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SYNAPTIN IN ACETYLCHOLINE- AND CATECHOLAMINE-CONTAINING SYNAPTIC VESICLE FRACTIONS FROM BRAIN CORTEX

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Summary

The nervous system specific membrane protein, synaptin, was determined in acetylcholine- and catecholamine-storing synaptic vesicles isolated from guinea-pig cerebral cortex. Synaptin was enriched more than 10-fold in both types of vesicle preparation. The enrichment was independent of the nature of the transmitter.

Introduction

By immunization with a crude rat brain synaptic vesicle fraction as immunogen, an antiserum against a membrane protein called synaptin (initially designated as antigen C1) has been raised. By quantitative immunoelectrophoresis it was shown that this protein was enriched more than 10-fold in crude synaptic vesicle fractions as compared to whole brain homogenates; it was also present in synaptosomal plasma membrane fractions [1—3]. Synaptin has also been demonstrated in fractions of chromaffin granule membranes isolated from bovine adrenal medulla [4]. The regional distribution of synaptin in the central nervous system of the rat has been determined, and the protein was shown to be present in all areas studied [5]. No correlation was found to known and putative neurotransmitters, neurotransmitter-synthesizing enzymes, or neurotransmitter receptors. The function of synaptin has not been clarified, but it has been hypothesized that the protein may be involved in the exo/endocytosis of the synaptic vesicles [4,5].

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It has been realised that the synaptic vesicle fractions isolated from the brain by the methods currently in use are not only rich in acetylcholine but contain other putative transmitters such as noradrenalin, dopamine and 5-hydroxytryptamine [6—9]. Recently, a method has been reported which gives a reasonably good separation of acetylcholine- and catecholamine-storing vesicles from mammalian cerebral cortex [10]. On the basis of the transmitter content the two vesicle fractions were about 10—15% contaminated with each other, probably less. The present study was undertaken in order to determine whether the high enrichment of synaptin in brain synaptic vesicle fractions could be attributed to either cholinergic or adrenergic vesicle populations.

Methods and Materials

The synaptic vesicles were isolated from guinea-pig (R9, 3—4-weeks old) cerebral cortex and purified using a continuous density gradient separation in a swinging-bucket rotor (Beckman SW 25.2), utilizing a ^2H_2O-H_2O (1 : 1)-sucrose gradient ranging from 0.2 to 0.8 M, and controlled pore glass chromatography as previously described [10]. The 12 000 × g supernatant of the osmotically disrupted crude synaptosomal fraction is referred to as W_S; the synaptic vesicle fractions with high acetylcholine content and with high catecholamine content, respectively, are referred to as V_{ACh} and V_{CA}.

On each sample determination of protein, transmitters, marker enzymes and synaptin was performed. Protein was determined by using the method of Lowry et al. [11] and the method of Peterson [12]. Synaptin was quantified by crossed immunoelectrophoresis as previously described [4,5,13,14]. The starting cortical homogenate was used as reference. The concentration of synaptin was expressed in arbitrary units: 1 unit being the amount of synaptin per g protein in the reference. Acetylcholine was determined by a bioassay as modified by Whittaker et al. [15] using small strips of the dorsal muscle of the leech [16]. Catecholamines were determined using the method of Coyle and Henry [17]. The following marker enzymes were determined: lactate dehydrogenase (EC 1.1.1.27) [18]; acetylcholinesterase (EC 3.1.1.7) [19]; NADPH cytochrome c reductase (EC 1.6.2.4) [20]; p-nitrophenylphosphatase (EC 3.1.3.2) [21]; fumarate hydratase (EC 4.2.1.2) [22]; ATPase (EC 3.6.1.3) [23].

Special reagents were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.)

