Effect of Opioid Lymphostimulation on the Microcirculation in Pial Vessels of the Ischemized Rat Brain

V. K. Khugaeva, P. N. Aleksandrov, and V. V. Aleksandrin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 119, № 1, pp. 100-105, January, 1995
Original article submitted April 4, 1994

The response of pial vessels to i.p. administration of leu-enkephalin (at 40 μg/kg) is studied biomicroscopically in the control, before and after bilateral occlusion of the common carotid arteries. Leu-enkephalin causes chiefly the narrowing of pial arterioles and does not affect venule diameter. The effect of leu-enkephalin on occlusion of the common carotid arteries manifests itself in the preservation of circulation stability, the narrowing of some arterioles, and in the decrease of the degree of dilation. These changes occur against the background of lowered arterial pressure, bradycardia, increased local circulation in the brain cortex by 50-70%, intensification of the lymph flow in micro- and macrovessels, and the absence of mortality of animals in the first hours of occlusion of the arteries.

Key Words: *pial microvessels; brain ischemia; leu-enkephalin*

Lymphostimulation is effective method of treating not only severe toxicoses, but also ischemia [2,6,15]. However, the traditional methods of pharmacological stimulation, namely water loading, osmotic diuretics, as well as drugs improving hemodynamics, microcirculation, and rheological properties of the blood and lymph, produce an indirect and insufficient effect and difficulties in prediction [3,4]. The most effective immunostimulators are the opioid peptides (leu-enkephalin, dalargin, and their tyrosine-containing analogs), which possess a direct lymphostimulating effect [7-9]. The peripheral leu-enkephalin-induced lymphostimulation is attended by prevention or restoration of the damaged local circulation in the ischemized brain cortex [10]. However, the mechanisms of leu-enkephalin compensation for the 40% reduction of the blood flow in the brain occurring due to ligation of the carotid vessels have not been studied.

The aim of the present investigation was to study the microcirculatory mechanisms mediating the restoration of the blood flow in the ischemized brain for opioid stimulation.

MATERIALS AND METHODS

Experiments were carried out on 110 inbred male albino rats weighing 200-300 g anesthetized with chloral hydrate (at 0.6 g/kg i.m.). The brain was ischemized by bilateral occlusion of the common carotid arteries (OCCA). Biomicroscopy of pial microvessels (diameter 10-100 μ for arterioles and 20-200 μ for venules) was performed through a 5×3-mm trepanation opening in the right parietal region of the cranium using a device created by us [1] for 1 h before and for 3 h after ischemia. To maintain physiological conditions the dura mater was preserved and the animal was heated with cotton wadding and disperse heat from an incandescent lamp (100 W) from a distance of 25-

Laboratory of General Pathology of Microcirculation, Research Institute of General Pathology and Pathological Physiology, Russian Academy of Medical Sciences, Moscow. (Presented by G. N. Kryzhanovskii, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0001-0096$12.50

