MORPHOLOGICAL AND FUNCTIONAL STUDY OF EXPERIMENTAL STAPHYLOCOCCAL AND PYOCYANEA INFECTION IN RATS RECEIVING DALARGIN, A SYNTHETIC ENDOGENOUS OPIOID ANALOG

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Despite differences in the pathogenetic bases of development of infection, a key place in the initiation of infection is ascribed to the state of the primary local focus of infection (wound), which, under certain conditions, can have a significant influence on the onset of local septic infection and its transition to generalization [1, 8, 10]. To a significant degree the dynamics and outcome of an infectious process may be determined also by the species of microorganism inducing it, although similar clinical-anatomical and histological changes can be induced by different microorganisms [4] and, conversely, infection by strains possessing different degrees of virulence, but of the same species, may lead to completely unequal and sometimes opposite reactions of the host organism to infection [13]. In principle, for timely inactivation of the microorganism in a focus of inflammation the factors of natural resistance are sufficient, provided that the microorganism/phagocyte ratio does not exceed the balance of the latter's functional activity, i.e., any pathogenetic method of treatment of an infectious process can only be effective when it ensures an optimal level of adaptation, enabling progression of further complications and the development of toxic effects to be limited.

It has recently been established that natural peptide bioregulators, with opioid structure, or their stable enzyme-resistant synthetic analogs, such as, for example, the first Soviet leucine-enkephalin analog — dalargin [5, 7, 12, 14] — may be one of the factors increasing the powers of adaptation of the organism to extremal influences.

The aim of the present investigation was accordingly a morphological and functional (histological and bacteriological) study of the state of the primary focus in parenchymatous organs in experimental generalized infection induced by the ubiquitous microorganisms *Staphylococcus aureus* and *Pseudomonas pyocyanea*, with or without treatment by dalargin.

EXPERIMENTAL METHOD

Experiments were carried out on 104 male Wistar rats weighing 250-270 g. Infection was carried out with a washed 24-hour culture of clinically isolated *Staph. aureus* (strains 6567) or *Ps. pyocyanea* (strain 487). The animals were infected by intramuscular injection of Gram-positive or Gram-negative microorganisms in a dose of microorganisms in a dose of 10^{12} bacterial cells/100 μl (right thigh). Dalargin was injected into the left thigh in a similar volume (concentration of the peptide 100 μg/kg) 6 and 18 h, and then again on the 1st, 2nd, and 3rd days after infection. The degree of infection was judged by the survival rate of the animals and the results of bacteriological tests [6]. For morphological investigation of the spleen and the primary focus, biopsy specimens of tissues to be studied were fixed in neutral formalin and embedded in paraffin wax, after which histological sections were stained with hematoxylin—eosin and by Van Gieson's method.

