PURIFICATION OF PLASMA MEMBRANES FROM IMMATURE BRAIN

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1. Introduction

The uniqueness of excitable membranes has attracted a number of scientists to attempt their purification and characterization. This has been successful from the eel electroplax [1] and the lobster nerve fibers [2, 3]. From brain tissue, isolation of plasma membranes has met with serious difficulties due to the abundance of non excitable membranes present. In addition to synaptosomal plasma membranes [4, 5] methods have been devised for the isolation of perikaryal plasma membrane from dissociated brain cells [6, 7]. The dissociation of cells requires trypsinization, which may release important surface components.

This paper describes a procedure for the isolation of perikaryal plasma membranes from solid brain tissue by homogenization. Immature brain is used to minimize contamination with non-neuronal plasma membranes [8]. Plasma membrane markers indicate a purity comparable to the best preparations of synaptosomal plasma membranes, although the present procedure is much simpler.

2. Materials and methods

2.1. Isolation of plasma membranes

Cortices of 8 to 12 rats, 4 to 6 days-old, were washed with ice-cold 0.1 M Tris buffer, pH 7.5, for 5 min and then teased apart on ice with two forceps in 20 ml of the same buffer. After 5 min, 20 ml of 1.2 M sucrose was added and the cortices were homogenized with 12 strokes of a loosely fitting Teflon-glass homogenizer. The homogenate was centrifuged at 4000 *g* for 10 min. The supernatant was diluted with 1 vol of water and centrifuged at 25000 *g* for 20 min. The pellet was resuspended in 25 ml of 1.0 M sucrose and centrifuged in a Spinco SW-25 rotor at 63000 *g* for 60 min. The top layer (about 10 ml) was collected with a Pasteur pipette and diluted with 3 vol of water. The suspension was centrifuged at 25000 *g* for 20 min. The pellet was suspended in 20 ml of water and layered on top of 8 ml of 0.85 M sucrose followed by centrifugation in SW-25 rotor at 63000 *g* for 60 min. The interphase was collected, diluted with water and centrifuged at 40000 *g* for 15 min. The membrane pellet was stored in 0.25 M sucrose at −20°C.

2.2. Assays and determinations

Mg^{2+}-dependent ATPase and Na^+–K^+-activated ATPase were assayed as described [6]. Ouabain was used at 0.1 mM, and a detergent, Lubrol at 0.2% final concentration. 5'-nucleotidase was assayed according to Heppel and Hilmoe [9]. The activity of succinate dehydrogenase was determined to estimate mitochondrial contamination [10] and that of rotenone-insensitive NADH-cytochrome *c* reductase for microsomal contamination [11]. Rotenone was used at 5 μM concentration. Ca^{2+}-binding was determined by the Millipore filtration technique as described by Shlatz and Marinetti [12]. Electron microscopy was performed as described by Sabatini et al. [13].

3. Results

Plasma membranes were isolated from immature rat brain with 0.2% recovery as compared to the total

protein. Electron microscopy (fig. 1) showed that the sample consisted exclusively of membrane structures. Myelin was not detected in the preparation, which is in agreement with previous findings that myelination occurs in rat cortex after the second post-natal week [14].

Na^+-K^+-activated ATPase is a well-established plasma membrane marker in brain as well as in other tissues [7]. The specific activity of the enzyme was 9.2-fold higher in plasma membranes than in the homogenate (table 1). A high concentration of Na^+-K^+-activated ATPase in plasma membranes was also measured when the assay was performed in the presence of a detergent, Lubrol. Analogously ouabain-sensitive ATPase was concentrated in plasma membranes 9.5-fold as compared to the homogenate.

