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COMPOSITION AND SYNTHESIS OF PLASMA MEMBRANES AND SMOOTH ENDOPLASMIC RETICULUM IN ISOLATED CELLS OF NEWBORN RAT BRAIN

K. HEMMINKI

Department of Medical Chemistry, University of Helsinki, Siltavuorenpenger 10 A, SF-00170, Helsinki 17 (Finland)

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SUMMARY

Plasma membranes and smooth endoplasmic reticulum were isolated from a suspension of cells prepared from newborn rat brain. The patterns of proteins and glycoproteins of the membrane fractions were studied by sodium dodecyl sulphate gel electrophoresis. Phospholipid classes were analyzed by thin-layer chromatography. Biosynthesis of membrane proteins was studied by incubating the cells with labelled leucine *in vitro*.

1. Sodium dodecyl sulphate gel electrophoresis revealed three major polypeptides in plasma membranes corresponding to 45000, 50000 and 58000 daltons. Smooth endoplasmic reticulum had prominent polypeptides of 45000 and 53000 daltons.

2. Plasma membranes were enriched in Schiff-positive material as compared to smooth endoplasmic reticulum. Both fractions contained lipid-extractable material of low molecular weight in sodium dodecyl sulphate gels, while only plasma membranes showed glycoprotein bands ranging in apparent molecular weights from 40000 to over 100000.

3. Both membrane types contained phosphatidylcholine as their major phospholipid. Plasma membranes were enriched in phosphatidylcholine and phosphatidylethanolamine while smooth endoplasmic reticulum showed higher concentration of phosphatidylserine. No sphingomyelin was detected in plasma membranes.

4. Incorporation of [^{3}H]leucine into plasma membranes and smooth endoplasmic reticulum was only 6 and 14%, respectively, of the radioactivity in the cell homogenate in a 60-min incubation. A 2-h chase with nonradioactive leucine increased the specific radioactivity of proteins in plasma membranes while decreased the labelling of smooth endoplasmic reticulum.

INTRODUCTION

The maintenance of highly specific cell-to-cell contacts in the central nervous system requires cooperation of a number of specialized membrane structures. Two kinds of membranes, myelin and synaptosomal plasma membranes, involved in the

integrative processes of the brain, have been isolated and characterized[1–5]. By contrast, the properties and functions of cellular plasma membranes are poorly known in the brain as suitable isolation methods have been lacking.

Recently we have described a procedure for the isolation of plasma membranes from a suspension of immature brain cells[6]. In this paper the plasma membrane fraction is characterized by protein, glycoprotein and phospholipid composition and compared to smooth endoplasmic reticulum of immature cells. Additionally, data are presented on synthesis of membrane proteins in isolated cells.

MATERIALS AND METHODS

Isolation and labelling of cells

Cells were prepared from cerebral cortices of 4–5-day-old rats as described previously[7]. Dissociated cells were washed twice with Eagle's minimal essential medium by centrifuging at $300 \times g$ for 10 min and suspended in "fortified minimal essential medium" containing leucine-free minimal essential medium, 200 mM *N*-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid, 100 mM sodium phosphate (pH 7.5) (8:1:1, by vol.), penicillin, 100 units/ml and streptomycin, 50 μg/ml. L-[4,5-$^3H_2$]Leucine, 52 Ci/mmole, was added (10 μCi/ml) and incubations were performed at 37 °C under air. Incubations were terminated by the addition of 5 vol. of cold 0.16 M NaCl followed by centrifugation at $300 \times g$ for 5 min.

Isolation of membrane fractions

Newly dispersed, nonradioactive or labelled cells were homogenized in a hypotonic medium, and plasma membranes[6] and smooth endoplasmic reticulum[8] were isolated according to a recently described procedure. The membrane fractions isolated were centrifuged to pellets and suspended in 0.25 M sucrose for further analysis.

Protein was measured according to Lowry *et al.*[9]. For the determination of radioactivity the samples were washed three times with cold 12% trichloroacetic acid heating once to 90 °C for 15 min, once with ethanol, and dissolved in Soluene-100 and toluol PPO–POPOP. Liquid scintillation counting was performed at about 20% efficiency.

