EFFECT OF DALARGIN ON THE BLOOD AND LYMPH MICROCIRCULATION

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The compound dalargin, synthesized in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR (Head, Professor M. I. Titov), is a synthetic analog of the opioid peptide Leu-enkephalin. Data on the high therapeutic activity of this preparation in experimental pancreatic necrosis, pancreatitis, peritonitis, gastric ulcer, and myocardial infarction have recently been published [1-6]. Meanwhile the mechanisms of action of dalargin have remained virtually unstudied. The stimulating action of Leu-enkephalin on the lymph flow in the mesenteric microvessels observed by the author previously [7, 8] suggests that its analog, dalargin, may have a similar mechanism of action.

The aim of this investigation was to study the effect of dalargin on the microcirculation in blood and lymphatic microvessels (LM), which play an important role in the maintenance of tissue homeostasis in various diseases.

EXPERIMENTAL METHOD

Experiments were carried out on 30 noninbred male albino rats weighing 250 g, anesthetized with pentobarbital (0.1 g/kg intramuscularly). The intravital study of the mesenteric microvessels of the rats was carried out by the method in [9]. Blood microvessels 6-60 μ in diameter and LM 40-200 μ in diameter were studied. An ISSh-400 flash tube was used for microfilming. Dalargin was applied to the surface of the microvessels or injected intramuscularly in doses of 0.001 to 10 μg in 0.1 ml of 0.14 M NaCl, equivalent to 0.004-40 μg/kg body weight. Independently of the method of administration of the drug, similar changes were observed in the microcirculation. After intramuscular injection the effects observed appeared 5-10 min later than after application. The data were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

The response of the mesenteric microvessels to administration of dalargin depended on the dose given: small doses (0.001-0.1 μg) caused slowing of the blood flow 7-10 min later in some of the microvessels, with the result that stasis developed in individual microvessels in the course of 17 min after administration. Later the number of arterioles, capillaries, and venules with stasis and the area occupied by them increased. Larger doses of dalargin (from 0.1 to 1 μg) as a rule did not slow the velocity of the blood flow or induce stasis in the microvessels; moreover, to judge from such indirect signs as a decrease in pavementing of the leukocytes in the venules, lengthening of the erythrocytes on the photomicrographs, reproducing a frame-stop effect, they led to an increase in the velocity of the blood flow. The highest dose (10 μg) had no visual effect on the velocity of the blood flow. In venules 20-60 μ in diameter dalargin (0.001-0.1 μg) caused an increase in pavementing of the leukocytes 5-15 min after administration. In 75% of cases in which dalargin was used in various doses, diapedesis of leukocytes was observed into the perivascular space (Fig. 1a, b). With an increase in the dose diapedesis of the leukocytes was accelerated: after 5-34 min in response to injection of 0.001-1 μg, after 4-8 min to injection of 10 μg. In the course of 5-51 min the number of leukocytes in the tissue could increase or decrease, or they may even disappear altogether, i.e., the process was reversible.

