HISTOCHEMICAL CHARACTERISTICS OF TRANSPORT ATPase IN HUMAN BRAIN CAPILLARIES

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Transport ATPase is one of the main enzymes involved in regulation of ionic homeostasis in cells and a very precise marker of its age changes [4, 9]. This enzyme takes part in transport of sodium ions against the concentration gradient and in accumulation of potassium ions in endothelial cells of brain capillaries, and it is thus a true indicator of endothelial premeability [6]. Transport ATPase activity has been demonstrated by histochemical methods only in microvessels of the rat and guinea pig spinal cord [8], but its changes during the postnatal development of these animals have been demonstrated by biochemical methods [7].

This paper describes an attempt to determine whether transport ATPase can be detected histochemically in human brain capillaries and to study changes in its parameters in old age.

EXPERIMENTAL METHOD

Capillaries from area 17, 41, and 4 of the cerebral cortex of men belonging to four age groups, dying accidentally, were studied: 9 were aged 22-44 years, 7 aged 55-64 years, 8 aged 65-74 years, and 7 were aged 75-86 years. Samples of tissue were studied not later than 8 h after death, when changes in enzyme activity in the capillaries were negligible.

Frozen sections 50 μ thick were mounted on coverslips, dried for 15-20 min, and transferred to incubation medium of the following composition: 5 mM p-nitrophenyl phosphate, 10 mM $MgCl_2$, 30 mM KCl, 20 mM $SrCl_2$, and 100 mM Tris-HCl buffer (pH 9.0-9.2). The intensity of the reaction was enhanced with 25% dimethyl sulfoxide solution. Nonspecific processes were inhibited by L-tetramisole (1 mM). The specificity of the reaction was verified by addition of 1 mM ouabain to the medium. After incubation (1-3 h at 37°C) the sections were treated with 2% $CoCl_2$ for 3-5 min, washed in three changes of distilled water and buffer, put into ammonium sulfide for 1-3 min, and mounted in glycerin-gelatin. Morphometric parameters of the capillary system, adopted in morphologic [1, 2] and histochemical [5] investigations, were studied in three fields of vision, in each of six sections separately, and for each subject individually. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1a, b that addition of dimethyl sulfoxide to the incubation medium increased the sensitivity of the reaction by substantially increasing the optical density of the precipitate deposited in the vessel walls, and the number of capillaries. It was thus possible to differentiate them by degree of enzyme activity. Photometric studies showed that the greatest optical density and, consequently, transport ATPase activity, was possessed by capillaries staining pale brown. Lower values of this parameter were obtained in vessel walls stained yellow and pale yellow, which were distinguished by moderate and low enzyme activity. The reaction was completely suppressed by ouabain.

Removal of L-tetramisole from the incubation medium leads to increased deposition of reaction product and an increase in the number of capillaries (Fig. 1c). In this case, however, the use of ouabain had no appreciable effect on the results. Since L-tetramisole is a specific blocker of alkaline phosphatase in brain microvessels [6, 7], revealed under simi-

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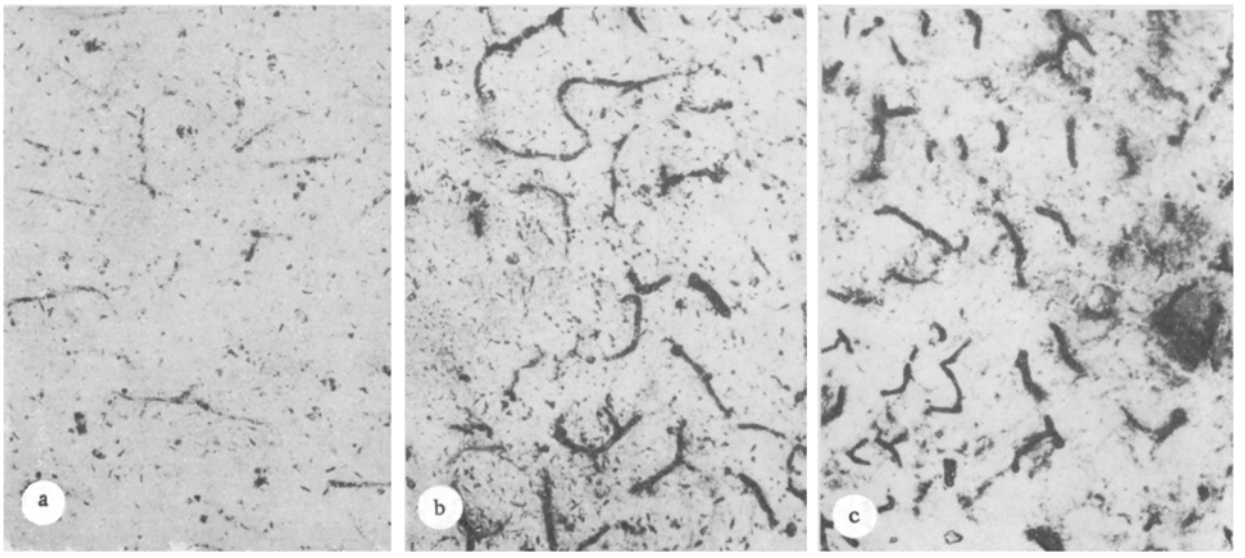

