INVESTIGATION OF THE BLOOD CAPILLARIES IN THE FUNCTIONING DIAPHRAGM

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UDC 611.26 : 611.161.068

To detect changes in the ultrastructure and dimensions of the blood capillaries connected with functioning of the muscle the rat diaphragm was frozen during various phases of respiration, fixed, and examined in the electron microscope. In the relaxed muscle the lumen of the capillaries was almost twice its area in the contracted muscle, while the thickness of the endothelium was significantly less. During relaxation of the muscle the external diameter of the capillaries increases, but the area of the endothelium in transverse sections is unchanged. In the relaxed muscle the increased blood flow is thus accompanied by improved conditions for transendothelial exchange. The results do not support the hypothesis that changes in the lumen of the capillaries take place as a result of swelling and projection of the endothelium.

EXPERIMENTAL METHOD

The test object was the diaphragmatic muscle of rats. To study the ultrastructure of its blood capillaries, the tissue was first frozen [5]. The diaphragm was frozen rapidly through an incision in the abdominal wall of a rat anesthetized with ether, during contraction or relaxation of the diaphragm corresponding to inspiration or expiration. The depth of anesthesia was so controlled that the animal breathed regularly at a rate of 60-64/min. The material was fixed successively in formol-sucrose and a 1% buffered solution of osmic acid and then embedded in Araldite. During embedding and cutting the pieces of tissue were so arranged that the transverse sections would be obtained through the muscle fibers. Ultrathin sections were stained with uranyl acetate and lead citrate by Reynold's method.

On examination of the sections in the electron microscope all capillaries discovered in the transverse section were photographed under the same electron-optical magnification. The resulting negatives were projected by means of an enlarger onto paper of uniform density, the outlines of the capillaries were traced, the paper was cut around these outlines, and the pieces weighed. The resulting weights were converged into square microns by comparison with a standard. The capillaries during contraction and relaxation of the muscle were compared with reference to the following parameters: the mean area of cross section of the lumen, endothelium, and nucleus. The following mean relative dimensions also were compared: the external diameter of the capillary, the diameter of its lumen, the area and thickness of the endothelium obtained by reducing the mean area of the capillary, and its lumen to the area of a circle.

EXPERIMENTAL RESULTS

The study of the blood capillaries after preliminary freezing of the diaphragm showed that their structure was well preserved and differed only a little from that found after the use of the ordinary methods of fixation (Fig. 1).

Comparison of the capillaries of the muscle frozen during contraction or relaxation revealed the following morphological differences: the capillaries in the contracted muscle were smaller in size, the

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Group for Electron Microscopy, Institute of Normal and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 77, No. 1, pp. 84-87, January, 1974. Original article submitted April 16, 1973.

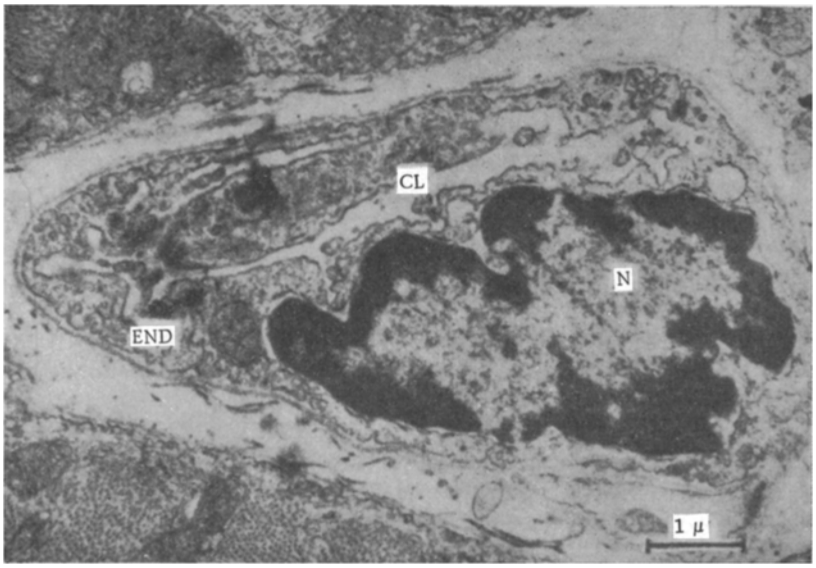

Fig. 1. Blood capillary from rat diaphragm after preliminary freezing: END) endothelial cell; N) nucleus of endothelial cell; CL) capillary lumen.

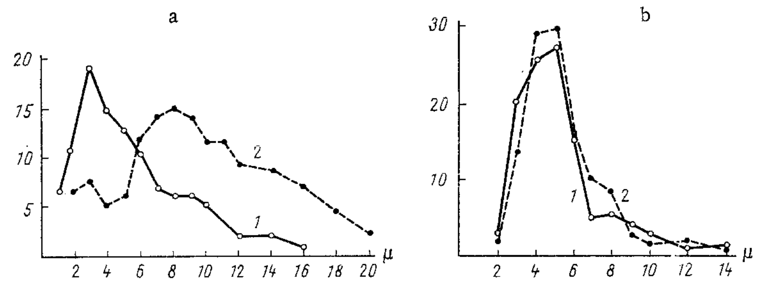

Fig. 2. Distribution of blood capillaries of the rat diaphragm by area of lumen (a) and area of endothelium (b) in the contracted (1) and relaxed (2) muscle. Abscissa, area (in μ^2); ordinate, number of capillaries (in %).