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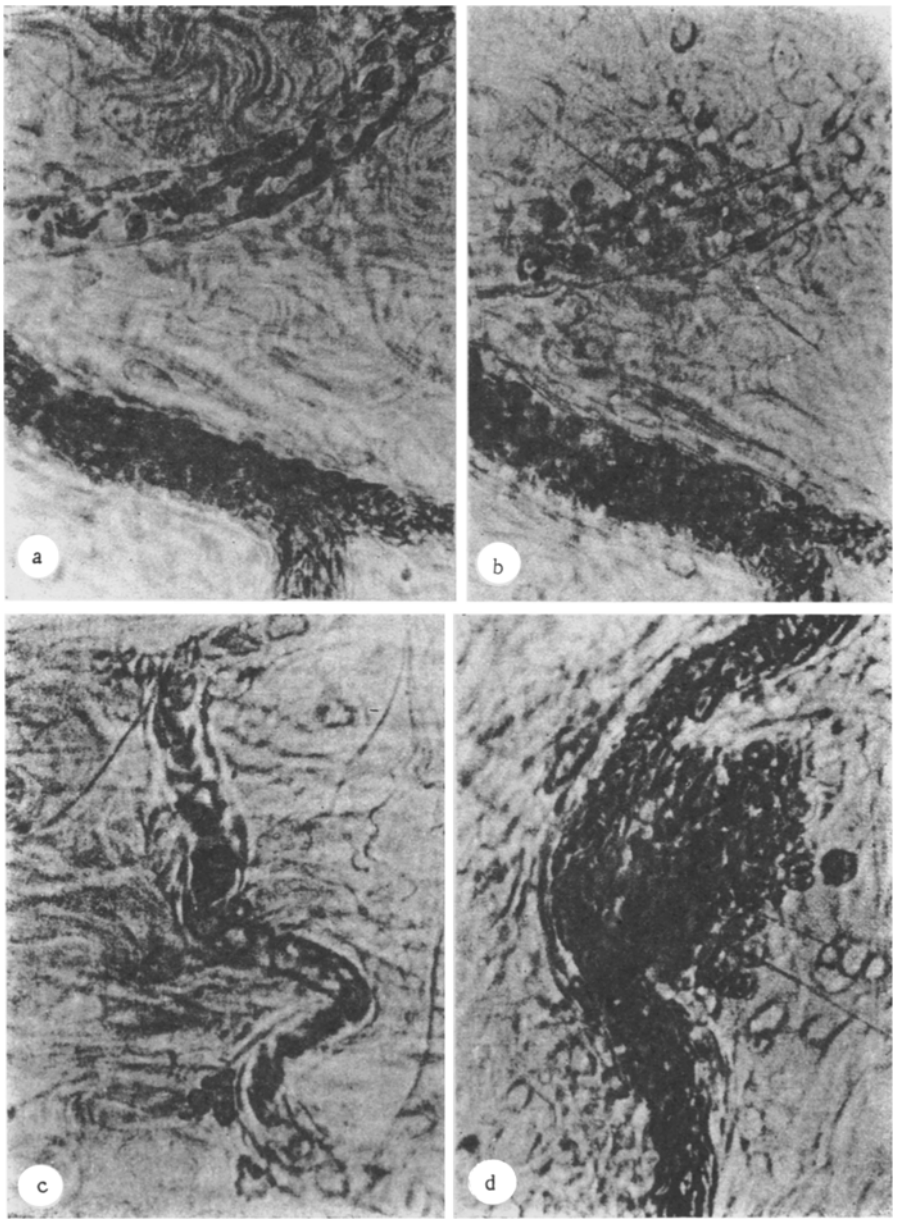

Fig. 1. Effect of dalargin on permeability of wall of mesenteric microvessels of a rat. a) Venules before application; b) 19 min after application of 0.001 μg dalargin: diapedesis of leukocytes, swelling of wall of of venule, slowing of blood flow — intermittent flow of round cells; c) diapedesis of erythrocytes from a postcapillary (24 min after application of 0.1 μg dalargin); d) microhemorrhage from a venule occurred for 1-2 sec 5 min after intramuscular injection of 0.001 μg dalargin. Biomicroscopy: magnification: objective 40 (a, b) and 70 (c, d); ocular 3.

Diapedesis of the leukocytes from the large venules into the perivascular space was preceded by a state known as the "leukocytic cuff." It appeared around the wall of a venule and consisted of tightly packed leukocytes, which formed a unique kind of membrane around the vessel. The "leukocytic cuff" did not last for more than 5-10 min. Its disappearance was due to passage of the leukocytes composing it into the perivascular space. A characteristic feature was the diffuse distribution of leukocytes in strictly definite areas and distances from the wall of the venule.

