EFFECT OF VENOUS STASIS ON STRUCTURAL ORGANIZATION OF COMPENSATION IN LYMPH NODES

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Intensification of transport of some components of the blood and lymph through the walls of blood capillaries and lymphatic sinuses during pathological processes and, in particular, in venous stasis, may be one mechanism of compensation of the latter [2, 3]. Structural manifestations of the formation of such a mechanism have not been adequately studied.

The aim of the present investigation was to study the morphological manifestations of formation of disturbances of the blood circulation in regional lymph nodes in the course of venous stasis at different structural levels.

EXPERIMENTAL METHOD

Experiments were carried out on 45 female Wistar rats weighing 150-160 g. Animals in the stage of diestrus were selected by the vaginal smears method. Venous stasis was produced by ligation of the caudal vena cava below the point of entry of the renal veins. The popliteal lymph nodes were studied by transmission electron microscopy 1 and 6 h, 3, 7, and 14 days, and 1 month after ligation. Lymph nodes of animals undergoing mock operations were used as the control. Each of the control and experimental groups consisted of five animals, from each of which one right popliteal lymph nodes was obtained. The dissection procedures, preparation of the material for study in light and electron microscopes, and morphometry were carried out as described previously [1]. To differentiate between the arterial and venous portions of the capillaries, the mean diameter of cross section of the capillary was determined: this was 2-7 μ in the arterial part and over 7 μ in the venous part [5]. To differentiate the venous part of the capillary from the postcapillary venule, the presence of high endothelial cells in the wall of the latter was utilized [4, 6, 7]. When the number of microvesicles was counted in the cytoplasm of the endotheliocytes, microvesicles with a structural connection with the luminal part of the plasmalemma were described as "luminal," those with a connection with the basal part as "basal," and those with no visible structural connection with the plasmalemma as "cytoplasmic."

Differences between mean values were considered to be significant at the $P < 0.05$ level, using Student's t test. For scanning electron microscopy specimens of lymph nodes from five control rats and from five rats 1 h after ligation of the caudal vena cava were investigated. The dissection procedures and preparation of the specimens followed the recommendations in [8]. The preparations obtained were studied in the JEM 100S/ASID electron microscope.

EXPERIMENTAL RESULTS

The study of interendothelial junctions in the above-mentioned parts of the blood capillaries showed that interaction between the contacting membranes was of the "tight junction" type and no sign of separation of them was observed either under physiological conditions of the hemodynamics or during venous stasis. At different periods of venous stasis excess fluid was transported from the microcirculatory blood stream evidently mainly through fenestrae in the venous part of the capillary. Under physiological conditions of the hemodynamics, for

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TABLE 1. Results of Investigation of Number of Microvesicles in Endothelial Cells of Arterial and Venous Parts of Capillaries of Popliteal Lymph Nodes of Rats at Different Times after Ligation of Caudal Vena Cava (M ± m)

Time after ligation of caudal vena cava	Arterial part			Venous part		
	N_l	N_b	N_c	N_l	N_b	N_c
Control	21,9±0,92	22,8±1,26	112,3±9,97	15,8±0,96	53,9±1,98	146,0±9,45
1 h	22,4±0,55	23,2±1,04	119,6±12,07	13,2±0,57*	25,0±1,46*	79,8±6,02*
6 h	26,8±0,71*	25,1±0,72	173,6±13,38*	15,3±0,54	26,8±0,62*	93,6±9,08*
3 Days	38,6±3,05*	36,1±3,70*	238,7±24,62*	20,1±1,77*	30,4±2,13*	135,2±12,22
7 Days	46,6±3,57*	38,6±2,10*	238,7±21,48*	31,3±2,22*	32,8±2,04*	244,2±17,57*
14 Days	33,4±2,16*	31,2±1,83*	168,5±14,6*	24,3±2,22*	31,9±2,58*	193,2±11,60*
7 Months	23,2±1,92	23,8±1,80	211,1±15,04*	23,8±1,80*	26,2±1,95*	141,0±12,13

Legend. N) Number of microvesicles connected with luminal (l) and basal (b) parts of plasmalemma, and also number located in cytoplasm (c), counted per cross section of all endothelial cells per section through blood capillary. *) Difference from control is significant.