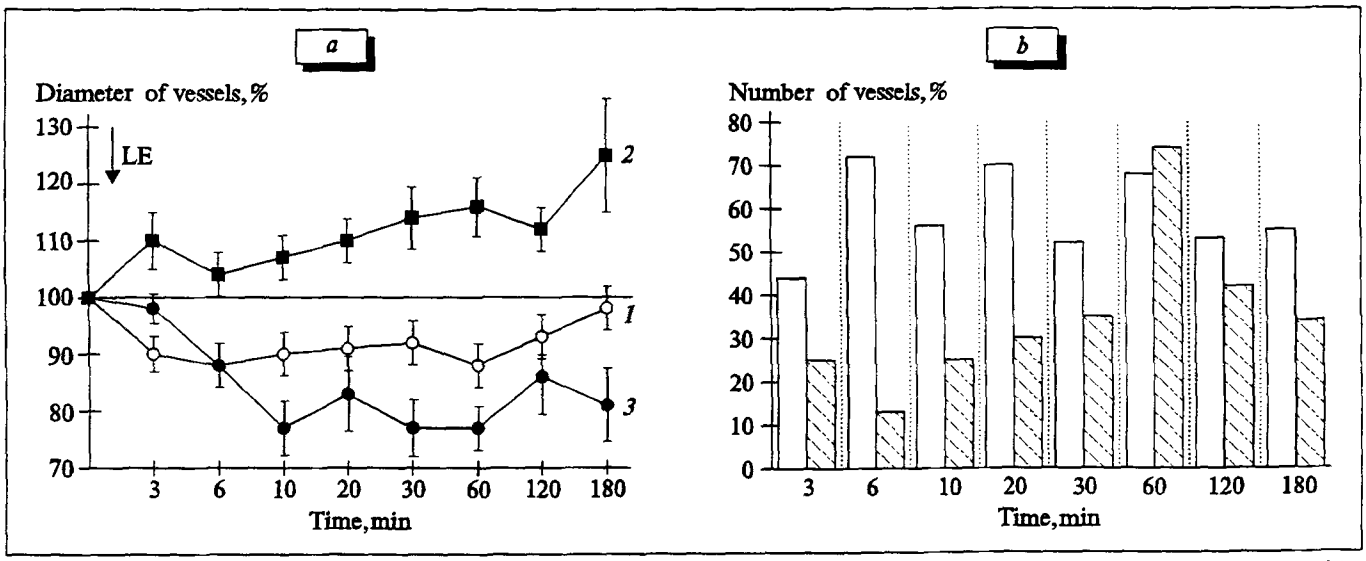

Fig. 1. Effect of i.p. administration of leu-enkephalin (*LE*, 10 μg/ml) on diameter of pial arterioles of rat brain. Here and in Figs. 2, 3: *a*) *1* – reaction of all arterioles, *2* – dilated vessels, *3* – constricted vessels; *b*) shaded bars show the frequency of dilation, white bars, the frequency of constriction.

30 cm. The rectal temperature was kept at the level of 36.9±0.1°C. A Russian-manufactured contact objective 10× (LOMO) was used for biomicroscopy. The diameter of pial microvessels was measured on photomicrographs. The local brain flow (BF) in the cortex was measured using the method of hydrogen clearance in the same region as the microcirculation in the pial vessels. The arterial pressure (AP) was taken in the right femoral artery and right common carotid artery using an electric manometer. The AP in the carotid sinus was recorded using a catheter mounted in the cranial direction, and the distal part of the right common carotid artery was ligated. Ten micrograms of leu-enkephalin (40 μg/kg animal weight) (Serva) in 1 ml 0.14 M NaCl were administered i.p. The reaction of pial vessels was examined in six experimental series. A study of spontaneous changes of the diameter and blood flow was performed in the 1st series in control animals; the effect of 1 ml 0.14 M NaCl administered i.p. was assessed in the 2nd series; OCCA was evaluated in the 3rd; the effect of i.p. administration of leu-enkephalin was investigated in the 4th series; in the 5th series leu-enkephalin was administered for preventive purposes 10 min prior to OCCA; and, finally, the therapeutic effect of leu-enkephalin injected 10 min after OCCA was examined in the 6th series. The lymph was sampled during 10 min after puncture of the thoracic lymphatic duct before it empties into the left venous angle. The velocity of the lymph flow was calculated in liters per kg in 1 sec. The contractile activity of mesenteric lymphatic vessels of the small intestine was studied using a method devised by us [11]. Results were processed statistically using the Student *t* test.

RESULTS

The diameter of the control pial arterioles and venules is stable during the 4 h of observation (variations do not exceed 3%). Changes of blood flow direction may occur in arterioles and arteriolo-arteriolar anastomoses in regions of adjacent blood supply. The systemic AP was 98.1±0.3 mm Hg and intrasinus pressure was 57.2±1.3 mm Hg. The BF in the cortex was 54.4±1.4 ml per 100 g in 1 min [10]. The lymph flow velocity in the thoracic lymphatic duct was $(2.8\pm0.5)\times10^{-7}$ liter/kg/sec. Mesenteric lymphatic microvessels of the small intestine did not contract.

