BIOCHEMICAL CHARACTERISTICS OF MEMBRANE FRACTIONS FROM VARIOUS TYPES OF CEREBRAL CORTICAL SYNAPTOSOMES

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Functional specificity of light (C) and heavy (D) synaptosomes isolated by centrifugation in a sucrose density gradient as described by De Robertis et al. [9] has been observed by several workers. For instance, under conditions of light deprivation (keeping animals from birth in darkness) more marked depression of Na,K-ATPase and acetylcholinesterase (AChe) activity and a greater disturbance of the intensity of binding of serotonin with receptors were found in the D subfraction from the visual cortex and superior colliculus of rabbits than in the C subfraction, and this was considered to be due to the effect of visual deafferentation on cortical inhibitory synapses [4-6].

Biochemical changes observed in the study of individual synaptosome fractions thus reflect to some extent the principles of synaptogenesis and characteristics of the functioning of different brain nerve endings *in vivo*.

The object of this investigation was to study the distribution of certain enzymes in subfractions of synaptic membranes isolated from C and D separately.

EXPERIMENTAL METHOD

The visual cortex from 42 rabbits was used. C and D were isolated by centrifugation in a sucrose density gradient. They were disintegrated by hyposmotic shock combined with a single freezing [7, 8, 15]. During repeated centrifugation of the disintegrated synaptosomes in a sucrose density gradient (0.4-1.4 M) subsynaptic fractions (layers 1-6) were isolated, of which layers 2-4 consist of fractions of synaptic membranes.

Activity of the following enzymes was determined in the subfractions: AChE and Na,K-ATPase as markers of synaptic membranes and for their investigation; monoamine oxidase (MAO, substrate p-nitrophenylethylamine) as a marker of mitochondria; and lactate dehydrogenase (LDH) as marker of synaptoplasm and for use as an indicator of the degree of disintegration of the synaptosomes.

Enzyme activity and protein content were determined by the following spectrophotometric methods in micromodifications: AChE after Hestrin [10], Na,K-ATPase after Samson and Quinn [13], LDH after Johnson [11], MAO after Gorkin [2], and protein by Lowry's method. Enzyme activity was expressed per gram wet weight of tissue and per milligram protein (specific activity — SA), and the relative specific activity (RSA) also was calculated as the ratio of enzyme activity, in percent, to the distribution of protein in the corresponding fraction, in percent.

EXPERIMENTAL RESULTS

The results of all determinations are given in Table 1.

The protein content in the fractions of synaptic membranes was relatively equal as regards its percentage distribution, namely 13-15% in C and 8-14% in D, and total protein accounted for 43 and 33%, respectively, of all subsynaptic components.

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TABLE 1. Distribution of Protein and Enzyme Activity among C and D Subfractions from Rabbit Visual Cortex (M ± m)

	Subfraction of synaptosomes	Protein		LDH				AChE	
		mg/g tissue	% of distribution	mmoles NADH/g/min ($\times 10^{-2}$)	% of distribution	SA	RSA	μmoles acetylcholine/g tissue/h	% of distribution
C	0	1,31±0,17	44	140±18	88	107±13	2,00	9,21±1,55	36
	1	0,21±0,07	7	6,0±2,0	4	24±4	0,57	1,22±0,29	5
	2	0,44±0,06	15	1,6±0,6	1	18±15	0,07	5,66±0,85	22
	3	0,38±0,04	13	2,7±1,1	2	15±7	0,15	4,49±0,49	18
	4	0,45±0,07	15	3,4±1,6	2	8±4	0,13	3,97±0,48	16
	6	0,22±0,06	7	5,0±2,0	3	9±4	0,43	1,52±0,18	6
Σ		3,01	100	159	100			25,5	100
D	0	0,83±0,12	53	69±14	90	83±11	1,70	5,38±1,23	42
	1	0,13±0,02	8	3,4±0,6	4	26±3	0,50	0,86±0,20	7
	2	0,13±0,02	8	1,2±0,5	2	9±4	0,25	2,45±0,47	19
	3	0,17±0,03	11	1,5±0,4	2	9±5	0,18	2,08±0,40	16
	4	0,22±0,03	14	1,2±0,4	2	5±2	0,14	1,57±0,33	12
	6	0,10±0,02	6	0,5±0,2	1	5±2	0,17	0,44±0,08	3
Σ		1,58	100	76,8	100			12,8	100

