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Subcellular distribution of thiamine pyrophosphatase in rat cerebral cortex

The subcellular distribution of thiamine pyrophosphatase in brain has not been reported. In the present note the results of the study of the content of thiamine pyrophosphatase in several fractions of the rat cerebral cortex and particularly in those fractions commonly prepared for the isolation of synaptic structures are presented.

Thiamine pyrophosphatase was determined by a microtechnique based on a method described for histochemical assay[1]. The enzyme incubation was made at 37° for 60 min using 40 μl of buffer substrate and 20 μl of the homogenized fractions The composition of the mixture in final concentration was 55 mM Tris–maleate (pH 7.2), 6.6 mM $MnCl_2$ and 3.6 mM thiamine pyrophosphate (Sigma Chemical Co. St. Louis, Mo.). After incubation, the reaction was stopped by the addition of 30 % (w/v) trichloroacetic acid to achieve 7.5 % (w/v) and the orthophosphate was assayed[2]. A tissue blank, prepared by adding trichloroacetic acid before the addition of the homogenate, was used in each case. The unit of activity was defined as the amount of enzyme which catalyzed the liberation of 1 μmole of P_i per h. Proteins were determined by the method of LOWRY *et al.*[3] with bovine plasma albumin as standard.

Groups of 4–6 Wistar rats (about 120 g body weight) were decapitated and the cerebral cortices were homogenized in 0.32 M sucrose adjusted to pH 7.0 with Tris base (final concentration, 50 μM). When the primary fractions were isolated according to the methods previously described in this laboratory[4,5], most of the thiamine pyrophosphatase was associated with the fractions containing the isolated nerve endings. Furthermore, the above-mentioned methods were modified to obtain in a single pellet most of the nerve endings (see Table I). This fraction was osmotically shocked and subfractionated for the isolation of the nerve-ending membranes[6] and synaptic vesicles[7].

The assay of monoamine oxidase (EC 1.4.3.4)[8] and choline acetyltransferase (EC 2.3.1.6)[9] as markers for mitochondria[10] and intact nerve endings[11] and the electron microscopy of the fractions indicated the concentration of mitochondria and nerve endings in two separated fractions (Fig. 1).

The assay of thiamine pyrophosphatase has shown that it is a particulate enzyme, not associated with the mitochondrial fraction (M) and that most of the activity is found in the nerve ending (NE) and microsomal (MIC) fractions. After osmotic shock of fraction NE, 85 % of thiamine pyrophosphatase appeared with the bulk fraction NE_1 which contained mainly nerve-ending membranes and mitochondria (Table I). By purifying the synaptic vesicles by centrifugation of the supernatant of NE_1 (NE_2 + NE_3) on a sucrose gradient[7], it was found that the thiamine pyrophosphatase present in fraction NE_2 was due to the contaminating membranes.

At variance with the lack of thiamine pyrophosphatase in the synaptic vesicles it is interesting to mention that the regenerating vesicles isolated from the proximal stump of the sciatic nerve above a ligature were rich in thiamine pyrophosphatase[12].

The centrifugation of NE and NE_1 fractions on discontinous sucrose gradients[6] showed that the TPPase was more concentrated in the fractions separated at the interfaces of 0.8–1.0 M sucrose (Table II). The ultrastructural study of these fractions has shown the presence of nerve-ending components and in addition a concentration

TABLE I

PROTEIN AND THIAMINE PYROPHOSPHATASE IN SUBCELLULAR FRACTIONS OF THE RAT CEREBRAL CORTEX

Absolute values per g cerebral cortex for TH: 160.2 ± 14.2 mg for protein and 59.8 ± 15.9 units for thiamine pyrophosphatase. Results are expressed per g fresh tissue in mg for protein and in units for thiamine pyrophosphatase. The percentages recovered (considering 100% the sum of the particulated fractions) for protein and thiamine pyrophosphatase are also included. The relative specific activity represents the ratio between the percentage of thiamine pyrophosphatase and that of protein recovered in each fraction. Results are the mean of 10 experiments for the primary fractions and 4 experiments for the subfractions of NE.

