long-term immobilization stress.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-220 g. There were four series of experiments (with 10 animals in each series). In series I (absolute control) functions of the isolated perfused heart (IPH), taken from intact rats, were studied. In series II (control) the rats were given intramuscular injections of a solution of placebo after immobilization by binding in the supine position under superficial ether anesthesia for 3, 8, and 23 h 30 min. After 24 h of immobilization the heart was quickly removed under pentobarbital anesthesia (50 mg/kg) and connected to a perfusion system. Retrograde perfusion of the hearts was carried out for 10 min with Krebs—Henseleit solution, oxygenated with carbogen, through the ascending aorta without a load on the left ventricle (LV), and this was followed by perfusion through the left atrium with a load on LV [8] for 15 min, with recording of parameters of function of the IPH every 5 min. In the experiments of series III, which was similar in the procedures to series II, a solution of dalargin was injected intramuscularly at the same times during immobilization in a dose of 10 μg/kg, dissolved in the same volume of Krebs—Henseleit solution, used as the placebo, as in series II. In series IV dalargin was injected in a dose of 3 μg/kg. Parameters of function of the IPH in series III and IV were compared with those in series I and II and with each other. In all experiments, besides a study of the function of IPH in the rats the stomach was excised and, after exposure for 3 min in a 10% solution of neutral formalin, the area of ulceration and erosion was counted in square millimeters under a microscope with magnification of 56 times, using a special grid mounted in the ocular; the ulcer index was calculated as the ratio of the number of ulcers to the number of animals in each series of experiments. To study the function of IPH the following parameters were taken into consideration: the heart rate (measured by two electrodes fixed in the region of the right atrium and the apex of LV), the coronary flow (CF) in ml/min (by direct measurement of the outflow from the coronary sinus), the aortic flow

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TABLE 1. Effect of Different Doses of Dalargin Given by Intramuscular Injection on Function of IPH and on UI after Immobilization Stress in Rats for 24 h

Parameter	Series I	Series II	Series III	Series IV
HR	273,4±21,8	177,6±24,1**	226±61	281±37
CF	84,5±6,2	54,2±13,9*	79,4±19,7	83±14,5
AF	151,6±8,4	0	220,7±10,4**	169±33
CO	236,1±7,3	54,2±13,9***	300,1±10,7**	243±35
BP_{syst}	114,8±8,1	70,3±16,8***	102±5	110,2±7,4
BP_{av}	85,3±4,2	51,5±9,0***	75±2,7	79±4,5
A_0	20 139±792	2792±516***	22 683±876	19 132±2 545
A_{str}	73,6±6,8	15,7±3,9***	102,8±3,3**	69,78±16,7
SV	0,867±0,075	0,305±0,051***	1,363±0,4**	0,885±0,207

Legend. *p < 0.05, **p < 0.01, ***p < 0.001 compared with series I.

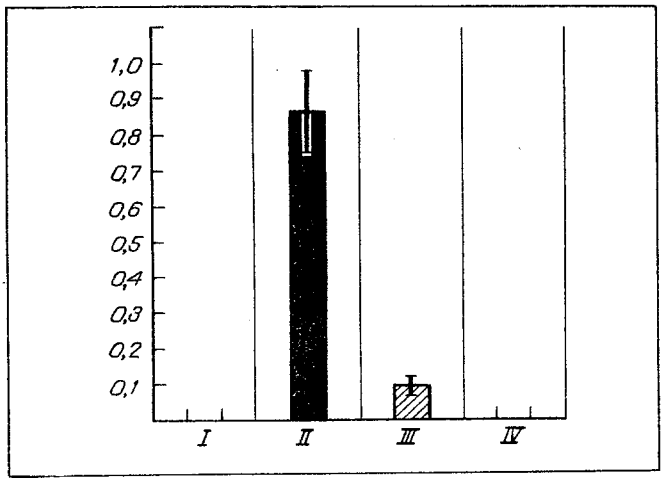

Fig. 1. UI after immobilization stress in rats for 24 h in four series of experiments. Abscissa, series of experiments; ordinate, value of UI.

