INVESTIGATION OF THE VESICULAR SYSTEM OF THE CAPILLARY ENDOTHELIUM OF THE DIAPHRAGM IN VARIOUS FUNCTIONAL STATES

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Micropinocytotic vesicles in the endothelial cells of capillaries in the diaphragm were counted during indirect repetitive stimulation of the muscle at a frequency of 50/sec, after division of the phrenic nerve, and during natural contraction and relaxation of the muscle during respiration. Changes in the total number of vesicles and even more marked changes in the ratio between vesicles fixed to the plasma membranes and lying freely in the cytoplasm of the cells were discovered reflecting changes in transendothelial transport of materials. The method of fixation used may affect the state of vesicle formation.

KEY WORDS: capillaries; micropinocytotic vesicles; endothelium; diaphragm.

It is now firmly established that micropinocytotic vesicles are part of the transport system of endothelial cells responsible for transcapillary exchange [1, 3-5, 8, 12].

Meanwhile some workers have expressed the view that since under certain experimental conditions no changes can be found in the number of vesicles, they are permanent organelles and do not change with changes in the functional state of the endothelial cells [6, 7, 9, 10].

The object of this investigation was to count the micropinocytotic vesicles in the capillary endothelium of the diaphragm in various functional states.

EXPERIMENTAL METHOD

August albino rats were used. The capillaries in the diaphragm were studied during tetanic contraction induced by supramaximal stimulation of the phrenic nerve at a frequency of 50/sec (series I), during relaxation of the muscle caused by division of the phrenic nerve 10-20 min before the beginning of fixation (series II), and during natural contraction (series III) and relaxation (series IV) of the diaphragm during respiration.

In the experiments with tetanization and division of the phrenic nerve material was fixed with formol–sucrose in situ. For this purpose the rats were anesthetized with ether, cold fixative was injected into the pleural and peritoneal cavities, and 10 min later the diaphragm was removed and immersed in the same fixative. Tetanization of the nerve began immediately before the beginning of fixation and continued for several minutes.

The method of preliminary freezing [2] was used to fix the diaphragm during natural contraction and relaxation.

In every case the muscle was postfixed in a 1% buffered solution of osmium tetroxide, dehydrated, and embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate by Reynold's method.

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TABLE 1. Characteristics of Vesicular System in Capillary Endothelium in Various Functional States (M ± m)

Functional state of muscle	Number of vesicles					
	total	free	bound with basal surface of capillaries		bound with luminal surface of capillaries	
			opening	forming	opening	forming
Fixation with formol–sucrose						
Tetanization, "pale" cells	122±4,1	74,3±6,4	22,1±2,6	14,3±2,3	4,9±0,8	5,8±0,6
Division of nerve:						
"pale" cells	106±5,6	50,0±6,3	21,0±2,2	19,4±5,2	9,6±1,4	6,3±1,5
"dark" cells	119±6,3	73,0±6,8	19,6±1,8	13,9±1,5	8,0±1,6	4,6±0,54
No additional treatment	110±5,7	41,0±3,9	18,9±2,9	24,4±2,6	11,2±1,8	12,8±1,2
Preliminary freezing						
Inspiration (contraction)	80,6±5,9	23,6±3,1	39,0±4,0		18±4,2	
Expiration (relaxation)	74,0±5,1	24,0±5,8	32,0±3,2		17±3,5	

In each series of experiments 10 capillaries, photographed in transverse section, each with a perimeter 11-13 μ in length, were chosen and the total number of micropinocytotic vesicles, both free and bound with the plasma membranes, was counted in them. In some cases the number of forming and opening vesicles was counted separately, their differentiation being based on accepted morphological criteria [3-6].

EXPERIMENTAL RESULTS

Data on some parameters and the ultrastructure of the capillaries during contraction and relaxation of the diaphragm were published previously [2].

Counting the micropinocytotic vesicles in the capillary endothelium showed that the total number of vesicles changes depending on the functional state of the diaphragm (Table 1), either by uniform changes in all groups of vesicles or by a change in their number predominantly in certain groups. The total number of vesicles bound with the basal plasma membrane of the cells was always greater than their number on the luminal surface. The number of forming and opening vesicles on the basal surface of the cells was greater than the number of corresponding vesicles on the luminal surface. After fixation with formol–sucrose in situ the total number of vesicles in the endothelial cells was always greater than after fixation by the preliminary freezing method.

During tetanic stimulation of the diaphragm the number of vesicles in the endothelium was greater ($P = 0.05$) than after division of the phrenic nerve, mainly on account of an increase in the number of free vesicles. Meanwhile a decrease in the number of forming vesicles was observed on the basal surface of the cells and in the number of opening vesicles on the luminal surface.

Comparison of the state of the vesicular system of the endothelial cells during contraction (inspiration) and relaxation (expiration) of the diaphragm during natural respiration showed a tendency toward a decrease in the number of vesicles bound with the basal surface of the cell during relaxation of the muscle compared with contraction ($P = 0.05$).

During fixation of the muscle with formol–sucrose in situ some of the endothelial cells in the capillaries had increased electron density ("dark" cells). The total number of vesicles in these cells was greater ($P < 0.05$) than in ordinary cells, chiefly because of an increase in the number of free vesicles. They also had fewer forming vesicles on the basal surface and fewer opening vesicles on the luminal surface, i.e., the same tendency was shown as during tetanic contraction of the muscle.

The investigation thus showed that in different functional states of the diaphragm, vesicle formation in the capillary endothelium varies. Both the total number of vesicles and the relative numbers of free vesicles and vesicles bound with the plasma membrane vary under these circumstances. Meanwhile, it is difficult to judge the intensity and direction of endothelial transport purely on the basis of a study of changes in the number of micropinocytotic vesicles.

Changes in the state of the vesicular system are also linked with the functional state of the endothelial cells themselves, as shown by differences in the number of vesicles and their distribution in ordinary and

"dark" cells when the muscle is in the same functional state. It is interesting to note that these cells may alternate in the capillary, so that different areas of the capillary wall are heterogeneous, from the standpoint of the functional state of the cells composing them. If, as has been postulated, the increased electron density of the cytoplasm is evidence of intensified synthesis in the cells and a lowering of their "external" function, the increase in the total number of vesicles as the result of an increase in the number of free vesicles must indicate a lowering of the level of transendothelial exchange processes.

Comparison of the state of the vesicular system of the endothelium after fixation with formol–sucrose in situ and after preliminary freezing of the muscle indicates that the process of vesicle formation can continue or even become intensified for some time after the beginning of aldehyde fixation.

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