Fraction	*Conditions of centrifugation*	*Ultrastructure*	*Protein*		*Thiamine pyrophosphatase*		*Rel. specific activity*
			mg/g	%	*units/g*	%	
NUC	900 × *g*·10 min, 2 washings	Nuclei, capillaries, myelin	8.3 ± 2.6	9.1	4.9 ± 1.2	11.4	1.25
M	7500 × *g*·20 min, 1 washing	Mitochondria, some nerve endings	36.4 ± 3.8	39.7	6.5 ± 2.5	15.1	0.38
NE	20000 × *g*·30 min, 1 washing	Nerve endings, membranes	30.9 ± 7.9	33.7	18.1 ± 3.9	42.1	1.25
MIC	100000 × *g*·60 min	Microsomes	16.0 ± 3.8	17.5	13.5 ± 4.8	31.4	1.79
SUP		Soluble fraction	31.0 ± 3.6				
Recovery (%)				77		72	
NE_1	20000 × *g*·30 min	Bulk fraction	13.3 ± 0.4	86.4	10.2 ± 2.5	85.0	0.98
NE_2	100000 × *g*·60 min	Synaptic vesicles, membranes	2.1 ± 0.9	13.6	1.8 ± 0.6	15.0	1.10
NE_3		Soluble	4.4 ± 0.5				
Recovery (%)				84		81	

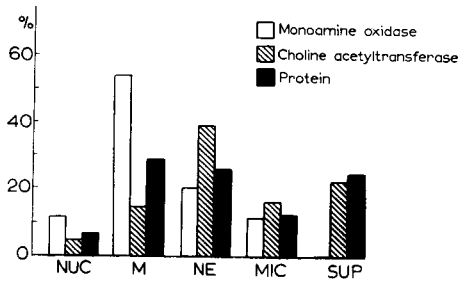

Fig. 1. Histogram of the percentage of monoamine oxidase, choline acetyltransferase and protein in the primary fractions of the rat cerebral cortex, taking as 100% the sum of the fractions.

of tubular structures containing dense material (Fig. 2). The same type of structures has been reported as arising from synaptic structures[13]. Our results indicate that the true nerve-ending membranes are separated in the same fractions, being identified by their size and the presence in most cases of the postsynaptic attachment (see ref. 6), and that these curve elements do not belong to the synaptic region.

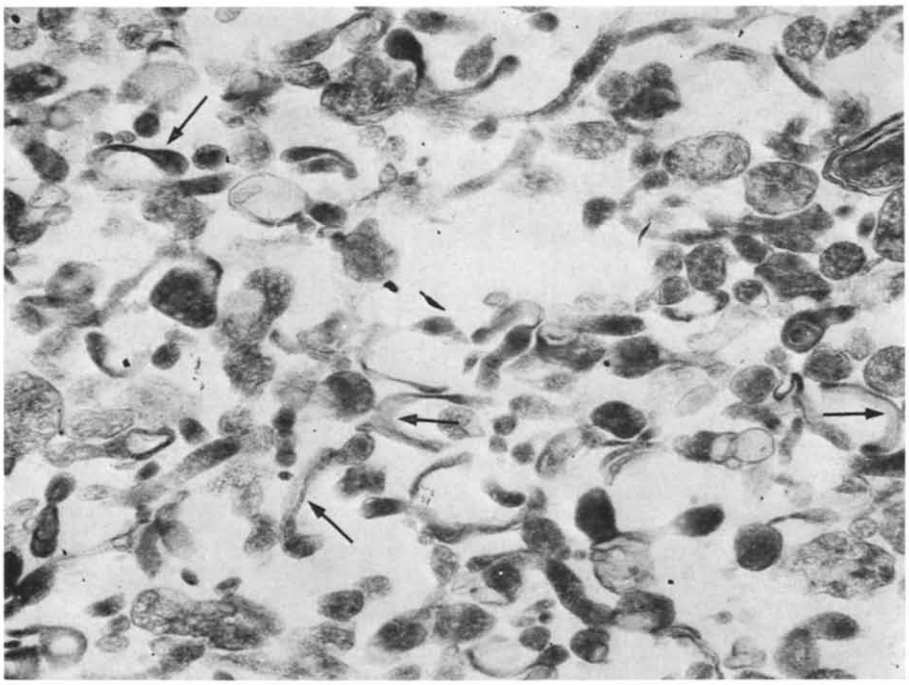

Fig. 2. Electron micrograph of the pelleted subfraction separated from NE at the interface 0.8–0.9 M sucrose fixed in a mixture of glutaraldehyde and paraformaldehyde, post-fixed in osmium tetroxide and embedded in Epon 812. It shows dense tubular structures, vesicles and typical curved auricular-shaped membranes (arrows). 24000 ×.