Results

Synaptin concentration, transmitter content and marker enzyme activities in the synaptic vesicle fractions of different purity are shown in Table I. The enrichment of acetylcholine relative to the starting material (W_s) was 3.1 for density gradient V_{ACh} and 14.5 for controlled pore glass chromatography V_{ACh}. The enrichment for catecholamine concentration relative to W_s was 4.5 for density gradient V_{CA} and 13.8 for controlled pore glass chromatography. These enrichments are in accordance with previously reported results [10]. It can also be seen that the two vesicle populations are clearly separated. Synaptin

TABLE I

TRANSMITTER CONTENT, MARKER ENZYME ACTIVITIES AND SYNAPTIN CONTENT IN SYNAPTIC VESICLE FRACTIONS WITH DIFFERENT PURITY

1 unit of synaptin is by definition the amount of synaptin per g protein in whole brain cortex homogenate. The data of columns 3 to 7 show the enrichments of synaptin, marker enzyme activities and transmitter concentrations in each fraction as compared to the whole brain cortex homogenate. The data are the mean of four experiments ± S.D. n.d., non-detectable.

Component	Brain cortex homogenate ($\mu mol \cdot mg^{-1} \cdot h^{-1}$) *	Differential centrifugation W_s	Density gradient centrifugation		Controlled pore glass chromatography	
			V_{ACh}	V_{CA}	V_{ACh}	V_{CA}
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Lactate dehydrogenase	33.6 ± 5.6	1.2 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	0.1 ± 0	n.d.
NADPH cytochrome c reductase	150.6 ± 22.6	0.9 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.1 ± 0	0.1 ± 0
Acetylcholinesterase	2.8 ± 0.3	1.2 ± 0.2	0.2 ± 0	0.6 ± 0.2	n.d.	0.1 ± 0
Fumarate hydratase	4.5 ± 0.8	0.9 ± 0.1	0.3 ± 0	0.1 ± 0	n.d.	n.d.
p-Nitrophenylphosphatase	0.7 ± 0.1	1.3 ± 0.2	0.3 ± 0.1	0.6 ± 0.1	n.d.	n.d.
Ouabain sensitive ($Na^+ + K^+$)-ATPase	71.4 ± 11.4	1.0 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	n.d.	0.1 ± 0
Acetylcholine	$\frac{pmol \cdot mg^{-1}}{108.2 \pm 26.2}$	1.8 ± 0.4	5.5 ± 0.7	0.9 ± 0.1	26.1 ± 6.4	0.2 ± 0.1
Catecholamine	13.2 ± 3.8	1.5 ± 0.4	1.1 ± 0.2	6.7 ± 2.5	0.7 ± 0.3	20.7 ± 4.8
Synaptin	1.0	1.4 ± 0.2	7.6 ± 0.7	7.1 ± 0.6	11.3 ± 1.5	13.0 ± 1.2

* Except fumarate hydratase ($\Delta Ext \cdot mg^{-1} \cdot h^{-1}$) (Ext, extinction).

was enriched approx. 7-fold in vesicles prepared by density gradient centrifugation and 11—13-fold in vesicles prepared by controlled pore glass chromatography relative to brain cortex homogenate. No statistically significant difference between V_{ACh} and V_{CA} with regard to synaptin was found.

Discussion

The demonstrated enrichments of synaptin in the various synaptic vesicle fractions in the present study correlate closely with previously reported enrichments in brain synaptic vesicle fractions [1—3]. The present data indicate that the synaptin concentration is uncorrelated to the nature of transmitter, acetylcholine or catecholamine, respectively, since similar concentrations of synaptin could be demonstrated in the two types of vesicle. The synaptin enrichment was relatively smaller than the transmitter enrichments in the vesicle fractions prepared by controlled pore glass chromatography. However, synaptin is not restricted to synaptic vesicle membranes, but is also present on synaptosomal plasma membranes, enriched approx. 4-fold when compared to whole brain homogenate [1]. This probably explains the relatively small synaptin enrichment in vesicle fractions. The concentration of synaptin in controlled pore glass chromatography V_{CA} (13 units) was more than 7-fold higher than the concentration previously reported for synaptin in chromaffin granule membranes (1.7 units). This difference cannot alone be attributed to difference in size between the two types of organelle, since Fried and Bock could only demonstrate trace amounts (0.1 unit) of synaptin in small noradrenergic vesicles isolated from the vas deferens of castrated rats (Fried, G. and Bock, E., unpublished data). Therefore, even though synaptin has been demonstrated on the membrane of transmitter-storing organelles of the peripheral nervous system, the protein seems to be preferentially enriched in transmitter-storing synaptic vesicles isolated from the central nervous system, the enrichment being independent of the nature of the transmitter.

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