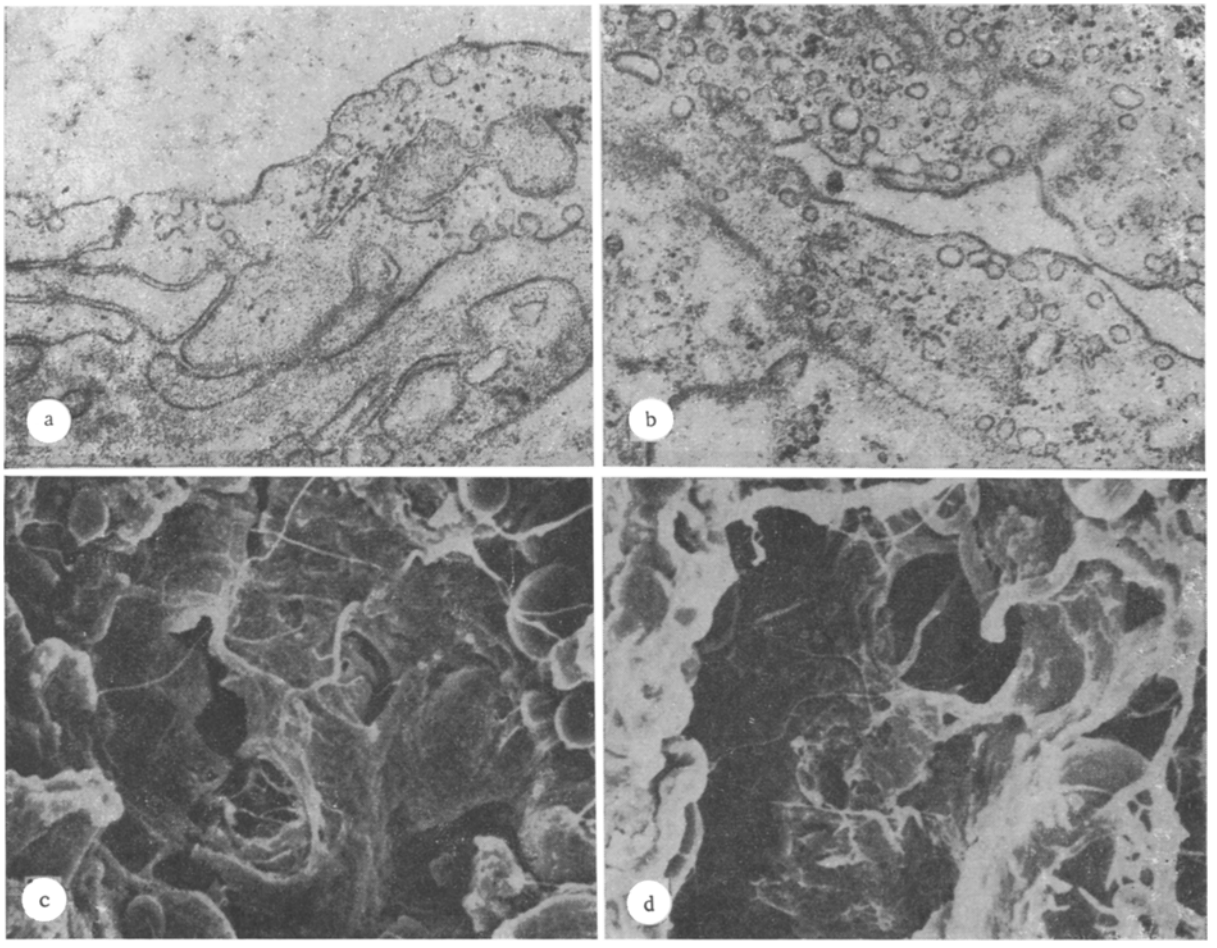

Fig. 1. Boundary structure of lymph node as revealed by transmission and scanning electron microscopy: a) microvesicles in endothelial cell of arterial part of capillary under physiological conditions of hemodynamics, 35,000 ×; b) microvesicles in endothelial cell of arterial part of capillary 7 days after ligation of caudal vena cava, 35,000 ×; c) space between littoral cells of inner wall of marginal sinus under physiological conditions of hemodynamics, 8000 ×; d) space between littoral cells of inner wall of marginal sinus 1 h after ligation of caudal vena cava, 8000 ×.

instance, fenestration of the arterial part of the capillary was not observed, whereas endotheliocytes of the venous part of 14% of the sections contained one or two fenestrae. Marked fenestration was found in 84% of endotheliocytes of the venous part 1 h after ligation of the caudal vena cava; on average 7.3 ± 0.51 of these formations were counted per section. In the arterial part of the capillary 18% of sections of endotheliocytes had one or two fenestrae. Six hours after ligation of the caudal vena cava fenestration of the endothelium of these parts of the capillaries was much less marked. In the venous part 20% of sections of endotheliocytes had 1-4 fenestrae, whereas in the arterial part 8% of sections had one fenestra. Later fenestration of the endothelium of these parts of the capillary reached its initial value.

The number of "luminal" microvesicles in endotheliocytes of the arterial part of the capillary 1 h after ligation of the caudal vena cava was unchanged, but their number in the endotheliocytes of the venous part was reduced to 83.5 ± 6.23% of normal. After 6 h, the number of microvesicles in this situation in the endotheliocytes of the arterial part was 122.4 ± 6.93% of the control, but in endotheliocytes of the venous part it reached its original values (Table 1). Later there was an increase in the number of microvesicles of this type in the endotheliocytes of these parts of the capillaries, which reached a maximum 7 days after ligation. The number of "luminal" microvesicles in endotheliocytes of the arterial part at this time was 212.8 ± 18.59% of the control, compared with 198.1 ± 18.50% in the endotheliocytes of the venous part (Table 1).