The administration of 1 ml 0.14 M NaCl did not cause reliable changes of the studied parameters. The bilateral OCCA was accompanied by a stable decrease of the intrasinus pressure, reflecting the pressure in the vascular system of the brain, to 34.1±2.7 mm Hg, or 59% of the baseline level. The BF in the cortex decreased to the same degree [10], while the systemic AP rose by 15%. The changes of microcirculation occurring in pial vessels manifested themselves in a short-term (for 1-2 sec) circulation arrest with subsequent restoration but a lesser velocity. The BF in the cortex dropped by 41% on average [10]. Continuous changing of the blood flow direction in arterioles and arteriolo-arteriolar anastomoses was characteristic for the first 30 min after OCCA onset. The blood redistribution occurring in the arteriolar bed is compensatory and is related to a reduced

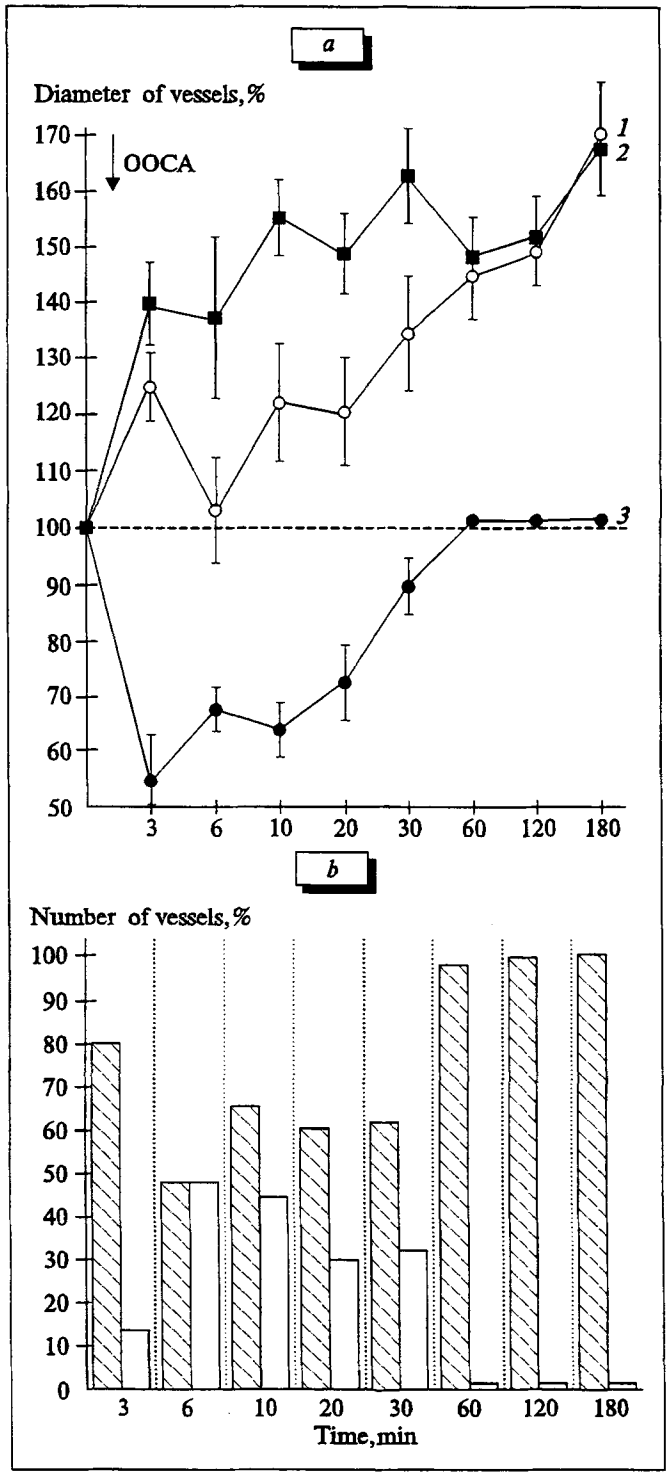

Fig. 2. Changes of the diameter of pial arterioles of the rat brain in dynamics of bilateral OCCA.