Gel electrophoresis

Polyacrylamide gel electrophoresis was performed in sodium dodecyl sulphate according to Weber and Osborn[10]. 200 μg of membrane protein dissolved in 1% sodium dodecyl sulphate were separated on 15 cm × 0.5 cm gel columns at pH 7.9 and stained for protein in 0.5% amido black. By contrast, 700 μg of protein were applied on 7 cm × 0.5 cm gel columns to detect carbohydrates by Schiff staining[11].

Separation and quantitation of phospholipids

Phospholipids were extracted by the system of Folch *et al.*[12] and individual phospholipid classes were separated by two-dimensional thin-layer chromatography on silica gel H plates. The first dimension was developed in chloroform–methanol–7 M ammonia (120:70:11, by vol.). The plates were dried at 120 °C for 15 min and the second dimension was run in chloroform–methanol–acetic acid–water (200:60:

12:4, by vol.). The phospholipid classes were detected with iodine vapor and assayed for phosphorus as described by Kankare and Suovaniemi[13].

RESULTS

The protein compositions of plasma membranes and smooth endoplasmic reticulum isolated from immature neurons are shown in Fig. 1. Plasma membranes appear to have three major polypeptides corresponding to molecular weights 45000, 50000 and 58000 as determined with marker proteins. Smooth endoplasmic reticulum has two prominent protein bands in this molecular weight region as well, but appears to have a different set of proteins in the high molecular weight region.

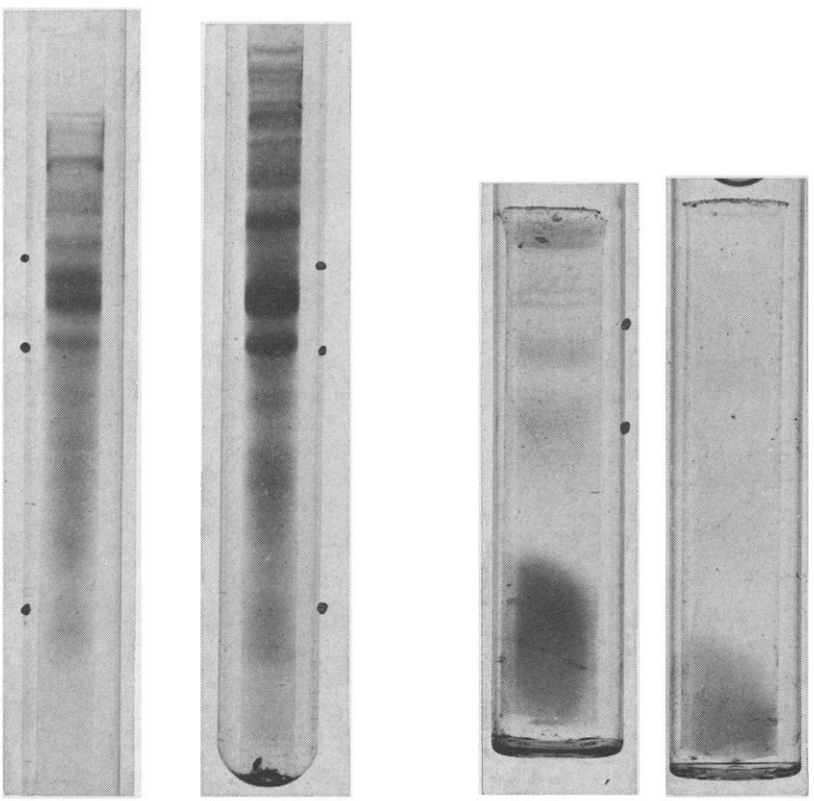

Fig. 1. Sodium dodecyl sulphate gels of plasma membranes (left) and smooth endoplasmic reticulum (right) isolated from immature neurons. The membrane fractions were dissolved in sodium dodecyl sulphate, separated on 7% polyacrylamide gels and stained with amido black. The migration front is at the bottom. The dots indicate the positions of marker proteins run on parallel gels: bovine serum albumin (68000 daltons), ovalbumin (43000 daltons) and lysozyme (17000 daltons).