TABLE II

DISTRIBUTION OF THIAMINE PYROPHOSPHATASE IN SUBCELLULAR FRACTION AFTER GRADIENT CENTRIFUGATION

For the amount of thiamine pyrophosphatase and protein in fractions NE, NE_1 see Table I. In parentheses, the number of experiments.

Sucrose molarity	*Relative specific activity**	
	NE (3)	NE_1 (3)
0.32–0.8	1.72	1.22
0.8 –0.9	2.12	2.22
0.9 –1.0	1.00	2.00
1.0 –1.2	0.59	0.75
Pellet	0.65	0.32

* See Table I.

Histochemical evidence has indicated the presence of thiamine pyrophosphatase in the Golgi complex[14,15]; the fractions in which we found a concentration of thiamine pyrophosphatase have the same specific gravity (1.12–1.14) as the Golgi membranes of other tissues[16,17,12]. Furthermore similar curved membrane fragments have been observed in Golgi material isolated from liver and other cell types[18].

From our results and the above-mentioned arguments we suggest that the membranes accompanying the synaptic structures may be identified as disrupted Golgi-complex membranes of the cell.

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1 S. S. LAZARUS AND B. J. WALLACE, *J. Histochem. Cytochem.*, 12 (1964) 729.
2 O. H. LOWRY AND J. A. LOPEZ, *J. Biol. Chem.*, 162 (1946) 421.
3 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
4 E. DE ROBERTIS, A. PELLEGRINO DE IRALDI, G. RODRIGUEZ DE LORES ARNAIZ AND L. SALGANICOFF, *J. Neurochem.*, 9 (1962) 23.
5 K. KATAOKA AND E. DE ROBERTIS, *J. Pharmacol. Exptl. Therap.*, 156 (1967) 114.
6 G. RODRIGUEZ DE LORES ARNAIZ, M. ALBERICI AND E. DE ROBERTIS, *J. Neurochem.*, 14 (1967) 215.
7 E. G. LAPETINA, E. F. SOTO AND E. DE ROBERTIS, *Biochim. Biophys. Acta*, 135 (1967) 33.
8 R. E. MCCAMAN, N. W. MCCAMAN, J. M. HUNT AND M. S. SMITH, *J. Neurochem.*, 12 (1965) 15.
9 R. E. MCCAMAN AND J. M. HUNT, *J. Neurochem.*, 12 (1965) 253.
10 G. RODRIGUEZ DE LORES ARNAIZ AND E. DE ROBERTIS, *J. Neurochem.*, 9 (1962) 503.
11 E. DE ROBERTIS, G. RODRIGUEZ DE LORES ARNAIZ, L. SALGANICOFF, A. PELLEGRINO DE IRALDI AND L. M. ZIEHER, *J. Neurochem.*, 10 (1963) 225.
12 A. PELLEGRINO DE IRALDI AND G. RODRIGUEZ DE LORES ARNAIZ, *J. Neurochem.*, in the press.
13 A. H. KOEPPEN, K. D. BARRON AND J. BERNSOHN, *Biochim. Biophys. Acta*, 183 (1969) 253.
14 A. B. NOVIKOFF AND S. GOLDFISCHER, *Proc. Natl. Acad. Sci. U.S.*, 47 (1961) 802.
15 S. GOLDFISCHER, E. ESSNER AND A. B. NOVIKOFF, *J. Histochem. Cytochem.*, 12 (1964) 72.
16 C. W. SCHNEIDER AND E. L. KUFF, *Am. J. Anat.*, 94 (1954) 209.
17 B. FLEISHER, S. FLEISHER AND H. OZAWA, *J. Cell Biol.*, 43 (1969) 59.
18 D. J. MORRÉ, R. L. HAMILTON, H. H. MOLLENHAUER, R. W. MAHLEY, W. P. CUNNINGHAM, R. D. CHEETHAM AND V. S. LEQUIRE, *J. Cell Biol.*, 44 (1970) 484.

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