blood flow in brain vessels, accomplished chiefly through vertebral arteries after OCCA. In the long term after OCCA (after 1-3 h) the direction of the blood flow in pial vessels becomes more stable. One of the constant phenomena associated with brain ischemia is a dilation of pial arterioles (Fig. 1), while the diameter of venules varies insignificantly. The first 30 min of OCCA, accompanied by a changing direction of the blood flow in pial microvessels, is characterized not only by dilation but by a brief constriction of some pial arterioles as well (Fig. 1, *a*, *b*). The arteriolar constriction is most intensive and frequent during the first 3-6 min of brain ischemia. The constriction of pial arterioles may be of two kinds, namely strong and short-term (over 2-fold lumen narrowing for 1-1.5 min) and weak and longer-lasting (10% reduction of the lumen for 20-30 min). The strong kind of constriction affects not just a particular arteriole but its branches as well. The weak type encompasses certain vessels of the pial bed or their parts. These different reactions of pial arterioles, constriction and dilation, are probably local mechanisms of blood flow redistribution aimed at maintaining the brain hemodynamics in ischemia. At later times of brain ischemia (1-3 h) the degree of dilation of arterioles steadily rises. The progression of the process is related more to the involvement of previously constricted vessels than to an enlargement of the lumen of already dilated ones. One hour after onset of OCCA all pial vessels are dilated and just isolated vessels exhibit no reaction. Despite the autoregulatory nature of the increase of the arteriole lumen (by 70% maximally) aimed at improving the brain flow, the local BF in the cortex and intrasinus pressure remained lowered for 3 h of OCCA, indicating that one vasomotor mechanism is not enough to compensate for the flow deficiency in moderately severe brain ischemia.

OCCA was attended by a progressive fall of the lymph flow velocity in the thoracic lymphatic duct. Thus, 50 min after OCCA onset the lymph flow velocity attained only 43% of the baseline level (100%). Mesenteric lymphatic vessels of the small intestine did not contract and lymphostasis was noted in many of them.

Leu-enkephalin injected i.p. at 40 μg/kg induced two types of reaction of pial arterioles (Fig. 2, *a*, *b*). A constriction by 20% on average of the majority of arterioles (43-74% of vessels) was noted during various periods after leu-enkephalin administration. The degree of constriction and number of constricted vessels showed wavelike variations during 3 h of observation. Dilation developed in the lesser proportion of arterioles (13-42% of vessels), progressing in the course of time. The diameter of these vessels was increased by 27% on average 3 h after leu-enkepalin administration. The total reaction of microvessels was characterized by a decrease of the lumen of the arteriolar bed of the pia mater (Fig. 2, *a*). The change of the pial venule lumen in response to leu-enkephalin did