(AF) in ml/min (by direct determination of ejection from LV by means of a measuring vessel and stop-watch), cardiac output (CO) in ml/min (the total of CF and AF), the pressure in the main vessel due to ejection from LV, with distinction between its maximal (BP_{syst}) and average (BP_{av}) values in mm Hg (by means of an electromanometer), the total work of the IPH (A_0) in conventional units (by the formula: $A_0 = CO \times BP_{av}$), the stroke work in conventional units (by the formula $A_{str} = A_0/HR$), and the stroke volume (SV) in ml/cycle (by the formula SV = CO/HR). All parameters except HR and BP were calculated per gram dry weight of IPH. The electrogram of IPH and BP were recorded on a "Salyut" polygraph. The numerical results were subjected to statistical analysis by the Student—Fisher test.

EXPERIMENTAL RESULTS

The results of the study of function of IPH in the different series are given in Table 1. In the experiments of series II (control) immobilization stress for 24 h led to marked inhibition of the function of IPH compared with the absolute control (series I). All the parameters studied were significantly depressed, and the most important parameter of the contractile and pumping function of IPH, namely AF, was not available because the hearts were unable to overcome the resistance of the main ejection vessel and the column of liquid. On the basis of the results of previous investigations into dependence of the effect of dalargin on its dose, the optimal dosage of the preparation was found to be between 3 and 10 μg/kg body weight. Accordingly, these doses were used in the experiments of series III and IV. Three intramuscular injections of dalargin in a dose of 3 μg/kg not only completely prevented stress-induced depression of the myocardium of IPH after immobilization stress for 24 h, but also led to statistically significant stimulation of the pumping function of IPH:

the most important parameters of function of IPH, namely AF, CO, A_{str}, and SV were statistically significantly higher in series III than in the absolute control in intact animals. Moreover, the increase in AF and CO compared with the absolute control was due to an increase in SV and A_{str} accompanied by a tendency toward a decrease of HR, evidence of increasing extensibility and contractility of the myocardium of IPH in series III. An increase in the dose of dalargin to 10 μg/kg (series IV) also gave complete protection to IPH against stress-induced depression, which was observed in the control series of experiments. The level of the functional parameters in this case was a little lower than in series III, but did not differ from that in the absolute control. Comparison of the ulcer index (UI), as an indicator of stress-induced damage to the mucosa of GIT, is shown in Fig. 1. In the control group, receiving the placebo (series II), UI was close to unity, evidence of almost 100% stress-induced damage to the gastric mucosa. The use of dalargin in a dose of 3 μg/kg led to a ninefold reduction of UI, and after injection of the drug in a dose of 10 μg/kg stress-induced damage to the gastric mucosa of the rats was completely prevented. During stress activation of the sympathicoadrenal system takes place, with elevation of the circulating blood levels of catecholamines and other stress hormones [3], and in connection with their histotoxic action on the myocardium, this is accompanied by electromechanical uncoupling, increased vulnerability of the ventricles [4], and depression of the contractile and pumping function of the myocardium, whereas in connection with their damaging action on the mucosa of GIT, the microcirculation in it is disturbed with the formation of erosions and ulcers [5].

Thus dalargin, a synthetic analog of the endogenous opioids, has a marked cardioprotective and antiulcerogenic effect in rats with immobilization stress. Predominance of one or other protective effect of the preparation, moreover, is dose-dependent. Considering the leading role of hypercatechol-aminemia in the pathogenesis of stress-induced cardiodepression and microcirculatory disturbances in the mucosa of GIT, it can be tentatively suggested that the cardioprotective and antiulcerogenic effects of dalargin are connected with its ability to inhibit activity of the sympathicoadrenal system [1].

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