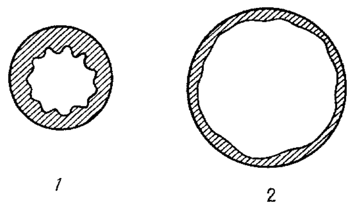

Fig. 3. Schematic representation of capillaries of the diaphram during contraction (1) and relaxation (2).

endothelial membrane surrounding their lumen formed numerous gulf-like investigations; the nuclei of the endothelial cells were irregular in outline, and the nuclear membrane formed deep invaginations. During relaxation of the muscle the capillary lumen was circular or oval in shape and the endothelial membrane was smooth in outline. The nuclei of the endothelial cells were elongated, and their outlines also were smooth. The thickness of the endothelial layer bounding the capillary lumen was smaller in the relaxed muscle.

The dimensions of the capillaries and of their lumen varied considerably. Histograms of the area of the capillary lumen showed that during contraction of the muscle most capillaries have a lumen of between 2 and 7 μ^2, whereas during relaxation the area of the lumen increased and varied from 5 to 16 μ^2 for most capillaries (Fig. 2a). The character of the histogram shows that the lumen of the capillaries of all sizes was increased.

The lumen in some capillaries was virtually absent or so small that they could be considered as "closed." When the muscle was contracted, 12.7% of the total number of capillaries were of this type, compared with 2.16% during relaxation.

TABLE 1. Parameters of Blood Capillaries of Rat Diaphragm during Contraction and Relaxation (M ± m)

Character of sections	State of muscle	No. of observations	Area of lumen (in μ^2)	Area of endothelium (in μ^2)	External diameter (in μ)	Diameter of lumen (in μ)	Thickness of endothelium (in μ)
Sections without nuclei	Contraction	65	6,81 ± 0,47	4,22 ± 0,42	3,53 ± 0,1	2,72 ± 0,1	0,4 ± 0,018
	Relaxation	95	10,49 ± 0,44	4,43 ± 0,17	4,06 ± 0,08	3,37 ± 0,08	0,35 ± 0,013
Sec. with nuclei	Contraction	51	3,57 ± 0,37	5,12 ± 0,28	3,86 ± 0,09	1,86 ± 0,11	—
	Relaxation	45	7,69 ± 0,091	5,18 ± 0,23	4,54 ± 0,13	2,85 ± 0,14	—

A statistical analysis of some parameters of the capillaries in the diaphragm in different functional states is given in Table 1. When the section passed through the body of an endothelial cell the capillary lumen was considerably narrower. Since the relative number of these capillaries varied during contraction and relaxation of the muscle, the data for sections through the capillaries with or without nuclei were analyzed separately. It is clear from this table that significant differences were found in several parameters of the capillaries depending on the functional state of the muscle. For instance, during contraction and relaxation the area of the lumen of the capillaries, their external and internal diameters, and the thickness of the endothelium all varied significantly. These changes were similar in direction in sections of the capillaries with or without nuclei. The greater increase in area of the lumen of the capillaries with nuclei during relaxation of the muscle is explained by the fact that these include both closed and half-closed capillaries. The absence of changes in the area of the capillary endothelium during contraction and relaxation of the muscle, despite changes in the external and internal diameters of the capillaries under these circumstances, is interesting. The histogram (Fig. 2b) shows that this conclusion applies to all capillaries studied.

On the basis of the morphological and statistical analysis of these results the changes taking place in the capillaries during contraction and relaxation of the diaphragm can be represented schematically (Fig. 3).

During natural functioning of the diaphragm changes thus take place in the lumen of its capillaries and these are synchronized with the phases of its activity. These changes take place chiefly as a result of dilatation and constriction of the capillaries but partially through their opening and closing. In view of observations showing that during contraction of the muscle the volume of blood flowing through it decreases whereas during relaxation it increases [3, 6], the increase in lumen of the capillaries during relaxation of the diaphragm and its decrease during contraction can be considered to be connected with the increase and decrease in the capillary blood flow. Bearing in mind, in addition, that during relaxation of the muscle the thickness of the capillary endothelium is reduced, the conditions for transendothelial exchange can be considered to be improved in the relaxed muscle.

The question naturally arises of the mechanisms of the changes in the capillary lumen. The cause is possibly a change in the blood pressure in the capillaries as a result of compression of the intramuscular arteries during contraction of the muscle and their dilatation during relaxation [6]. However, the last decrease in pressure caused by compression of the abdominal aorta or by massive blood loss, resulting in changes in the diameter of the afferent and drainage vessels, did not cause changes in the lumen of the capillaries in the resting muscle [10].

Some investigators nowadays accept that active changes can take place in the lumen not only of vessels applied with smooth muscle cells, but also of capillaries [1, 7]. The active elements are considered to be pericytes [4, 12], and endothelial cells [9, 11]. No fibrillary structures could be found in the endothelial cells, although the shape of their nuclei when the lumen was reduced was typical of the cells in a state of contraction.

The absence of changes in the area of the capillary endothelium during changes in the size and lumen of the capillaries is also interesting. Since it is very probable that under these circumstances there was no change in the volume of the endothelial cells, these results do not support the view that swelling and projection of the endothelium is the mechanism responsible for the changes in the lumen of the capillaries [2, 8, 13].

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