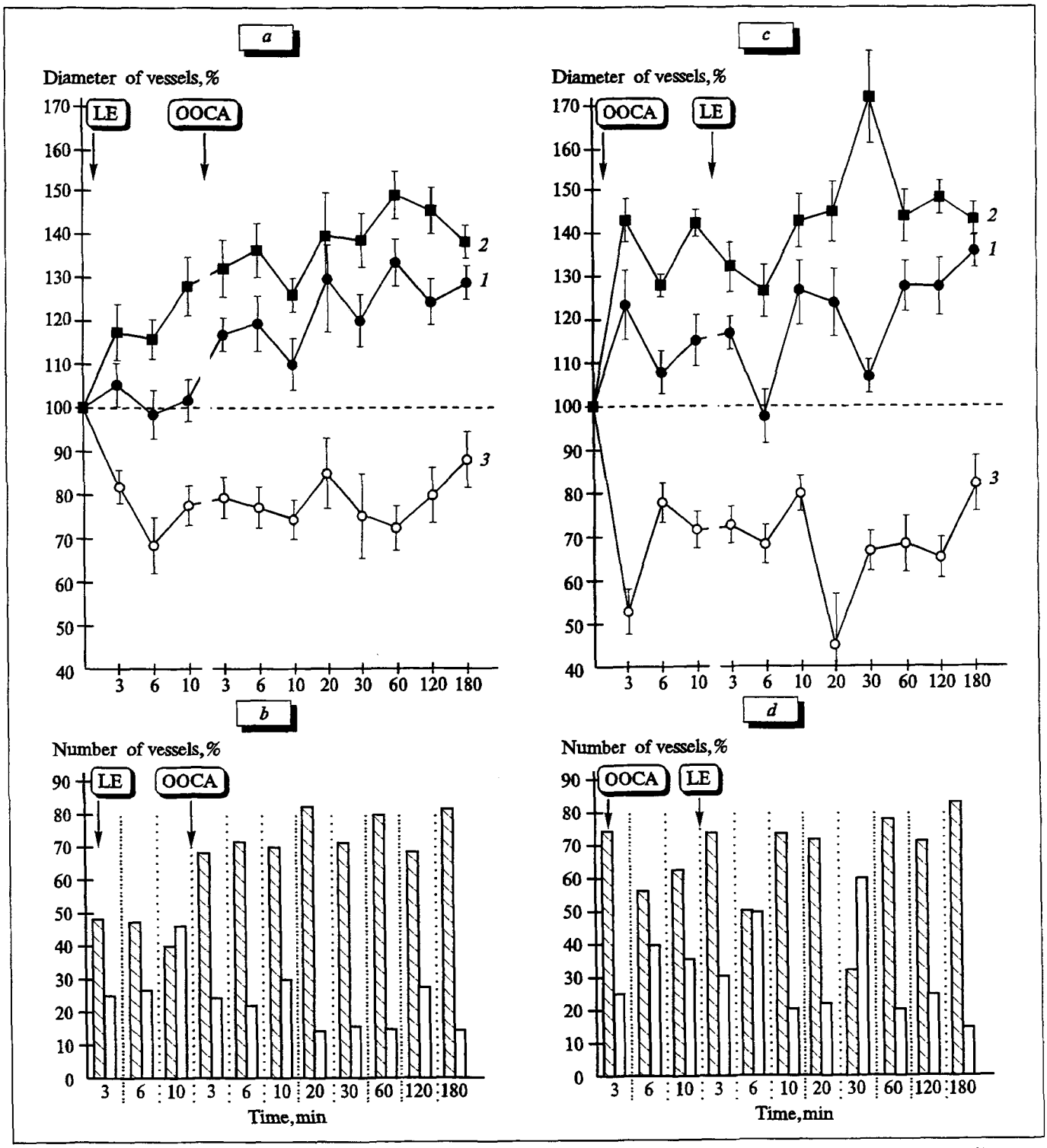

Fig. 3. Effect of preliminary (*a*, *b*) and therapeutic (*c*, *d*) i.p. administration of leu–enkephalin (*LE*, 10 μg/ml) on diameter of pial arterioles of brain in bilateral OCCA.

not exceed 2-3 μ (no difference from the baseline level). Even though arterioles were mainly constricted, the velocity of the blood flow in pial microvessels was accelerated. The local flow in the brain cortex rose progressively under the same conditions, attaining the maximal value of 171% 2.5 h after the administration of the drug [10]. The slight short-term two-phase fluctuations of the central AP and bradycardia could not have produced the acceleration of the blood flow velocity in pial and cortical microvessels in response to the administration of leu-enkephalin, whereas a strong

stimulation of the lymph flow in micro- and macrovessels could markedly stimulated the blood flow [7,9]. Initially inactive mesenteric microvessels of the small intestine (blood depot) began to contract with a frequency reaching 33 contractions per min and the duration of activation was over 40 min (in the control the spontaneous contraction of lymphatic microvessels ceased after 5-10 min and the frequency did not exceed 5-10 contractions/min). Intensified contractile activity of lymphatic microvessels was accompanied by acceleration of the lymph flow velocity in mesenteric microvessels. The flow velocity in the thoracic duct was 293, 561, 621, and 468% 10, 20, 30, and 70 min after leu-enkephalin administration, respectively ($p<0.001$).

