MORPHOLOGICAL ANALYSIS OF THE FUNCTIONAL STATE OF THE VASCULAR ENDOTHELIUM IN HYPOKINESIA

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The length of the free surface of the capillary endothelium in the soleus muscle of rats whose mobility was restricted for between 1 and 60 days was studied on electron micrographs of transverse sections through the capillaries. On the first day of the experiment the length of the free surface of the endothelial cells was increased by 1.6-1.8 times over the control. On the 30th-60th day of limited movement these changes were no longer significant.

KEY WORDS: hypokinesia; capillary endothelium.

It is generally considered that the membranes bounding cell surfaces as a rule possess low permeability. In the living organism this property of the cell membranes is overcome by various anatomical devices, one of which is increasing the surface area through the formation of multiple processes [2]. The plasma membrane of the endothelial cells of blood vessels under normal conditions forms small invaginations and villi on its free (inner) surface [3]. Depending on the functional state, the character of metabolism, or the action of pathogenic factors on the surface of the endothelial cells, many cytoplasmic outgrowths and invaginations may be formed, so that the area of contact between the blood and the cell is increased and the intensity of the exchange of materials between the cell and the blood is altered [6]. A quantitative assessment of the increase in the area of the cell surface can thus be used as a criterion of the functional state of the vessel. It is assumed that the ratio between the length of the inner and outer surfaces of the endothelial lining of the capillary or other type of vessel, expressed numerically, can reflect the state of transcellular transport systems.

Line and point methods are nowadays widely used for quantitative analysis in morphology in order to determine the number and volume of cells and their components [1].

A method of determining the ratio between the length of the inner cell membrane, measured planimetrically, and the length of the circumference drawn at the level of the epithelial lining of the vessel without allowing for outgrowth and invaginations of the cytoplasm (K), is accordingly suggested.

EXPERIMENTAL METHOD

Transverse sections of blood vessels were photographed in the electron microscope under low power (6000-8000). The length of the free surface of the capillary endothelium (l_0) and the length of their inner (l_1) and outer (l_2) circumferences were measured on enlarged projections. The circumferences l_1 and l_2 were drawn arbitrarily at the outer and inner borders of the plasma membrane on the free surface of the capillaries, cutting off the outgrowths and invaginations of the cytoplasm of the endothelial cells. The mean between l_1 and l_2 was calculated as the length of the free surface of the capillary under normal conditions. The length of the free surface of each capillary and circumference was measured 3 or 4 times and the mean calculated. The numerical value of K was calculated by the equation

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TABLE 1. Quantitative Determination of Cell Surface Area of Capillary Endothelium of Soleus Muscle of Rats Kept under Conditions of Restricted Mobility

Time of observation	No. of cells	1			2			3			4			5			6			Mean
		l_0	l_1	l_2	K	l_0	l_1	l_2	K	l_0	l_1	l_2	K	l_0	l_1	l_2	K	l_0	l_1	l_2	K	K
Control		18,3	17,5	17,3	1,02	5,5	23	22	1,13	22,5	22	21	1,0	36,75	36	34	1,0	20	20	17,5	1,00	1,0
Hypokinesia:																						
1st day		68	43	31	1,83	26	21	12,6	1,6	28	21,3	16,3	1,5	43,6	31	22	1,6	35,3	21	14,5	1,38	1,8
3rd "		27,66	22	18,5	1,36	18,3	13	11	15,25	8,5	7,	6	13,07	19	13	12	1,52	20,5	15	13	1,38	1,38
14th "		18	14	13	1,33	27	19	20	1,34	48	43	40	1,13	65	59	53	1,16	26	23	22	1,13	1,23
30th "		23,66	22,66	21	1,1	28	21	20	1,12	25	24,5	22	1,1	24	22,3	21,3	1,1	71	65	63	1,1	1,05
60th "		69,3	65	64	1,07	57,6	51	50	1,14	21	20	18	1,15	28	26,3	24	1,1	33	28,6	27,3	1,18	1,0

Time of observation	No. of cells	1			2			3			4			5			6			Mean
		l_0	l_1	l_2	K	l_0	l_1	l_2	K	l_0	l_1	l_2	K	l_0	l_1	l_2	K	l_0	l_1	l_2	K	K
Control		18,3	17,5	17,3	1,02	5,5	23	22	1,13	22,5	22	21	1,0	36,75	36	34	1,0	20	20	17,5	1,00	1,0
Hypokinesia:																						
1st day		68	43	31	1,83	26	21	12,6	1,6	28	21,3	16,3	1,5	43,6	31	22	1,6	35,3	21	14,5	1,38	1,8
3rd "		27,66	22	18,5	1,36	18,3	13	11	15,25	8,5	7,	6	13,07	19	13	12	1,52	20,5	15	13	1,38	1,38
14th "		18	14	13	1,33	27	19	20	1,34	48	43	40	1,13	65	59	53	1,16	26	23	22	1,13	1,23
30th "		23,66	22,66	21	1,1	28	21	20	1,12	25	24,5	22	1,1	24	22,3	21,3	1,1	71	65	63	1,1	1,05
60th "		69,3	65	64	1,07	57,6	51	50	1,14	21	20	18	1,15	28	26,3	24	1,1	33	28,6	27,3	1,18	1,0

$$K = l_0 : \left(\frac{l_1 + l_2}{2}\right).$$

The suggested method was tested by analyzing the state of the blood vessels (capillaries) of the soleus muscle of rats kept in a state of severe hypokinesia, attained by keeping the animals in small cages for periods of between 1 and 60 days.

EXPERIMENTAL RESULTS

During exposure to hypokinesia, structural changes in the vascular wall and, in particular, in the endothelium appeared and developed in the red soleus muscle. By the end of the first day of the experiment the cytoplasm formed numerous outgrowths and invaginations, with a consequent substantial increase in the area of endothelium facing the lumen of the vessel. In the cytoplasm of the endothelial cells numerous pinocytotic vesicles, electron-transparent vacuoles, and cavities were found. Later these manifestations of edema subsided and the structural organization of the cytoplasm changed: the number of pinocytotic vesicles and vacuoles fell sharply. The outlines of the lumen of the vessels were less indented in appearance. In the later stages of observation the number of pinocytotic vesicles in the endothelial cells and the character of the outlines of the capillary lumen likewise did not remain unchanged. A few pinocytotic vesicles were found in the cytoplasm of the endothelial cells and the outlines of the lumen of the blood vessels appeared smooth, similar to those in the control animals [4, 5].

Measurement of the length of the free surface of the endothelium in transverse sections through the capillaries showed (Table 1) that on the 1st day of hypokinesia K was between 1.6 and 1.8; in other words, a significant ($P < 0.05$) increase was found in the relative length of the free surface of the capillary endothelium of 1.6-1.8 times compared with the control animals. On the 3rd day of the experiment K = 1.37. On the 14th day the relative length of the free surface of the capillary endothelium was increased by 1.2 times in the experimental animals. On the 30th-60th day of the experiment the changes were not significant and the length was only 1.12-1.15 times greater than in the control rats.

The results of the quantitative analysis of capillary endothelial cells of the soleus muscle of rats exposed to hypokinesia thus demonstrate an increase in the length of the free surface of the capillaries between the 1st and 60th day of restricted mobility. Significant differences in the values of the coefficient K under normal conditions and on the 1st day of hypokinesia evidently indicate a marked increase in the exchange of materials between the endothelial cell and the pericapillary tissue elements and also between the endothelial cell and the blood plasma in the early stages of hypokinesia. The results are in full agreement with those of previous morphological investigations [4, 5].

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