DISTRIBUTION OF ENZYME ACTIVITY IN SUBCELLULAR FRACTIONS FROM VARIOUS PARTS OF THE RAT BRAIN ISOLATED BY GRADIENT CENTRIFUGATION

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The mitochondrial fraction and its five subfractions isolated by ultracentrifugation in a sucrose density gradient differ in their protein content and levels of specific activity of cytochrome oxidase, succinate-cytochrome c-oxidoreductase, and acetylcholinesterase.

There is experimental evidence of differences in the biochemical properties of mitochondrial fractions isolated from different parts of the brain [4, 5, 14, 15]. Studies with electron microscopes have shown that mitochondrial fractions isolated from the brain by differential centrifugation may contain, in addition to free mitochondria and mitochondria bound with nerve endings, other structural components, such as synaptic vesicles, myelin, microsomes, and so on [7, 8, 12].

In the investigation described below, the biochemical properties of mitochondrial fractions obtained by differential centrifugation were studied.

EXPERIMENTAL METHOD

Mitochondrial fractions were isolated from the cerebral cortex and brain stem of 5-6 rats in each experiment by differential centrifugation by Biesold's method [1]. A fraction of the mitochondria from whole rat brain also was used for comparison. These fractions were divided into five subfractions in a continuous sucrose gradient, with concentrations of 0.8, 1.0, 1.2, and 1.4 M, by the method of De Robertis and co-workers [6]*. A suspension of mitochondria in 0.25 M sucrose solution was layered on to the gradient and centrifuged at 120 000 g for 45 min. Five subfractions were isolated (A, B, C, D, and E), and their components were sedimented in 0.25 M sucrose solution at 100 000 g for 30 min.

The subfractions were examined under the electron microscope [2]. The mitochondrial fraction and subfractions were then investigated to determine the distribution of protein and oxidative enzymes: cytochrome oxidase (CO), succinate-cytochrome c-oxidoreductase (SCOR), and acetylcholinesterase (ACE). Activity of these enzymes was measured spectrophotometrically: of CO by the method of Hess and Pope [9], of SCOR by the method of Potter and Schneider [13], and of ACE by Hestrin's method [10]. Protein was determined by Lowry's method [11]. The results were expressed in milligrams of cytochrome c and micromoles acetylcholine, respectively, per milligram protein per hour. The results of 18 experiments were analyzed by statistical methods.

*The subfractions were isolated by the use of a Superspeed-50 ultracentrifuge at the Laboratory of Immunology, V. P. Serbskii Central Research Institute of Forensic Psychiatry.

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TABLE 1. Distribution of Protein and Enzyme Activity among Mitochondrial Fractions and Subfractions from Whole Brain (I), Cerebral Cortex (II), and Brain Stem (III) of Rats

Test object	Protein (in mg/g tissue)			Cytochrome oxidase (in mg cytochrome c/mg protein)			Succinate-cytochrome c-oxidoreductase (in mg cytochrome c/mg protein)			Acetylcholinesterase (in μmoles acetylcholine/mg protein)	
	I (5)	II (7)	III (6)	I (5)	II (7)	III (6)	I (5)	II (7)	III (6)	II (7)	III (6)
Mitochondrial fraction	25,8±2,50	24,3±2,80	24,1±2,10	72±8,5	96±5,2	192±21,0	47±2,6	60±1,4	68±1,7	9,4±0,2	12,1±0,6
Subfractions:											
A	3,4±0,20	2,5±0,30	8,8±0,24	30±3,9	25±3,9	25±0,90	52±2,1	36±0,6	50±2,3	13,2±2,7	12,8±0,7
B	2,6±0,13	3,0±0,07	1,6±0,25	41±3,5	70±1,6	98±6,0	95±1,2	40±2,6	60±1,4	16,2±1,2	20,0±1,2
C	5,3±0,38	6,3±0,35	3,7±0,28	75±8,4	152±1,2	152±14,0	62±6,8	63±7,0	58±2,1	10,0±0,7	18,2±0,8
D	5,3±0,48	4,5±0,24	4,5±0,18	156±3,0	242±3,0	190±10,0	72±8,5	145±14,0	120±6,0	10,8±1,0	10,8±1,0
E	1,8±0,25	1,8±0,08	1,5±0,12	546±8,0	630±16,0	342±15,0	227±6,0	334±38,0	224±20,0	10,4±1,3	14,5±1,1

Note. Figures in parentheses give number of determinations.