AchE		Na, K-ATPase				MAO			
SA	RSA	μmoles P_{inorg}/g tissue/h	% of distribution	SA	RSA	E_{450}/g tissue/h ($\times 10^{-2}$)	% of distribution	SA	RSA
6,46±1,45	0,82								
7,94±1,20	0,71	0,18±0,10	0	3,46±1,79	0	0	0	0	0
14,2±1,08	1,47	13,5±2,50	35	39,6±7,93*	2,33	7±9	10	15±19	0,67
10,6±1,27	1,38	13,6±2,72	35	38,7±5,87*	2,69	13±6	18	33±15	1,38
7,69±1,09	1,07	10,8±2,65	28	25,0±9,58	1,87	27±5	37	59±6	2,47
6,92±1,41	0,33	0,27±0,15	1	5,59±3,82	0,14	26±8	36	117±11*	5,17
		38,4	100			73	100		
6,85±2,40	0,79	0,12±0,12	1	2,07±2,60	0,13	0	0	0	0
6,98±0,98	0,88	5,28±1,15	27	40,6±6,29*	3,38	3±3	8	24±21	0,88
13,6±1,98	2,38	6,49±1,84	33	38,2±8,50*	3,00	7±5	17	29±21	1,55
8,17±1,16	1,45	7,79±1,63	39	35,4±5,59*	2,79	16±4	39	74±16	2,79
5,51±1,16	0,86	0,12±0,09	1	2,70±2,45	0,17	15±5	37	153±45	6,17
6,55±0,21	0,50	19,8	100			41	100		

*Subfraction 5 (partially disintegrated synaptosomes) was not investigated.

Note: The symbol Σ denotes optical density.

LDH activity in fractions of synaptic membranes of both types of synaptosomes was virtually absent. A peak of LDH activity was found in the synaptoplasm fraction (fraction 0).

MAO activity was present in the fractions and RSA of the enzyme increased from the upper layers to the lower. Most MAO activity was located in the fraction of synaptic mitochondria (layer 6), in which RSA was 5.14 and 6.17 in C and D, respectively. Incidentally, the presence of MAO in the membrane fractions was due to the fact that partial liberation of mitochondrial membranes occurred as a result of the technique of freezing and thawing which was used, and these were probably found in layers 2-4 [14].

SA of AChE was somewhat higher in the C fractions when homonymous subfractions were compared. The pattern of the percentage distribution of AChE among subfractions was practically identical in C and D. Maximal SA of AChE was found in the upper membrane layers, and it was lower in the heavier layers.

Of the total AChE activity 56% was found in the synaptic membrane fractions in C and 47% in D. There is evidence that AChE is transported with the flow of axoplasm from the neuron body, and this evidently may explain the presence of the enzyme in fractions of the synap-

toplasm (36% in C and 42% in D). Partial solubilization of AChE in a medium with high ionic strength is also possible [1].

As might be expected, Na,K-ATPase activity was discovered for practical purposes only in the synaptic membrane fractions (99% of total activity of the enzyme). The levels of SA of Na,K-ATPase were practically identical in C and D. The maximum of SA occurred in the synaptic membrane fractions with low sedimentation density.

It is difficult to compare these results with data in the literature because of differences in the techniques used. Membrane fractions were isolated either from the total fraction of synaptosomes isolated on a Ficoll density gradient or directly from fractions of unpurified synaptosomes [8, 12].

Studies involving isolation of different types of synaptosomes demonstrate the cholinergic nature of C [3, 9]. According to our own observations, the C subfractions also are more cholinergic than the D subfractions, but when homonymous subfractions were compared the difference was very small. Similarity of the synaptic membranes of C and D, both layer by layer as reflected in SA values and also by the distribution of the two enzymes in these fractions, is more demonstrative with respect to Na,K-ATPase and to AChE. This fact is noteworthy because we know that metabolic changes (including changes in Na,K-ATPase and AChE activity) have been found to differ quantitatively during functional loading [5, 6].

Synaptic components were isolated separately from C and D from one particular brain region for the first time in the present investigation. As a result of such a biochemical analysis of subsynaptic components it is possible to study the fine morphochemical organization and to investigate its connection with the activity of the CNS in various functional states.

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