Fig. 2. Sodium dodecyl sulphate gels of plasma membranes (left) and smooth endoplasmic reticulum (right) stained for carbohydrate by Schiff reaction. The migration front is at the bottom. The dots indicate the positions of marker glycoproteins run on parallel gels: transferrin (82000 daltons) and phosvitin (40000 daltons).

Schiff staining was used to expose carbohydrates in gels containing plasma membranes and smooth endoplasmic reticulum (Fig. 2). As equal amounts of protein were used for the gels the quantitative differences of carbohydrate-containing material between the fractions are striking. The amount of glycoprotein is small in smooth endoplasmic reticulum, while the gel containing plasma membranes shows several Schiff-positive bands in the 40000, 60000 and 100000 dalton regions. The heavily stained zone migrating close to the buffer front and appearing both in plasma membrane and smooth endoplasmic reticulum gels varies in intensity in relation to the lipid content of the fraction and can be completely extracted with lipid solvents. Hence, it contains lipids and glycolipids rather than glycoproteins[11].

The contents of phospholipid in subcellular fractions of immature neurons have been previously published[6]. The protein/phospholipid ratios for plasma membranes and smooth endoplasmic reticulum were 1.3 and 1.9, respectively, as compared to 3.3 for the homogenate. In Table I the proportions of phospholipid classes in these membrane fractions are shown. Phosphatidylcholine is the major phospholipid in all fractions studied comprising 1/2 of all phospholipids. Phosphatidylethanolamine makes 1/3, phosphatidylserine, phosphatidylinositol and sphingomyelin generally 5–10% of the total phospholipid of immature brain cells. Plasma membranes differ from smooth endoplasmic reticulum by their higher concentration of phosphatidylcholine and lower concentration of phosphatidylserine and sphingomyelin.

TABLE I

COMPOSITION OF PHOSPHOLIPIDS IN IMMATURE NEURONS, THEIR PLASMA MEMBRANES AND SMOOTH ENDOPLASMIC RETICULUM

Each figure represents the means ± S.E. of 3 independent determinations.

	Cell homogenate (%)	*Plasma membranes* (%)	*Smooth endoplasmic reticulum* (%)
Total phospholipids	100	100	100
Phosphatidylcholine	48.3 ± 6.0	53.5 ± 3.4	47.6 ± 3.1
Phosphatidylethanolamine	31.1 ± 1.2	33.6 ± 0.6	31.0 ± 1.4
Phosphatidylserine	9.5 ± 0.5	8.4 ± 0.6	13.2 ± 1.6
Phosphatidylinositol	4.8 ± 0.6	4.5 ± 1.4	3.5 ± 0
Sphingomyelin	6.3 ± 4.7	n.d.*	4.7 ± 2.6

* n.d., not detected.

Immature neurons incubated *in vitro* in the presence of a labelled amino acid show a high rate of incorporation in protein but relatively low labelling of plasma membranes and smooth membranes is observed (Table II). Within 1 h plasma membranes attain a specific radioactivity of only 6% and smooth membranes 14% of that of the total homogenate. If an excess of nonlabelled precursor is added to the incubation medium in order to stop additional synthesis of labelled proteins, the specific radioactivity of the homogenate decreases by 20% in 2 h indicating continuous turnover of proteins. Analogously, proteins of smooth endoplasmic reticulum appear

TABLE II

INCORPORATION OF [^{3}H]LEUCINE IN PROTEINS OF PLASMA MEMBRANES AND SMOOTH ENDOPLASMIC RETICULUM OF IMMATURE NEURONS *IN VITRO*

Cells were isolated from newborn rat brain and incubated at 37 °C in fortified minimal essential medium containing [^{3}H]leucine (10 μCi/ml). After 1 or 3 h incubations were terminated and subcellular fractions were isolated as described in Methods. In the 3-h incubations 5 mM nonradioactive leucine was added at 1 h to follow the decay of labelled proteins. Each figure represent the means ± S.E. of 4 experiments.