EXPERIMENTAL RESULTS

With the same protein content in the original mitochondrial fraction, the protein distribution in the homonymous subfractions differed in the two parts of the brain. The protein content in the mitochondrial subfractions of the brain stem, for instance, was much higher in A (myelin), but lower in C and D (mitochondria and nerve endings), than in the corresponding subfractions of cortical mitochondria and mitochondria from the whole rat brain. However, the protein content in E (free mitochondria) was almost identical in the structures tested. The enzyme activity of these subfractions can therefore be compared.

Activity of oxidative enzymes was found chiefly in subfractions C, D, and E (Table 1). However, the specific activity of these enzymes, calculated per milligram protein of the fractions, was highest in E, in which it was 2-5 times greater than in the other subfractions. Activity of the enzymes in subfraction E of the cortex was considerably higher than in the brain stem or the whole rat brain. A similar relationship between the cortex and other parts of the brain was observed for enzyme activity in subfraction D (mitochondria of nerve endings). No such differences were found, however, in subfraction C (Table 1).

The distribution of activity of oxidative enzymes among the various subfractions also differed in each part of the brain tested. The ratio between the specific activity of cytochrome oxidase in A, B, C, and D and its activity in E, for instance, was much lower in the cortical mitochondria than in those of the brain stem. With respect to SCOR, however, this rule did not apply (Table 2).

Investigation of the distribution of ACE among subfractions of the cortex and brain stem showed that activity of this enzyme was present in all subfractions, but was maximal in subfraction C. In subfractions C + D (nerve endings) from the cortex and brain stem, 47% and 40% of the total ACE activity, respectively, was found. The specific ACE activity in the subfraction of nerve endings was much lower in the cortex than in the brain stem (10.4 and 14.5, respectively).

The electron-microscopic findings [2] showed that the mitochondrial subfractions isolated by gradient centrifugation corresponded morphologically to those described previously by De Robertis and co-workers [7].

In a comparative study of the subfractions of pure mitochondria from different parts of the brain, no significant difference was found in their protein content. The slightly lower protein content in the myelin subfraction (A) and the higher content in subfractions of nerve endings (C and D) in the cortex than in the corresponding subfractions of the brain stem and whole brain correlate with the morphological data as regards the presence of conducting structures in these parts of the brain. This fact is also in agreement with results obtained by investigation of mitochondrial fractions from the bovine cerebral cortex and from the whole rat brain [6, 7].

TABLE 2. Ratio between Enzyme Activities of Subfractions in Mitochondria from Cortex and Brain Stem of Rats (in %)

Subfraction	Cytochrome oxidase		SCOR	
	cortex	brain stem	cortex	brain stem
A	9,8	18,6	18,7	11,4
B	11,4	31,5	22,7	13,5
C	23,2	43,8	21,7	19,0
D	39,0	57,0	50,6	45,6
E	100,0	100,0	100,0	100,0
C	97,0	100,0	113,0	100,0
D	128,0	100,0	120,0	100,0
E	184,0	100,0	149,0	100,0

The material described, indicating higher activity of oxidative enzymes in the subfractions of purified mitochondria (E), and also in the mitochondria of nerve endings (D) of the cortex compared with the corresponding subfractions of the brain stem, are in agreement with earlier findings obtained with heterogeneous fractions of mitochondria from these regions [4, 5]. The greater affinity of CO for free mitochondria (E) of the cortex than of the brain stem (184 and 100%,respectively), and the less marked differences between these subfractions in their SCOR activity (149 and 100%), correspond to the view that the succinate oxidase system is relatively more important in the peripheral portions of sensory systems in the mammalian brain [3]. The smaller differences of enzyme activity between subfractions C and D of the cortex and brain stem compared with the subfractions E evidently reflect some degree of similarity between the mitochondria of the nerve endings of these brain structures. At the same time, these results indicate biochemical differences between mitochondria of different types (free, and bound with nerve endings).

No significant differences were found between the distribution of acetylcholinesterase among the subfractions of the cortex and brain stem. Activity of this enzyme was mainly associated with the subfractions of nerve ending (C). This is in agreement with the findings of De Robertis, obtained on the whole rat brain [6]. The differences discovered in the present experiments between the level of specific ACE activity in the subfractions of nerve endings in the parts of the brain studied are evidence of the importance of cholinergic mechanisms in the brain stem. No other comparative data of this kind relating to metabolism of the subfractions are present in the literature.

The results described above evidently reflect differences in the organization of mitochondrial metabolism in parts of the central nervous system which differ in their structure and functions. They demonstrate the morphochemical heterogeneity of the mitochondria, which is independent of the presence of other components in the fraction of these organelles.

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