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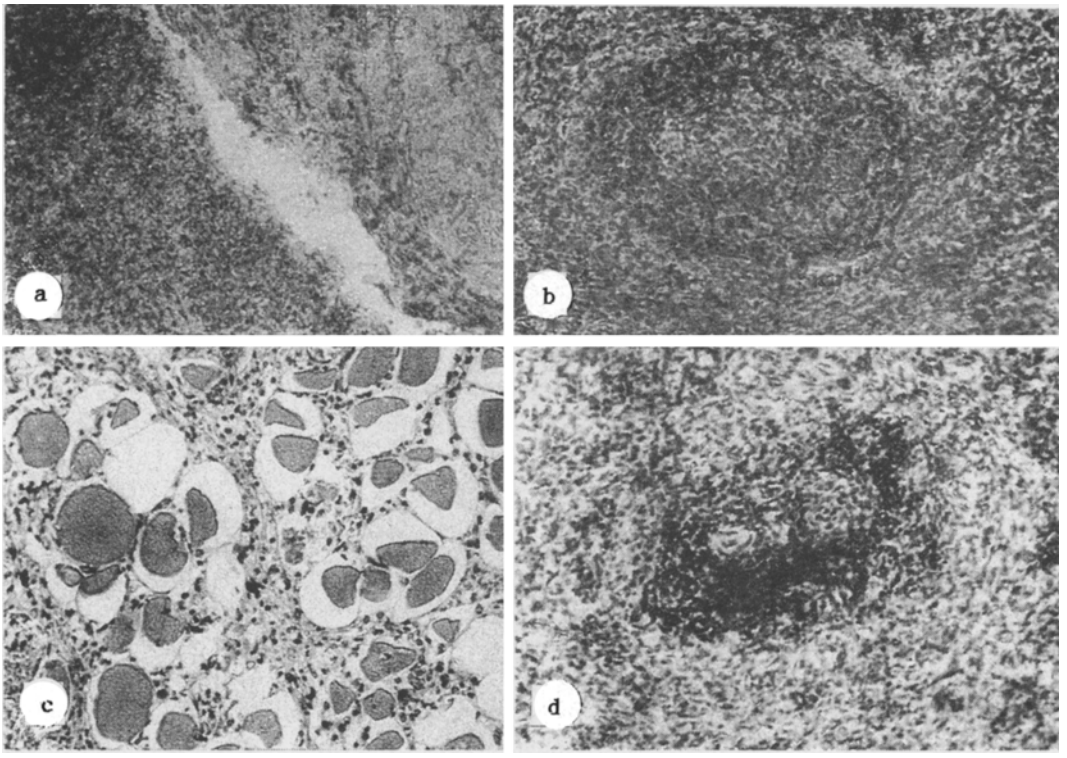

Fig. 1. Morphological picture of changes in muscles in focus of injection and in spleen of rats infected with *Staph. aureus* and *Ps. pyocyanea*. a) Necrosis and suppuration of muscles after injection of *Ps. pyocyanea*. b) Hypoplasia of germinal center of spleen during infection by *Ps. pyocyanea*. c) Necrosis and suppuration of muscles following injection of *Staph. aureus*. d) Hypoplasia of periarteriolar lymphoid cuff in spleen after injection of *Staph. aureus*. Here and in Fig. 2: hematoxylin and eosin. 160×

EXPERIMENTAL RESULTS

The time course of experimental septic infection induced by *Staph. aureus* and *Ps. pyocyanea* was virtually identical, but in the latter case the changes and visible signs of the local response in the form of edema, infiltration, followed by dysfunction of the limb, were more marked although all ultimately led in both groups of animals to the formation and spontaneous opening of an abscess, accompanied by a mortality of 60%.

Parallel quantitative studies of the microflora revealed that despite the initially equal infecting dose, in the case of infection caused by the Gram-negative microflora the level of generalization, judged by the degree of dissemination of infection in the spleen, was lower than in the case of staphylococcal infection. This also was combined with fewer microorganisms in the wound induced by *Ps. pyocyanea* compared with Gram-positive microorganisms, indirectly pointing to higher virulence in the case of already established infection, i.e., the ability of *Ps. pyocyanea*, present in lower concentration, to exert an action similar to that of *Staph. aureus*, or even a damaging action on the host. This conclusion is directly confirmed by morphological assessment of the state of the primary septic focus (wound) in the parenchymatous organs (spleen). In infection with *Ps. pyocyanea* compared with *Staph. aureus* destructive changes in the wound were deeper and took the form of necrotic myositis with total necrosis of a considerable part of the muscles. In other muscles, outside the zone of total necrosis, changes in staining properties were observed, indicating changes preceding necrosis and necrobiosis. Signs of hypoplasia of lymphoid tissue, reactive histocytosis in the sinuses, and virtually total disappearance of the germinal centers were observed in the splenic tissue. Staphylococcal infection was characterized by suppuration of the floor of the wound with signs of necrosis of muscle fibers in the form of cytoplasia and homogenization of the structures, and also leukocytic infiltration of the intermuscular spaces by polymorphonuclear neutrophils, with proliferation of the latter in granulation tissue. In the spleen a characteristic reaction of the microcirculatory bed to injury was observed in the form of congestion of the vessels and capillaries and some degree of hypoplasia of the red pulp. Hypoplasia also was observed in

