LIMITING DISTURBANCES OF FUNCTION BY THE REGULATORY PEPTIDE DALARGIN IN BURNED RATS INFECTED WITH *Staphylococcus aureus*

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Disturbances of the normal activity of the cardiovascular system aggravate the course of burns. This is largely due not only to the cardiodepressive action caused directly by thermal injury, but also to the superaddition of infection against a background of the secondary immunodeficiency which develops as a result of the acute stressor effect of burn trauma and its sequelae. However, the combined action of burn and microbial toxemia on the basic functional parameters of the work of the heart have so far remained virtually unstudied, as is also the case with methods of protecting the heart against the damage indicated above. Yet the specific nature of burn trauma requires new and unusual approaches to the optimal correction of functional disturbances, including activation of regulatory mechanisms determining adaptation to stressors, mediated by opioid neuropeptides [1-3].

The aim of this investigation was to study the ability of dalargin, a synthetic analog of the endogenous opioid neuropeptide D-Ala^2-Arg^6-Leu-Enkephalin (from the All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR), modified in order to enhance its biological activity and its resistance to proteolytic enzymes, to increase resistance of rats subjected to the stressor action of burn trauma, to subsequent infection with *Staphylococcus aureus*.

EXPERIMENTAL METHOD

All investigations were conducted on 139 male Wistar rats weighing 200-250 g. An electrical burn was inflicted under ether anesthesia for 45 sec with exposure of the animals lying in the supine position to a temperature of 340-360°C, affecting an area equal to 25-30% of the body surface. The level of stress was judged from the extent of ulcer formation in the gastric mucosa [4]. The intensity (in points) and the frequency of appearance of ulcers (FU) on the mucosa as the ratio of the number of animals with ulcers to the total number of animals in the experimental group, and the ulcer index (UI) were calculated for each group by the equation:

$$UI = 2 \times FU(\text{total number of points} + \text{number of ulcers})/\text{number of rats}$$

Three days after thermal burning the animals were infected by intramuscular injection (into the thigh) of a 24-h culture of *Staphylococcus aureus* strain 6567 (10^{11} microbial cells in 0.1 ml of suspension). The intensity of staphylococcal infection was estimated as the level of colony formation in splenic tissue on the 3rd day after infection. The basic parameters of cardiac function were studied on a model of the isolated perfused rat heart with functioning left ventricle [5]. Perfusion was carried out at 37°C through cannulas inserted into the left atrium and aorta, with initial values of filling pressure and resistance (10 and 100 cm water, respectively) with standard Krebs–Henseleit solution. The pH of the solution was 7.3-7.4 and oxygenation with carbo-

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TABLE 1. Parameters of Function of Isolated Perfused Heart in Different Groups of Rats ($M \pm m$)

Group of animals	AF, ml/min	CO, ml/min	A_{st}, conventional units	SV, ml
1 (n=6)	23,3±3,7*	37,3±6,4*	9,5±1,3*	0,134±0,02*
2 (n=7)	3,9±1,8	16,6±4,4	4,3±1,2	0,066±0,02
3 (n=7)	18,9±3,3*	34,1±3,9*	10,0±2,1*	0,128±0,02*
4 (n=5)	6,6±3,3	21,9±5,9	4,5±1,2	0,066±0,01
5 (n=7)	19,6±3,8	37,0±3,6	9,4±1,0	0,122±0,01
6 (n=7)	0	0	0	0
7 (n=6)	18,8±3,5**	35,0±6,3**	7,4±1,3**	0,123±0,02**
8 (n=7)	22,8±3,5	35,6±3,7	9,1±0,5	0,130±0,01
9 (n=16)	34,6±1,2	48,0±1,9	14,3±1,6	0,180±0,013

gen (95% O_2 + 5% CO_2) maintained a pO_2 of 550-600 mm Hg. Under these conditions of perfusion, the aortic and coronary flow (AF and CF, respectively) were determined and the cardiac output (CO) was calculated as the sum of AF and CF. The heart rate and systolic and mean arterial pressure were recorded with an EMT-35 transducer on a Mingograf-34 instrument, and the stroke volume (SV) and stroke work (A_{st}) of the heart were calculated from them.