The number of "basal" microvesicles in the endotheliocytes of the arterial part of the capillary was increased, to reach a maximum by the 7th day of venous stasis, when it was 169.3 ± 13.13% of the control. The number of "basal" microvesicles in the endotheliocytes of the venous part was reduced throughout the experiment and amounted to between 46.4 ± 3.20 and 60.9 ± 4.40% of the control. The number of "cytoplasmic" microvesicles in the endotheliocytes of the arterial part of the capillary increased during venous stasis, reaching a peak between 3 and 7 days after ligation of the caudal vena cava (Table 1; Fig. 1a, b). In the period from 14 days to 1 month after the operation an increase in the number of "cytoplasmic" microvesicles was observed in the endotheliocytes of the arterial part of the capillary, accompanied by a decrease in the number of "luminal" and "basal" microvesicles; this evidently indicated an increase in the time of movement of the microvesicles through the endothelial cell. The number of "cytoplasmic" microvesicles in the endotheliocytes of the venous part was below normal until 6 h after the operation, but later it increased, and by the 7th day of the experiment it amounted to 167.3 ± 16.19% of the control, after which it fell to the original level (Table 1).

According to the data of scanning electron microscopy, the lining of the medullary vessels and the inner wall of the marginal sinus had numerous fenestrae from 60 to 1000 nm in diameter. In addition, between the littoral cells in the wall of the sinus, wide intercelluar speaces were observed, their size and number being greater during venous stasis (Fig. 1c, d), and this was accompanied by fenestration of the littoral cells of the inner wall of the marginal sinus and medullary sinuses. No open intercellular spaces were found between the littoral cells of the medullary sinuses. These facts suggest that both under physiological hemodynamics conditions during venous stasis transport through the inner wall of the marginal sinus takes place along intercellular spaces and through fenestrae, whereas transport through the wall of the medullary sinuses takes place through fenestrae only.

Mechanisms of compensation of the blood circulatory disturbances in venous stasis thus operate through "discharge" of the excess fluid from the circulating blood stream into the lymphatic microcirculatory system of the lymph node and evidently in the following ways: in the early acute period (1 h after ligation of the caudal vena cava) mainly through the sharply increased number of fenestrated cells in the venous part of the capillary, in the next period (6 h to 3 days) by an increase in microvesicular transport through the endotheliocytes of the arterial part of the capillary and through fenestrae in the venous part, and in the period from 7 to 14 days by activation of microvesicular transport through both parts of the blood capillary. At all the above stages of venous stasis fluid from the parenchyma of the lymph node passes into the marginal sinus, evidently, through fenestrae and intercellular spaces, whereas it passes into the medullary sinuses only through fenestrae.

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EFFECTIVENESS OF MYOCARDIAL PROTECTION AGAINST ISCHEMIA BY NORMOTHERMIC CARDIOPLEGIC SOLUTION WITH CREATINE PHOSPHATE

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Recently a number of natural metabolic substrates whose action is directed toward regeneration and preservation of high-energy phosphates (HEP) have begun to be used as components of cardioplegic solutions [1, 3-5, 10]. As a rule the so-called metabolic protection of the myocardium is effected under hypothermic conditions, i.e., when activity of the metabolic processes on which these components of the cardioplegic solution must act is considerably depressed.

The aim of this investigation was to study the anit-ischemic action of creatine phosphate (CP) and adenosine triphosphate (ATP) as components of potassium-based cardioplegic protection, and to compare the protective effect of CP under normo- and hypothermic conditions.

EXPERIMENTAL METHOD

Experiments were carried out on a model of the isolated working rat heart [9]. Male Wistar rats weighing 220-350 g were used. Heparin was injected intraperitoneally into the animals in a dose of 3000 U with thiopental sodium in a dose of 0.2 mg/g body weight. The heart was excised from the anesthesized animals 5-8 min later and placed in cardioplegic solution which was cooled on ice. After the heart stopped beating the aorta was cannulated and retrograde perfusion of the aorta was carried out for 10 min by Langendorff's method with modified Krebs–Henseleit buffer [9] under a pressure of 82 cm water. In the course of this time the left atrium (LA) was cannulated and the left ventricle (LV) punctured to record the left-ventricular pressure (LVP) and heart rate (HR) by means of a P-50 electromanometer, coupled to a power supply, SP-1405 amplifier, and SP-2009 recorder (Gold Statham, USA). The heart was then perfused for 10 min through LA under a pressure of 17 cm water (Neely's method), and during this time LV, which contracted spontaneously, worked against a resistance and expelled 25-30 ml/min of perfusate. After the 10-min control period the cardioplegic solution was injected into the aorta for 3 min and the heart was exposed to the action of normothermic (36-36.5°C) ischemia for 30 min, after which retrograde reperfusion of the aorta for 10 min and reperfusion through LA by Neely's method for 30 min were carried out. During perfusion through LA, HR (beats/min), LVP (in mm Hg), the aortic ejection — AE (in ml/min), and the coronary blood flow K (in ml/min) were measured in the control and reperfusion period. Parame-

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