5′-nucleotidase is a reliable marker of liver plasma membranes, but it has not been concentrated in brain plasma membrane fractions to the same extent as Na^+-K^+-activated ATPase [7]. The present preparation confirmed these data (table 2). 5′-nucleotidase was only 2.3-fold concentrated in the plasma mem-

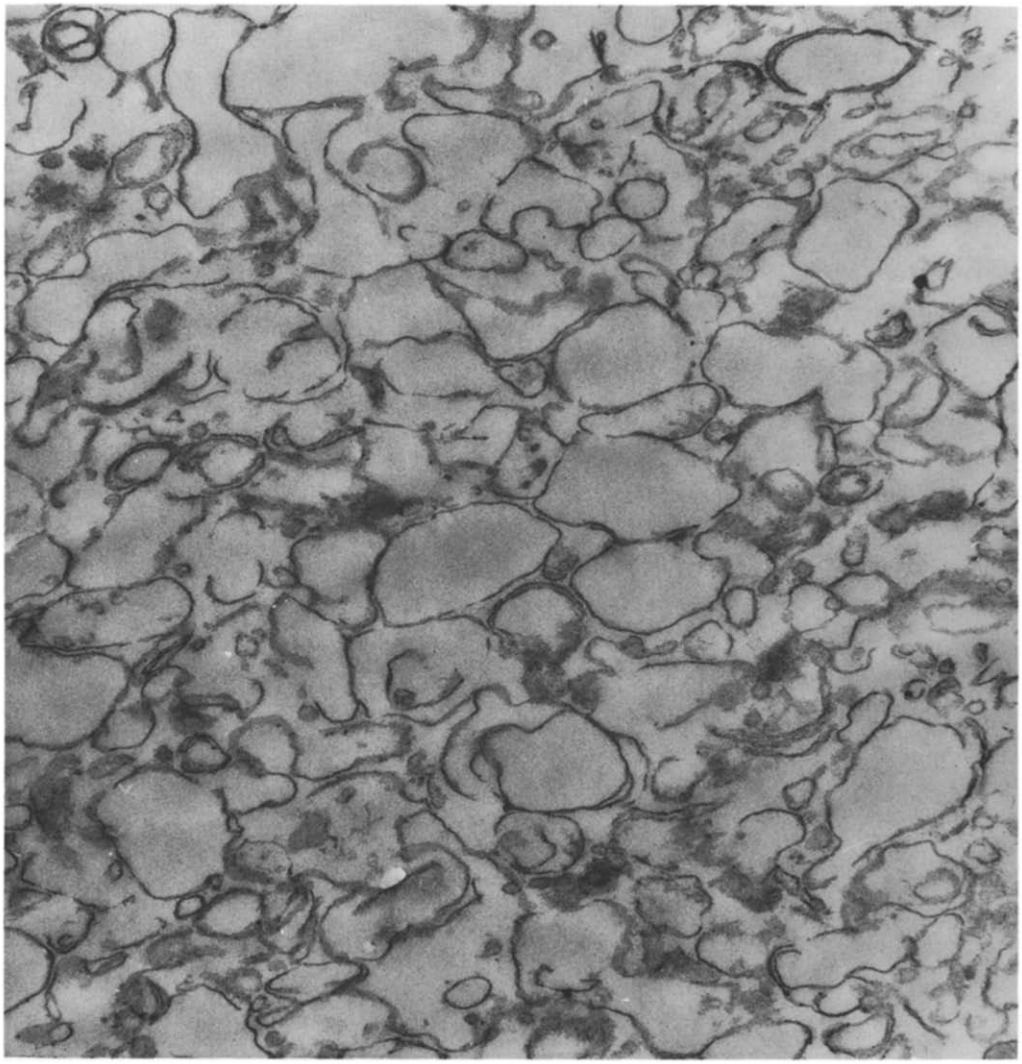

Fig. 1. An electron micrograph of plasma membranes isolated from immature rat brain, × 63 000 (reduced by 20%).

Table 1
ATPase activities of brain plasma membranes.