EXPERIMENTAL RESULTS

There were three series of experiments. In series I the antistressor action of dalargin was studied after a single intramuscular injection in a dose of 100 μg/kg, 6 h after infliction of the burn. On the 3rd day after burning, the sequelae of stress in the rats of the experimental group were less marked than in animals receiving a placebo (physiological saline). This was expressed primarily as considerable limitation of stress-induced lesions of the gastric mucosa. The reduction of UI in rats receiving dalargin to 1.09 conventional unit compared with 4.82 conventional units in the control took place on account both of the smaller number of ulcers per animal in the group and the reduced frequency of appearance of ulcers, namely 38% of the total animals in the group receiving dalargin compared with 94% of animals in the control. Higher values of ulcer formation in the latter also were combined with marked inhibition of the functional state of hearts isolated for the same periods after burning (3 days). For instance, the AF level, as an indicator of the pumping function of the myocardium, was reduced by more than 5 times, and A_{st} by more than half in the control rats compared with the experimental (Table 1). In the experiments of series II the state of the cardiac activity of intact animals (absolute control) and of infected rats after preliminary burn trauma, was studied. The results show that the infecting dose of *Staphylococcus aureus* used in the experiments caused only local sepsis in the intact rats with a very slight reduction of functional activity of the isolated heart (Table 1). In the burned rat the same dose of infection led to marked generalization of the infectious process (up to 10^8 CFU of staphylococcus/g spleen) and to considerable inhibition of the functional parameters of the myocardium. For instance, on subsequent switching from retrograde (through the aorta) perfusion of the isolated heart to working perfusion on the Neely principle, a progressive fall of cardiac activity was observed. In the experiments of series III the intensity of staphylococcal infection and functional parameters of the isolated heart were studied in rats exposed to burns and subsequent exogenous infection, depending on the schedule of dalargin administration. Intramuscular injection of dalargin in a dose of 100 μg/kg 6 h after burning and before infection, promoting adaptation to the stress of burn trauma, increased resistance to subsequent staphylococcal infection. This was manifested not only by limitation of generalization of infection ($0.29 \times 10^1 \pm 0.14 \times 10^1$ CFU/g staphylococci) on the 3rd day after infection and, correspondingly, on the 6th day after burning, but also by prevention of depression of the myocardium, the functional parameters of which were virtually indistinguishable from those of animals exposed to infection alone or to burning alone, but receiving dalargin subsequently, and they actually exceeded by several times the analogous parameters on the 6th day after burning in rats receiving the placebo (Table 1). It must be pointed out that a virtually identical picture was observed in animals receiving injections of dalargin 6 h after infection, preceded (by 3 days) by infliction of burn trauma. Just as in the group of animals with prophylactic injection of the neuropeptide, dalargin prevented generalized infection ($0.6 \times 10^1 \pm 0.2 \times 10^1$ CFU/g staphylococci), but also abolished disturbances of cardiac activity caused by burn toxemia and had developed previously.

Thus, the sequelae of stress due to burn trauma are manifested not only as serious disturbances of myocardial function and high frequency of appearance of ulcers in the stomach, but also as a marked decrease in the ability of the body to withstand factors of pathogenicity of burn and microbial toxemia. Correction of these disturbances with the aid of the regulatory peptide dalargin, a synthetic analog of endogenous opioids, takes place through the limitation or abolition of the damaging action of burn trauma followed by toxemia, and also the prevention of developing of infectious complications.

LITERATURE CITED

1. G. N. Kryzhanovskii, V. D. Slepushkin, and G. K. Zoloev, Patol. Fiziol., No. 6, 47 (1987).
2. F. Z. Meerson and M. G. Pshennikova, Adaptation to Stress Situations and to Physical Exertion [in Russian], Moscow (1988).
3. E. I. Chazov, M. I. Titov, V. A. Vinogradov, et al., Vopr. Med. Khim., No. 3, 47 (1984).
4. V. N. Shatalov, V. M. Polonskii, S. A. Bulgakov, et al., Modern Methods of Diagnosis and Treatment of Internal Diseases [in Russian], Moscow (1980), pp. 97-98.
5. J. R. Neely, H. Liebermeister, E. J. Battersby, and H. E. Morgan, Am. J. Physiol., **212**, 804 (1967).

INDEXATION OF MICROCIRCULATORY CHANGES IN ANIMALS WITH EXPERIMENTAL DEHYDRATION

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The microcirculatory system (MC) is one of the functional systems of the body responsible for maintaining the fluid balance [2-5, 15]. Much research into changes in working cells of different organs during dehydration has been published [1, 8-10, 15]. Recent investigations have dealt with the state of the blood vascular system during dehydration [6, 7, 9, 11, 14]. There is evidence on changes in the structure of lymphatic microvessels during dehydration [11, 13]. There is no information in the literature on the role of the interstitial component of MC. There has likewise been no research into the reactivity of MC as a whole to stages of dehydration. This is explained by the absence of any suitable methods for solving problems in the analysis of the system as a whole [3].

The aim of this investigation was to obtain an integral evaluation of the reactivity of MC to the stages of experimental dehydration.

EXPERIMENTAL METHOD

Experiments were carried out on 160 albino rats weighing 180-200 g. The animals had free access to dry food, but were completely deprived of water for 3, 6, and 12 days. MC was studied by a combined assessment of morphological, physiological, and biophysical parameters, determining the state of the fluid balance and the hydrodynamics and permeability of its interstitial, lymphatic, blood vascular, and cellular components. The morphological experiments were carried out on the trapezius muscle, pancreas, and mesentery of the small intestine, in histological preparations, by intravital microscopy, and by electron-microscopy. Functional and biophysical parameters were determined by investigating the total, extra- and intracellular body water, the circulating volume of the blood, and its viscosity. To assess changes in MC, each of its four compartments was characterized by the three most essential indicators.

The method of morphokinetic synthesis [12] provided a basis for generalization of parameters differing in phenomenology and dimensionality. Essentially, to compare objective values of heterogeneous parameters, it uses measured values of relative levels of their deviations from the norm, expressed in points. Values of parameters with no significant deviations from

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