SYNAPTIN, Na^+,K^+ ATPase, LDH, CNPase AND CYTOCHROME-*C* OXIDASE IN ADULT RAT BRAIN SYNAPTIC VESICLE FRACTIONS

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1. Introduction

By using crude rat brain synaptic vesicle fractions as antigen [4], an antiserum against a membrane protein called synaptin (initially designated as antigen C1) has been raised. This protein is enriched in crude synaptic vesicle fractions as compared to whole brain homogenates; it is also present in synaptosomal plasma membrane fractions [5,6] and appears to be absent in primary astroglial cell cultures [6]. Before concluding that brain synaptin is intrinsic to all or to one class of brain synaptic vesicles, it appeared necessary to rule out that the synaptin present in crude brain synaptic vesicle fractions is not intrinsic to membranes contaminating synaptic vesicles, in particular synaptosomal plasma membranes. Here we examine the enrichment of synaptin and the presence of contaminating membranes in synaptic vesicles fractions of different purity by determining the levels of four enzyme markers: Na^+,K^+-activated ouabain-inhibited ATPase (marker of plasma membranes), 2′-3′-cyclic nucleotide monophosphate-phosphodiesterase (CNPase) (marker of myelin and glial cell plasma membranes [15,16]), cytochrome-*c* oxidase (mitochondrial marker) and lactate dehydrogenase (LDH) (cytosol marker).

2. Materials and methods

Highly purified synaptic vesicles (SV) were obtained as in [14] by purification of a crude synaptic vesicle fraction (D fraction) obtained from adult rat forebrain (inbred Wistar strain). Whole forebrain homogenates, SV and D fractions were each separated into 6 aliquots One aliquot was used for protein determination as in [13] by using bovine albumin as standard. Another aliquot, labelled only by an arbitrary code number and by the protein content, was used for synaptin determination in a blind test. The other four aliquots were used for marker enzyme determinations. Na^+,K^+ ATPase (EC 3.6.1.3) was determined by a modification of the radiometric method in [3]. CNPase (EC 3.1.4.16) was measured, after activation with deoxycholate as in [16]. Cytochrome-*c* oxidase (EC 1.9.3.1) was determined by following the oxidation of reduced cytochrome-*c* at 550 nm [9]. LDH (EC 1.1.1.27) was measured by following the oxidation of NADH in the presence of pyruvate at 340 nm [11].

Synaptin was quantitated as in [4–7] by means of crossed immunoelectrophoresis [2,12] of membrane samples solubilized in Berol EMU-043 (MoDo-

Table 1
Synaptic vesicle fractions of different purity: enrichment of synaptin as compared to enrichment of enzyme markers

Experiment number	Whole brain homogenate (μmol substrate utilized/h/mg protein)				Enrichments (ratio of activity in each fraction to activity in corresponding whole brain homogenate)								
	Na^+,K^+ ATPase	CNPase	Cytochrome-*c* oxidase	LDH	Synaptin		Na^+,K^+ ATPase		CNPase		Cytochrome-*c* oxidase		LDH
					D	SV	D	SV	D	SV	D	SV	SV
1	5.43	178	138	188	n.m.	13.4	n.m.	n.d.	n.m.	0.27	n.d.	n.d.	0.004
2	5.84	n.m.	130	105	6.8	12.3	0.27	0.11	n.m.	n.m.	n.d.	n.d.	0.006
3	5.61	222	138	126	13.9	20.3	n.d.	n.d.	0.51	0.31	n.d.	n.d.	0.007
4	5.61	222	140	n.m.	13.0	11.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.m.

Abbrevitions: D, fraction D (enriched in synaptic vesicles); SV, purified synaptic vesicles derived from fraction D; n.d., not detectable; n.m., not measured

Kemi, Sweden). The specificity of the antiserum used in these experiments (code 0276) was identical to that of antisera [1] also raised against rat brain synaptic vesicle fractions as in [4]. In all cases, the antisera were produced and purified as in [10]. As detailed [4–7] the concentration of synaptin is expressed in arbitrary units (a.u.). Since 1 a.u. is, by definition, the amount of synaptin per gram of protein in the whole forebrain homogenate, the no. a.u./g protein of each fraction is a direct indication of the enrichment of synaptin in the fraction relative to the synaptin content of the whole forebrain homogenate. Lower limit of detection was, in the present set-up, 0.05 a.u. The analytical standard deviation coefficient was 10.4%.

3. Results and discussion

3.1. *Synaptin in synaptic vesicles*

In agreement with [5] the crude synaptic vesicle fractions (D fraction) are highly enriched in synaptic (table 1). Purification of synaptic vesicles contained in D fractions yielded the SV fractions which, on the average, are even more enriched in synaptin (table 1). SV fraction does not contain cytosol proteins as shown by the virtual absence of LDH, and both D and SV fractions contain negligible or undetectable amounts of Na^+,K^+ ATPase and CNPase and no cytochrome-*c* oxidase, thus the synaptin in the synaptic vesicle fractions is not due to contamination of SV by myelin, mitochondrial membranes, synaptosomal and other plasma membranes. The absence, in SV fractions, of putative enzyme markers of SER and Golgi membrane [14] also rules out these membranes as possible contaminants. However it is possible that a SER network, similar to that shown in the nerve terminals of chicken ciliary ganglion neurons [8], exists in rat forebrain nerve terminals, and that such a specialized structure might specifically lack the enzyme markers used. Fragments of this terminal reticulum vesiculated during the osmotic shock treatment and homogenization of the synaptosomal fractions might be present in our SV fractions and be undetected by electron microscopy [14] and enzyme markers. However the high enrichment of synaptin in SV fraction is not compatible with a unique localization of antigen in these hypothetical contaminating membranes. In addition synaptin is also present in another neurotransmitter storing organelle: the adrenal gland chromaffin granule [7].

We can conclude then that synaptin is intrinsic to synaptic vesicles, but we cannot exclude that it is also present in terminal reticulum membranes from which synaptic vesicles appear to derive [8].

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