RAT BRAIN SYNAPTIC VESICLES AND SYNAPTIC PLASMA MEMBRANES COMPARED BY CROSSED IMMUNOELECTROPHORESIS

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1. Introduction

A number of studies have suggested that during the synaptic transmission synaptic vesicles of the nerve terminal fuse with the presynaptic membrane and release their neurotransmitter content into the synaptic cleft [1]. After the physiological response has been elicited, the neurotransmitters are either inactivated by degradation or by reuptake [2], but little is known about the fate of the vesicular membrane material.

Based on electron microscopy of frog neuromuscular synapses treated with horseradish peroxidase, Heuser and Reese [3] have postulated that the synaptic vesicle membrane was added to the nerve terminal surface during exocytosis and then retrieved by means of coated vesicles via an endocytotic process, distant from the synaptic cleft. The coated vesicles formed at that site, were then recycled into new synaptic vesicles by way of the intermediate cisternae. Jørgensen and Mellerup [4] have shown that intracerebrally injected bovine serum albumin could be incorporated into a preparation of rat brain synaptic vesicles by an endocytotic process. A prerequisite for endocytotic models should be the presence of vesicular membrane material in the synaptic plasma membrane, and in order to detect this it is necessary to compare the composition of synaptic plasma membranes with that of the synaptic vesicles.

In this investigation we have compared by means of crossed immunoelectrophoresis synaptic vesicles of rat brain with preparations of synaptic plasma membranes. The synaptic vesicle membrane antigen Cl was also found in the synaptic plasma membrane which tends to confirm the exocytosis theory. On the other hand the synaptic plasma membrane antigens D1, D2 and D3 were not found in our synaptic vesicle preparation, suggesting that a reversal of this process, i.e. endocytosis, if it occurs, is mediated by a highly selective process.

2. Materials and methods

For the experiments we used the forebrains of 35-day-day-old female Wistar rats. The brains were homogenized in 0.32 M sucrose containing 1 mM phosphate and 0.1 mM EDTA pH 7.4 (hom) and subsequently subfractionated according to sedimentation rates in a discontinuous density gradient be a method essentially similar to that described by Morgan et al. [5] and by Whittaker et al. [6]. The synaptosomes, recovered at the 9/16% Ficoll interface layer of the density gradient, were aspirated and then subjected to osmotic shock. After a further density gradient separation of the osmolyzed synaptosomes, purified synaptic vesicles (ves) were obtained from the 0.2/0.4 M sucrose interface, the synaptic plasma membranes (spm) from the 0.8/1.0 M sucrose interface. The fractions were solubilized in a medium consisting of 2% v/v Triton X-100, 1 mM EDTA, 10 mM phosphate and 100.000 U/l Aprotinin = Trasylol (Bayer, Germany), pH 8.5, before the immunoelectrophoretic analysis. Electronmicroscopical examination of the ves and the spm fractions was performed as previously described [7].

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The polyspecific antibodies used for analysis were prepared from rabbits injected with either spm (anti syn-mem 0374) or ves (anti ves 1173) [7–9]. The ves antigen C1 and the spm antigens D1, D2 and D3 were quantified by crossed immunoelectrophoresis [10] with the above mentioned antibodies.

3. Results and discussion

Fig.1 demonstrates the immunoelectrophoretic patterns of ves and spm which are obtained using either anti syn-mem 0374 or anti ves 1173. As shown, ves was devoid of the spm antigens D1, D2 and D3, but contained a high concentration of the ves antigen C1 as shown by a large enclosed precipitation area. In contrast both the antigens D1, D2, D3 and the antigen C1 were present in spm. In table 1 we have given the quantitative results on the antigen content in the ves and spm fractions expressed as specific concentration in arbitrary units, calculated relative to protein concentration with hom taken as equal to one. Ves was free of the spm antigens D1, D2 and D3 although these antigens were enriched in spm compared to hom. The anitgen C1 was extremely enriched in the ves fraction compared to hom but it was also present in spm in rather large amounts. The presence of C1 in spm may represent a high degree of contamination of spm because of incomplete hypoosmotic release of vesicles. On the other hand this finding may indicate that C1 represents an integral constituent of spm, as suggested by Heuser and Reese [3].

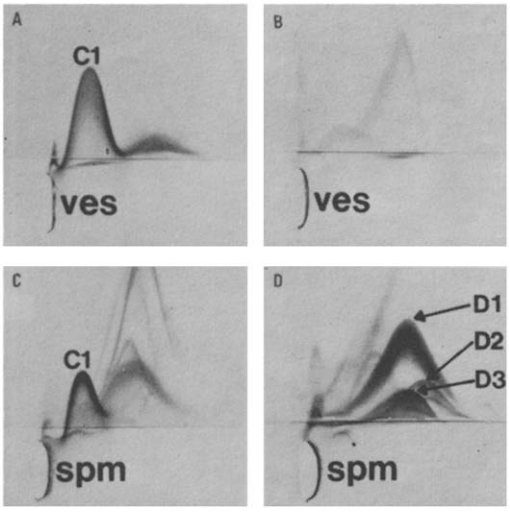

Fig.1. Extracts of rat brain synaptic vesicles (ves) and of synaptic plasma membrane (spm) subjected to crossed immunoelectrophoresis. In part A and C the antibodies were anti ves 1173 (8 μl/cm^2), in part B and D the antibodies were anti syn-mem 0374 (8 μl/cm^2). The amount of protein in the ves and spm extracts were 25 μg in A and B, 60 μg in C and D. It is seen, that the antigens D1, D2 and D3 were present only in spm, but C1 was present both in ves and spm.

Table 1
Specific activities of four antigens in different rat brain fractions calculated relative to protein concentration with hom arbitrary set to 1.0

Antigen	hom	ves	spm
Cl	1.0	13.3	2.9
D1	1.0	ND	3.8
D2	1.0	ND	2.7
D3	1.0	ND	4.1

The strong enrichment of antigen C1 in ves, and the moderate enrichment of D1, D2 and D3 in spm can be seen. ND: not detectable.

Morgan et al [11] have investigated ves and spm fractions by SDS-polyacrylamide gelelectrophoresis and found a number of proteins and glyco-proteins common to both structures, but the protein profile of ves was simpler than the profile of spm, supporting a mosaic model of spm. Also the lack of gangliosides in ves compared to spm adds further support for the differences in composition between the two membranes [12].

Our results show that the protein composition of ves differs in a specific manner with respect to spm, and therefore tend to support the exocytosis model. These data do not allow us to choose between the two alternative possibilities, namely whether there occurs an immediate retrieval of the vesicles, or whether an endocytosis from the synaptosomal plasma membrane occurs distant to the place of exocytosis. Although we have found the C1 antigen in the spm fraction, we cannot completely rule out a simple contamination of the plasma membrane material with vesicular constituents. We can conclude, however, that the endocytosis in any case must be a highly selective process, since the vesicular preparations did

not contain three brain specific plasma membrane proteins.

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