Fraction	*Incubation time*			
	1 h		*3 h, chase at 1 h*	
	Radioactivity (cpm/mg protein ×10^{-3})	%*	*Radioactivity (cpm/mg protein ×10^{-3})*	%*
Cell homogenate	106.0 ± 19.0	100	84.2 ± 21.0	80
Plasma membranes	6.9 ± 1.8	6	8.6 ± 0.4	8
Smooth endoplasmic reticulum	14.7 ± 6.2	14	6.6 ± 1.8	6

* Labelling of cell homogenate at 1 h is taken as 100%.

to turn over as their specific radioactivity decreases during the chase period. Plasma membranes, however, increase their specific radioactivity during the chase. This suggests a delayed flow of labelled proteins in plasma membranes after the synthesis of labelled proteins is finished.

DISCUSSION

The rat brain is an interesting example of brain development as the cellular differentiation takes place predominantly postnatally[14]. In order to perform experiments with immature brain *in vitro*, we have dispersed newborn brain tissue in a culture of cells[7]. The preparation used permits prolonged *in vitro* experiments, during which the cells show signs of differentiation.

The immature cells offer a unique chance to isolate cerebral plasma membranes. Two procedures have been described, one using homogenization[6] and the other chemical treatment[15] to separate plasma membranes. In this study homogenization was used to prepare plasma membranes, which were then compared to smooth endoplasmic reticulum by their molecular composition as well as labelling characteristics with [^{3}H]leucine.

Elsewhere we have presented data on the properties of surface membranes isolated by the chemical treatment[15]. Although this preparation appeared less pure than the present one, the results are in good agreement.

The animals used were 4–5-days-old and caution is required in attempts to relate the present data to those obtained with adult animals. The brain of a newborn rat is under a vigorous growth including a net synthesis of proteins[16] and phospholipids[17]. This may imply that plasma membranes and other organelles of a newborn animal are enriched in structural rather than functional constitutents as compared

to those of an adult animal. If this is the case, the data obtained may describe the basal requirements for an integral, but functionally rather primitive membrane structure.

Plasma membranes of immature neurons displayed three prominent protein bands at 45000, 50000 and 58000 daltons (Fig. 1). As the intensity of amido black staining is well related to the amount of protein in a band[18], the three major polypeptides obviously constitute a large fraction of the total plasma membrane protein. Levitan *et al.*[19] studied the polypeptide composition of synaptosomal plasma membranes and demonstrated two major bands at 42000 and 53000 daltons. Interestingly, Neville and Glossmann[20] found three prominent proteins corresponding exactly to ours by molecular weights in liver plasma membranes. They could also show by mixing experiments that some of these polypeptides were present in erythrocyte ghosts and kidney surface membranes. These data support the hypothesis that some protein components may be shared by plasma membranes of different origin[20]. By contrast, the gel containing smooth endoplasmic reticulum displayed major polypeptides at 45000 and 53000 daltons indicating nonidentity of the pattern.

Plasma membranes are generally enriched in glycoproteins and this obviously holds to brain plasma membranes as well. Using Schiff staining several carbohydrate-containing bands could be exposed in plasma membrane preparations, while a similar protein concentration in the gels containing smooth endoplasmic reticulum gave hardly any reaction.

The relative proportions of phospholipid classes showed some differences between plasma membranes and smooth endoplasmic reticulum (Table I). Phosphatidylcholine appeared to be enriched in plasma membranes while phosphatidylserine and sphingomyelin in smooth endoplasmic reticulum. Sphingomyelin is concentrated in plasma membranes of many cell types[21–23], but synaptosomal plasma membranes from adult rat brain contain rather small amounts of it[24]. Our failure to detect sphingomyelin in plasma membranes could be due to the age of the animals used[17].

The labelling of plasma membranes and smooth endoplasmic reticulum was low in a short time *in vitro* incubation with [^{3}H]leucine. They reached specific radioactivity of 6 and 14%, respectively, of the labelling of the cell homogenate. In a chase experiment the specific radioactivity of smooth endoplasmic reticulum rapidly decreased indicating that no net flow of labelled proteins occurred. By contrast, the specific radioactivity of plasma membranes increased during the chase, which suggests a continuous flow of labelled proteins in plasma membranes from polysomes. These data are in agreement with *in vivo* experiments with liver plasma membranes[25,26] showing that the peaks of radioactivity first occurred in endoplasmic reticulum and later in plasma membranes. A delayed appearance of labelled proteins in plasma membranes suggests that at least a portion of their proteins is transported slowly in an intracellular membrane system.

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