The most constant phenomenon arising in response to all doses of dalargin was diapedesis of erythrocytes from the various microvessels: arterioles, venules, and vessels of capillary type (Fig. 1c). It began 2-10 min after administration of dalargin, and was followed by an

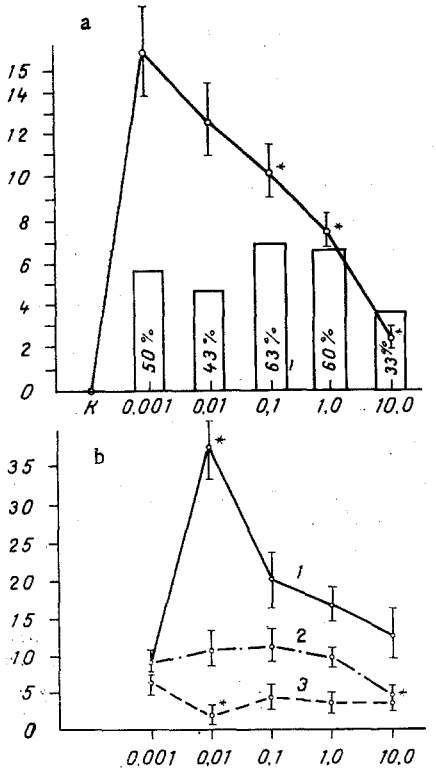

Fig. 2. Contraction of wall of LM in rat mesentery under the influence of dalargin. Abscissa, dose of dalargin (in μg/0.1 ml). a) Frequency of contraction of wall per minute (numbers in columns indicate percentage of LM activated by dalargin); K) control. b: 1) Duration of contraction, 2) time of maximal effect, 3) latent period to beginning of contraction; ordinate, time (in min). *p < 0.05 compared with dose of 0.001 μg.

increase in the number and size of the areas of diapedesis. The commonest location of these areas was at sites of branching and fusion and of greatest tortuosity of the microvessels. Microhemorrhages from arterioles and venules began to occur 3-10 min after injection of the various doses of dalargin, and during the next 15-30 min they increased in number and size. The microhemorrhages occurred as a result of increasing diapedesis of single cells, or rapidly, in the course of 1-2 sec, in which case they were particularly large (Fig. 1d).

Another phenomenon arising 8-30 min after administration of 0.001-1 μg dalargin was swelling of the walls of the microvessels (Fig. 1b). This was expressed as thickening and indistinctness of the outlines of the walls, which made accurate measurement of the diameter of the vessels difficult. This phenomenon, as we know, is evidence of increased permeability of the walls of the microvessels and it is reversible. For example, washing out the dalargin applied to the mesentery previously with physiological saline contributed to the rapid recovery of clarity of the outlines of the vessel walls. Spontaneous restoration of the clarity of outline of the walls occurred after 20-30 min. Swelling of the nuclei of single endothelial cells in the walls of the microvessels was found and is indirect evidence of swelling of the whole endothelial cell. Total swelling evidently took place both of the endothelium and of the membrane of capillary microvessels.

The lymph flow in the tissues is maintained by rhythmic contraction of the wall and valves of LM. Under normal conditions mesenteric LM contract periodically. The properties of dalargin were studied on initially uncontracted LM. Dalargin caused contraction, not of all, but only of some of the LM studied. Data for the activated LM are given in Figs. 2 and 3. The number of vessels which began to contract depended on the dose of the compound: small doses (0.001-0.01 μg) activated fewer LM (43-50% of all vessels) than doses of 0.1-1 μg (60-63%) of LM). The threshold concentration of dalargin was 0.001 μg/0.1 ml (0.004 μg/kg body weight). With an increase in the dose of dalargin from 0.001 to 10 μg the frequency of contraction of the wall of LM decreased (Fig. 2). The duration of the contraction effect was longest (37 min) and the latent period until the beginning of contraction was