Fig. 1. Differences in Na^+, K^+-ATPase activity and number of capillaries under different conditions of demonstration. a) Incubation of sections in medium without dimethyl sulfoxide; b) incubation of sections in medium with addition of dimethyl sulfoxide; c) incubation of sections in medium without L-tetramisole. 140 ×.

TABLE 1. Age Changes in Morphometric Parameters of Capillaries in Area 17 of Cerebral Cortex

Parameter studied	Age groups			
	22-44 years	55-64 years	65-74 years	75-86 years
Mean diameter, μ	5,7 ± 0.17	6,23 ± 0,2	6,06 ± 0,17	6,13 ± 0,16
Mean length, mm	107 ± 8,6	82,4 ± 7,2	94,6 ± 5,8	72,3 ± 8,4
Volume of vessels in 1 mm^3	$2,73 \cdot 10^6$	$2,5 \cdot 10^6$	$2,72 \cdot 10^6$	$2,12 \cdot 10^6$
Working (exchange) surface	$1,91 \cdot 10^6$	$1,64 \cdot 10^6$	$1,79 \cdot 10^6$	$1,39 \cdot 10^6$
Optical density of reaction product (activity):				
maximal values	7,2 ± 0,3	7,8 ± 0,2	7,3 ± 0,1	7,6 ± 0,3
average values	4,6 ± 0,2	4,0 ± 0,1	3,5 ± 0,2	3,4 ± 0,1
Proportion of capillaries (in percent) with different degrees of enzyme activity in their wall:				
high	23 ± 2,4	14 ± 1,2	7 ± 1,9	6 ± 1,0
moderately high	47 ± 3,4	29 ± 2,0	24 ± 3,0	20 ± 2,2
low	30 ± 4,3	57 ± 2,7	69 ± 3,2	74 ± 2,7
Intensity of metabolism in capillary wall	8,81	6,56	6,26	4,73

lar conditions, capillaries with activity of this enzyme also could be seen on the photomicrographs.

During aging all morphometric parameters used to assess the state of the capillary system change (Table 1). Age changes in the diameters of these vessels on the whole correspond to those found by morphological studies [3, 10], but fluctuations of length possess some features of their own. For instance, an increase in this parameter, and also in the level of the working (exchange) surface or volume of capillaries in 1 mm^3 of brain, occurs at precisely those age groups in which a reduction in their diameter is observed. The exception is the volume of the vessels in persons aged 65-74 years, which was higher than in persons aged 22-44 years. This state of affairs can probably be attributed not only to a compensatory increase in the number of capillaries, but also to reduction of the weight of the brain observed at this age, death of nerve cells, and an increase in brain density [3].

Toward old age there is a redistribution of the relative proportions of capillaries with different levels of enzyme activity: The number of vessels with high and moderately high in-

tensity of reaction in their wall falls whereas the number of vessels with low intensity of the reaction rises. Because of this, the mean values of optical density of the reaction product in the capillaries also are reduced, although in old age the highest optical density of precipitate among vessels with high transport ATPase activity actually increases a little, although admittedly, not significantly ($P > 0.5$).

One of the most important histophysiological characteristics of the capillary system is the level of intensity of exchange processes, which is a product of the area of working surface of the vessels and mean values of enzyme activity in their walls. Unlike parameters of working surface adopted usually by morphologists [1, 2], as one of the principal equivalents of metabolism effected by the capillary wall, the level of intensity of exchange processes diminishes steadily with age. In the visual cortex, by 75-86 years it falls by 46.3% (Table 1), a figure which corresponds quite closely to the results of biochemical tests [4], whereas the working surface decreases by almost half at this age.

Specificity of the histochemical reaction in human brain capillaries is thus proved by three groups of factors: 1) depression of transport ATPase activity in the vessels by ouabain; 2) activity of the reaction is preserved after addition of L-tetramisole, an inhibitor of alkaline phosphatase, to the incubation medium; 3) the conditions under which the best results of the histochemical reactions were obtained are optimal also for biochemical investigations with "pure" Na^+,K^+-ATPase [8].

This method is very sensitive also to age changes in active ion transport, and the histochemical parameters used are appropriate for quantitative evaluation of metabolic processes.

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