The administration of leu-enkephalin 10 min prior to OCCA resulted in a combined reaction of pial microvessels, including constriction due to leu-enkephalin and dilation induced by OCCA. During the first 10 min after leu-enkephalin administration the constriction of some and the dilation of other vessels was noted (Fig. 3, *a*). Subsequent OCCA promoted an increase of the number of dilated arterioles, without qualitatively affecting the vascular reaction (Fig. 3, *b*). The summary lumen of pial arterioles increased, but the degree of dilation was lesser as compared to the effect of OCCA alone. Thus, the maximal widening caused by OCCA was 170%, for the preventive use of leu-enkephalin the lumen was only 130% of the baseline diameter, whereas in the group of dilated vessels it was 150%. Secondly, this series differed from the OCCA series in the preservation of the constriction reaction in some arterioles during the entire experiment (Fig. 3, *a*, *b*). The velocity of the blood flow in pial microvessels did not drop, and the direction of the flow was stable. The local flow in the cerebral cortex was preserved at the baseline level [10]. The flow velocity in the thoracic lymph duct, as well as in the mesenteric lymphatic microvessels and their contractile activity were intensified and corresponded to the data obtained for control leu-enkephalin administration.

Treatment with leu-enkephalin 10 min after OCCA changed the lumen of pial arterioles (venules did not react) in the same way as with the preventive use of leu-enkephalin before OCCA. During 3 h of ischemia two kinds of arteriole reaction were also noted, namely monotonous constriction of the lesser part of vessels and dilation of the majority of them (Fig. 3, *c*, *d*). The reaction of the lymphatic system, central hemodynamics, and BF were the same as in experiments with the preventive use of leu-enkephalin.

Leu-enkephalin caused an increase of the survival of animals with chronic OCCA. The leu-enkephalin effect was most pronounced in the first few hours after the onset of brain ischemia, during which all animals remained alive. Without leu-enkephalin 12% of animals died during the first 10 h after OCCA. At later terms of OCCA the efficacy of leu-enkephalin dropped. Thus, 33% of animals survived 3.5 months after OCCA onset and leu-enkephalin administration, while without leu-enkephalin only 27% of animals stayed alive.

The results differed from data obtained by other authorities [12-14]. The difference lies in the constriction of some pial arterioles and stable, progressive, and long-term (over 3 h) intensification of BF in the cerebral cortex caused by i.p. administration of leu-enkephalin. The different route of leu-enkephalin administration (instead of the previously used intraarterial, intravenous, and intraventricular administration and application) lies at the root of such differences. Intraperitoneal administration of leu-enkephalin was accompanied by a strong stimulation of the contractility of peritoneal lymphatic microvessels, resulting in the acceleration of the lymph flow not only in mesenteric microvessels but also in the thoracic lymphatic duct, the major collector of the lymphatic system. The noted changes occurred against the background of the lowered central hemodynamic indexes (AP, pulse rate). Thus, the lymphostimulation induced by the opioid peptide exerted a protective and therapeutic effect on the microcirculation, BF of the ischemized brain, and animal survival. The preserved constriction and lesser degree of dilation of pial arterioles attest to the lowered autoregulatory reaction of brain vessels, which is known to be in proportion to the oxygen deficiency and metabolic disturbances in ischemized brain tissue. It has been experimentally proven that enkephalins reduce the oxygen uptake by tissues in myocardial infarction [5]. The same reaction probably occurs in the brain tissue under the action of leu-enkephalin. Since compensatory mechanisms of flow deficiency in our experiments cannot be explained solely by the vasomotor reaction of the pial vessels, a key role in this process may be played by the general lymphostimulation of the organism caused by leu-enkephalin, which normalizes the brain homeostasis.