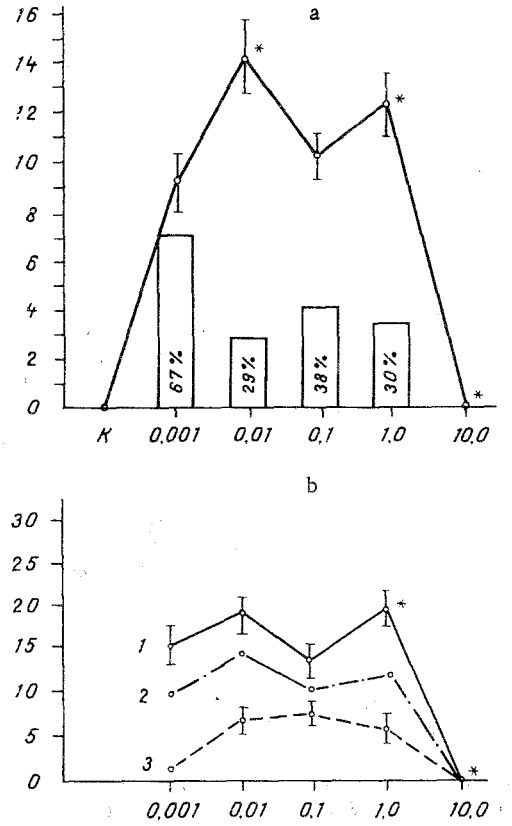

Fig. 3. Contraction of valves of mesenteric LM of rats under the influence of dalargin. a) Frequency of contraction of valve per minute. Remainder of legend as to Fig. 2.

shortest (2 min) when the dose given was 0.01 μg. The maximal frequency of contraction when different doses were used (except 10 μg) took place on average 10 min after administration of dalargin.

On the basis of these results it can be postulated that any dose from 0.001 to 1 μg causes the outflow of an equal volume of lymph from the tissues of the mesentery, for activation of fewer LM in response to small doses of dalargin was accompanied by a higher frequency of contraction of the wall of LM, whereas involvement of a larger number of LM in the process in response to larger doses of dalargin led to a lower frequency of their contraction. The total work done by LM in connection with lymph formation and drainage under these circumstances will be the same.

Valves of LM did not contract synchronously with contraction of the wall of LM. With an increase in the dose of dalargin, wave-like changes were observed in parameters of their activity such as frequency of contraction and time of the maximal effect (Fig. 3). Unlike the wall, the valves of LM did not respond to dalargin in a dose of 10 μg. Correlation between the frequency of contraction of the valves and the number of functioning valves was a common feature with the functioning of the wall of LM. The total work done by the valves in response to doses of 0.001-1 μg ought to be the same.

Dalargin activated contraction not only of LM, but also of the musculature of the small and large intestine of the rat 10-60 sec after administration of the compound. Contraction continued for 30 min and involved, not the whole intestine, but only certain parts of its wall, which may have had a better innervation and may have contained concentrations of nerve cells.

The changes in the blood and lymph microcirculations described above lead to the conclusion that there are three mechanism of action of dalargin at the microcirculatory level. The first is an increase in vascular permeability. This view is also confirmed by phenomena such as swelling of the walls and nuclei of the endothelium of the microvessels and increased

diapedesis of blood cells from the vessels into the tissue. A second important mechanism of action of dalargin is activation of the lymph flow, which is brought about in three ways: 1) as a result of the direct action of the compound on the contractile apparatus of LM with an increase in the frequency of contraction of the wall and valves of LM; 2) as a result of an increase in vascular permeability the volume of the interstitial fluid increases and, consequently, lymph formation and the lymph drainage also are increased; 3) stimulation of intestinal movements promotes mechanical expulsion of lymph from the splanchnic region, and this also makes a definite contribution toward activation of the lymph flow. The third important mechanism of action of dalargin is activation of diapedesis of leukocytes, which leads to activation of the phagocytic function of the leukocytes in the extravascular space, a matter of the greatest importance in the treatment of inflammatory diseases of varied etiology and for the acceleration of repair processes in the tissues. Dalargin, like β-endorphin, is perhaps bound by specific receptors of lymphocytes [10], and thus potentiates their diapedesis.

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