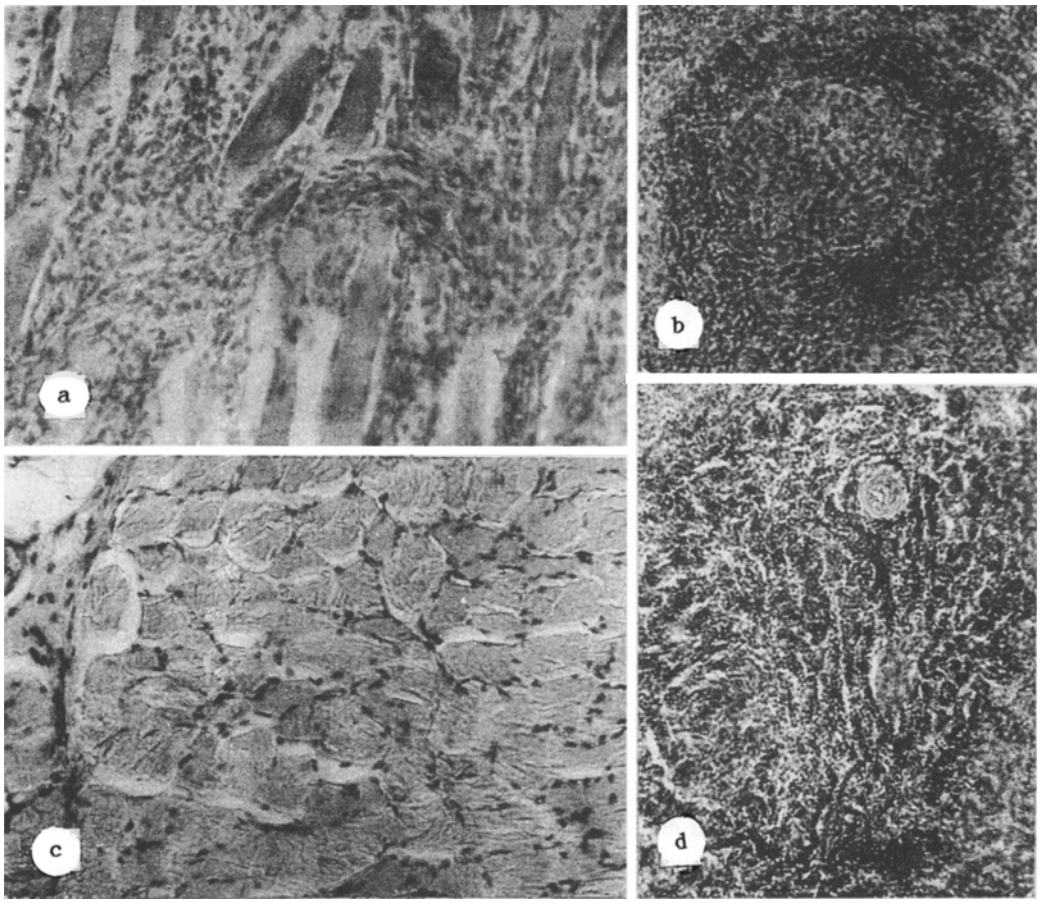

Fig. 2. Changes in focus of suppuration and in spleen following infection of rats with *Staph. aureus* and *Ps. pyocyanea*, accompanied by treatment with dalargin. a) Proliferation of granulation tissue with individual muscle fibers immured in its substance, after infection with *Ps. pyocyanea*. b) Preservation of germinal center of spleen following infection with *Ps. pyocyanea*. c) Single muscle fibers showing loss of cross-striation and of staining properties, absence of necrosis and suppuration. Staphylococcal infection. d) Marked periarteriolar lymphoid cuff in spleen in staphylococcal infection.