REFERENCES

1. P. N. Aleksandrov, V. V. Aleksandrin, and V. K. Khugaeva, *Pat. Fiziol.*, № 6, 54 (1989).
2. V. M. Buyanov and A. A. Alekseev, *Lymphology of Endotoxicosis* [in Russian], Moscow (1990).

3. Z. D. Ismailova, Ya. D. Mamedov, D. Ya. Guseinov, *et al.*, *Farmakol. Toksikol.*, № 4, 26 (1991).
4. Yu. M. Levin, *Fundamentals of Therapeutic Lymphology* [in Russian], Moscow (1986).
5. Yu. B. Lishmanov and I. G. Avten'eva, *Byull. Sibirsk. Otdel. Akad. Med. Nauk SSSR.*, № 4, 17 (1984).
6. Ya. D. Mamedov, *Myocardial Infarction* [in Russian], Moscow (1989).
7. V. K. Khugaeva and V. V. Suchkov, *Byull. Vsesoyuz. Kardiol. Nauch. Tsentra*, № 1, 95 (1980).
8. V. K. Khugaeva, *Byull. Eks. Biol. Med.*, **105**, № 3, 300 (1988).
9. V. K. Khugaeva, *Fiziol. Zh. SSSR.*, № 1, 92 (1990).
10. V. K. Khugaeva, *Byull. Eksp. Biol. Med.*, **112**, № 8, 117 (1991).
11. V. K. Khugaeva, *Fiziol. Zh. SSSR.*, № 6, 138 (1991).
12. W. M. Armstead, R. Mirro, D. W. Busija, *et al.*, *Circ. Res.*, **68**, № 4, 922 (1991).
13. J. H. Hanko and J. E. Hardebo, *Europ. J. Pharmacol.*, **51**, 295 (1978).
14. M. Kobari, F. Gotoh, N. Suzuki, *et al.*, *J. Cereb. Blood Flow Metab.*, **5**, 34 (1985).
15. E. Okada, *Heart Vessels*, **4**, № 4, 215 (1988).

A Quantitative Study of Dehydrogenase Activity of Hepatocytes in Systemic Endotoxinemia

O. D. Mishnev, A. P. Ettinger, A. I. Shchegolev, M. V. Anurov, and S. P. Yavolov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, No. 1, pp. 105-108, January, 1995
Original article submitted February 28, 1994

A histophotometric study of the liver dehydrogenase activity reveals the nature of changes of enzymatic homeostasis and its periodicity in the dynamics of endotoxinemia in dogs. A compensatory reaction to lipopolysaccharide administration develops during the first two hours. A decrease of dehydrogenase and diaphorase activity and the development of structural damage to hepatocytes appear later. It is shown that the activation of free-radical oxidation as well as an increase of the level of medium-sized molecules in the blood plasma play a key role in the pathogenesis of liver damage.

Key Words: *metabolism; liver; endotoxin*

Recent experimental studies have confirmed the importance of endotoxin from Gram-negative bacteria as a triggering element in the total reaction of the organism, which is accompanied by typical clinical-functional changes leading to the development of endotoxin shock and of multiple organ failure [1,13]. Being responsible for the development of the somatogenic stage of intoxication, the liver is the chief organ fulfilling a barrier and detoxication function. At the same time, the initial period of endotoxicosis may proceed asymptomatically due to pronounced adaptive-compensatory reactions of the hepatic cells [9]. The above considerations call for a study of the alterations in hepatocyte metabolism which determine the level of compensatory resources and which are an important pathogenic element in hepatic insufficiency.

The aim of the present study was a quantitative histoenzyme assay of hepatocytes in the dynamics of systemic endotoxinemia.

MATERIALS AND METHODS

The study was carried out on the 97 liver biopsies from 11 mongrel dogs weighing 13-18 kg which were injected i. v. simultaneously with *E. coli* lipopolysaccharide in a dose of 2 mg/kg. Bi-

Department of Pathological Anatomy, Medical Faculty, Department of Digestive Pathophysiology, Russian State Medical University, Moscow. (Presented by V. V. Kupriyanov, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0001-0101$12.50