Enzyme	Enzyme activity (nmoles/min/mg protein)		Ratio
	A Homogenate	B Plasma membranes	B/A
Mg-ATPase	131 ± 13	246 ± 21	1.9
Na, K-ATPase	42.7 ± 6.3	394 ± 40	9.2
Ouabain-sensitive ATPase	38.9 ± 5.9	369 ± 44	9.5
Mg-ATPase + 0.2% Lubrol	105 ± 16.3	143 ± 21	1.4
Na, K-ATPase + 0.2% Lubrol	75.0 ± 18.5	465 ± 78	6.2

Each figure represents means ± SE of 5 to 10 determinations.

Table 2
Activities of marker enzymes in brain plasma membranes.

Enzyme	Enzyme activity (nmoles/min/mg protein)		Ratio
	A Homogenate	B Plasma membranes	B/A
5'-nucleotidase	31.2 ± 8.1	73 ± 11	2.3
Succinate dehydrogenase	482 ± 36	209 ± 37	0.43
Rotenone-insensitive NADH-cytochrome *c* reductase	238 ± 33	41.5 ± 2.3	0.17

Each figure represents means ± SE of 4 to 10 determinations. The activity of succinate dehydrogenase in a purified mitochondrial fraction was 2875 nmoles/min/mg protein and that of rotenone-insensitive NADH-cytochrome *c* reductase in a microsomal fraction 664 nmoles/min/mg protein.

brane fraction. Contamination of the preparation with other membranous structures was estimated by assaying for mitochrondrial and microsomal marker enzymes (table 2). These data indicated that plasma membranes may be contaminated with mitochondria and microsomes by about 6% each. This is however an upper estimate, since it is difficult to prepare pure reference fractions from immature brain [15].

Calcium ions are thought to be bound to plasma membranes and they are supposed to be involved in the excitation process [16]. The present plasma membranes bound calcium twice as much as the brain homogenate (table 3). The binding was not increased if the membranes were washed with 2 mM EDTA, whereby they differ from liver plasma membranes [12]. Otherwise, the extent of binding in brain and liver plasma membranes appears similar.

Table 3
Binding of calcium to plasma membranes.

	Amount of Ca^{2+} bound (nmoles/mg protein)		Ratio
	A Homogenate	B Plasma membranes	B/A
No EDTA	16.1 (1.0)	32.4 (2.8)	2.0
Fractions washed with 2 mM EDTA	8.8 (0.7)	24.1 (1.2)	2.7

Each figure represents the means (ranges) of 2 experiments. Samples were incubated at 37°C in 0.1 M Tris buffer, pH 7.5, containing 1 mM $CaCl_2$ and 0.5 μCi $^{45}CaCl_2$ for 10 min. The binding was determined by collecting the samples on a membrane filter under a vacuum [12].

4. Discussion

Immature rat cortex is suitable material for the isolation of neuronal enriched plasma membranes, because the tissue contains few glial cells and little myelin [14, 17, 18]. Although the exact number of spongioblasts in immature rat brain has not been established, histological follow-up studies [17] and data on cell multiplication [18] indicate that the cells are predominantly developing neurons, which are forming their connections to other neurons. The presented procedure, which for the first time describes isolation of plasma membranes from immature solid brain tissue, may be applied in studies of the plastic membrane changes occuring during the development of the brain. The purification may be completed in 5 hr with a yield of 0.2% on a protein basis. It is thus simpler and as efficient as the procedure used to isolate highly purified synaptic plasma membranes [4].

The preparation is characterized by over 9-fold concentration of Na^+–K^+-activated ATPase and ouabain-sensitive ATPase. On this basis the degree of purity is comparable to the best preparations of synap-

tic plasma membranes [4, 5]. 5′-nucleotidase is moderately concentrated in plasma membranes. The fraction appears to be contaminated with mitochondria and microsomes maximally to 6%, as determined with marker enzymes. No contamination with myelin is detected by electron microscopy. The membranes bind calcium to the same extent as liver plasma membranes, treated with EDTA [12], and twice as much as the brain homogenate.

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