TABLE 1. Bacteriological Parameters and Mortality Among Rats from Experimental Generalized Infection Induced by Gram-Positive (*Staphylococcus aureus*) or Gram-Negative (*Pseudomonas pyocyanea*) Microorganisms Against the Background of Administration of Dalargin (100 μg/kg, intramuscularly) or a Placebo (3rd day after intramuscular injection in doses of 10^{12} bacterial cells) (M ± m)

Experimental conditions	Mortality, %		Rate of dissemination (number of microbial cells per gram tissue)			
			focus		spleen	
	Gram-positive	Gram-negative	Gram-pos.	Gram-neg.	Gram-pos.	Gram-neg.
Control—injection of physiological saline 6 and 18 h and on the 1st, 2nd, and 3rd days after infection	60	60	$5{,}6\times10^{7}\pm1{,}06$ ++	$2{,}7\times10^{6}\pm1{,}34$	$1{,}13\times10^{6}\pm1{,}15$ ++	$1{,}34\times10^{5}\pm1{,}19$
Experiment—injection of dalargin at the same times	10 +, ++	30 +	$1{,}05\times10^{4}\pm1{,}22$ +	$1{,}3\times10^{4}\pm1{,}25$ +	$1{,}17\times10^{2}\pm1{,}42$ +, ++	$1{,}31\times10^{3}\pm1{,}2$ +

Legend. $^{+}p < 0.05$ Compared with control. $^{++}p < 0.05$ Compared with Gram-negative.

cells of the white pulp, but mainly individual lymphatic follicles and with preservation of the germinal centers, but with some degree of aplasia, in both white and red pulp.

It can thus be postulated on the basis of these results that so far as the present experiments are concerned, the initially low pathogenicity of *Ps. pyocyanea* was due to a definite level of adaptive reactions of the agent, when penetrating into the host, into a medium differing greatly from its physiological medium. Many cells of the pathological agent perish under these circumstances, while the rest gradually adapt themselves to the new and unusual conditions (the "lag phase" of bacterial growth in vivo), and subsequently, when optimization of relations between microorganism and phagocyte in the focus is already disturbed, the infectious process, determined by species-specific features, follows a lightning type of course, and this may be associated both with high mortality and with the extremely difficult treatment of this type of septic infection, which arises as a rule in states linked with marked inhibition of natural resistance and, in particular, in burn trauma or other types of trauma, including operative [11]. It must not be forgotten, however, that besides the above-mentioned causes, the breakdown of the adaptive reactions of the host with "emergence" into pathological is possible also when there is relatively normal function of the system of phagocytes and/or other systems maintaining homeostasis such as, for example, in massive bacterial invasion, exceeding the powers of physiological protection, which cannot withstand the extremely powerful toxic action [3]. In such cases, moreover, just as in all other cases connected with the damaging action of the stress reaction, mobilization of functional systems and mechanisms responsible for adaptation to extremal factors is essential, through activation and/or the formation of a higher level of antibacterial resistance. Such an increase in resistance to infection was observed in the experimental groups of rats against the background of exogenous administration of the regulatory opioid peptide, dalargin; the efficacy of the peptide, moreover, was largely determined by the species of pathogenic agent. For instance, during infection induced by Gram-positive microorganisms, injection of dalargin limited mortality to 10% compared with 30% in the case of Gram-negative infection, i.e., in the first case the survival rate of the experimental rats increased sixfold whereas in the second case it increased only twofold (Table 1). The level of generalization also was lower in rats infected with *Staph. aureus* than with *Ps. pyocyanea*; moreover, the degree of dissemination of infection of splenic tissue with *Staph. aureus* was reduced almost by 4 orders of magnitude, whereas in the case of *Ps. pyocyanea*, it was reduced by 2 orders of magnitude. It must be pointed out that despite the approximately equal number of microorganisms in the septic focus of the experimental rats, even though smaller than in the control, nevertheless the rate of cleansing of the wound from Gram-positive microflora was higher than from Gram-negative. The results suggest the important role of the primary focus, which does not lose its influence on the course and outcome of the disease even at the climax of the infectious process, against the background of a high level of generalization, and also the no less great importance of dalargin, which can limit progression of the experimental generalized infection. As regards the present experiments the protective action of dalargin may be linked both with weakening of hormonal-metabolic disturbances and gross rheologic disorders, and also with improvement of the microcirculation and intensification of reparative regeneration of the damaged tissues, and also with its immunomodulating action which, in particular, is characteristic of many opioid peptides [7, 9, 12, 14]. This was reflected also in the results of the morphological investigations. For instance, the pathological picture of the experimental infection treated with dalargin consisted of reduction of the scale of destruction, limitation of the zone of damage, and weakening of the vascular disturbances. These changes were more characteristic of staphylococcal infection, in which the muscle tissue of the wound and spleen, unlike in the control, was virtually unchanged apart from an ill-defined edema of the intermuscular connective-tissue spaces in the wound and some degree of congestion of the vessels and capillaries in the splenic tissue. Treatment of pyocyanea infection was characterized by reduction of the number of muscle fibers with a change in their staining properties. The intensity of necrosis of the muscles also was reduced, and signs of homogenization of the sarcoplasm were observed only in individual groups of muscle fibers, whereas the remaining muscles were intact. Many germinal centers were observed in the spleen, with a lesser degree of hypoplasia of the lymphoid tissue than in the control. Reactive histiocytosis of the sinuses also was much less marked.

Combination of certain properties inherent in opioid peptides thus provides the opportunity for enkephalins to affect the pathogenesis of septic infection, and this may ultimately lead to more effective pathogenetic treatment in the therapeutic management of different types of septic-suppurative complications in clinical surgery

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AUTORADIOGRAPHY OF PROTEIN SYNTHESIS AS A METHOD OF EVALUATING MORPHOLOGICAL AND FUNCTIONAL CHANGES IN BRAIN STRUCTURES

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KEY WORDS: autoradiography; protein synthesis; rat brain; L-3,4-dihydroxyphenylalanine; motor activity.

Since protein metabolism in the nervous system is ascribed a fundamental, if not decisive, role in its functional activity [6, 9], determination of protein synthesis at different levels of structural and functional organization of the brain, both under normal and under control experimental conditions, provides an indication of the particular features of the role of these structures in activity of the CNS.

The aim of this investigation was to assess the method of autoradiography of protein synthesis, combining macro- and microversions, for a comprehensive study of morphological and functional changes in brain structures at the level of anatomical formations and of individual types of neurons.

EXPERIMENTAL METHOD

As an indicator of functional changes in the CNS we chose an experimental model of reduced motor activity of animals, created by giving them an injection of L-3,4-dihydroxyphenylalanine (L-dopa) [1, 2, 8]. For this purpose, male Wistar rats weighing 160 ± 10 g were given an intraperitoneal injection of L-dopa in a dose of 100 mg/kg. A stable reduction of motor activity, characterized by an anxiety state, was observed after 3-4 weeks in the animals. Six rats which received L-dopa (three animals received L-dopa for 3 weeks and another three animals for 4 weeks) and three control rats, which received only injections of physiological saline for 4 weeks, were given an intraperitoneal injection of D,L-leucine-2-[$^3H_1$] (specific activity 8.8 mCi/mmole, from "Izotop," USSR) in a dose of 2 mCi/100 g body weight. The rats were decapitated 2.5 h after injection of the isotope under ether anesthesia, their brain was fixed in Carnoy's fluid and, after embedding

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