BBA 75933

THE LIPID COMPOSITION OF ADULT RAT BRAIN SYNAPTOSOMAL PLASMA MEMBRANES

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(Received December 8th, 1971)

SUMMARY

The lipid composition of highly purified synaptosomal plasma membranes has been analyzed. The membranes contained cholesterol (0.158 mg per mg protein), phospholipids (0.714 mg per mg protein) and gangliosides (0.125 mg per mg protein) as major constituents while cerebrosides and sulphatides were present in only trace amounts. Analysis of the gangliosides by thin-layer chromatography showed that there was no selective enrichment of any individual type in the plasma membranes in relation to total brain. The major phospholipids were ethanolamine (34.2 %), choline (41.6 %) and serine (13.2 %) phosphoglycerides. Inositol monophosphoglyceride (3.5 %), sphingomyelin (5.1 %) and lysolecithin (1.0 %) were relatively minor components while phosphatidylglycerol was not present. Each phospholipid class showed a specific fatty acid composition which differed from that of the other phospholipids. This result extends the specific fatty acid compositions observed in grey matter and certain sub-fractions by other workers. In particular, choline phosphoglycerides contained high levels of saturated fatty acids while large quantities of docosahexaenoic acid were present in ethanolamine and serine phosphoglycerides.

INTRODUCTION

Lipids are of importance in the structure and function of cerebral membranes, as they are in fact for all membranes. Apart from this general role certain phospholipids have been more specifically implicated in brain function. The metabolism of phospholipids has been linked to the transmission of nerve impulses since phosphatidyl inositol turnover is stimulated in brain slices exposed to depolarizing agents[1–3]. In addition, by analogy with processes postulated to occur in the adrenal medulla[4] and other systems[5,6], it has been suggested[7] that lysolecithin acts as the mediating agent in the presumptive fusion of the synaptic vesicle and the neuronal plasma membrane during the exocytosis of transmitter substances. An understanding of the molecular basis of these biochemical processes depends upon the separation and subsequent analysis of the various subcellular components involved.

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Many observations[8] on regional differences in the lipid composition of brain (in particular the differences between grey and white matter) point to certain specificities in the lipid compositions of the cell types which form the central nervous system. But until recently the only fraction of defined cellular origin which could be isolated, was the oligodendrocyte-derived myelin sheath[9]. For this reason the lipid composition of this structure has been extensively studied[10–16]. It seems likely that cerebrosides and sulphatides are highly concentrated in myelin, and perhaps in the associated oligodendroglial cells. Less direct evidence suggests[17,18] that gangliosides may be localized in the neuronal plasma membrane. However, there is some evidence[19,20] that the monosialoganglioside GM_1 may be present in the myelin sheath and very recently it has been reported that isolated glial cells[21,22] contain gangliosides.

With the development of methods for the isolation and subfractionation of synaptosomes[23–25], it became possible to study the composition of synaptic vesicles, synaptic mitochondria and synaptosomal plasma membranes[10,13,14,26]. Unfortunately, with the exception of the work of Cotman and co-workers[25,26], the fractions studied were not sufficiently characterized with regard to possible contamination by other types of membrane. This paper reports a detailed study of the lipid composition of a synaptosomal plasma membrane preparation which has been thoroughly characterized enzymatically and morphologically[27].

METHODS

Materials

All solvents were reagent or analytical grade. The solvents and flasks were thoroughly flushed with high purity nitrogen immediately before use. This step was essential for quantitative recoveries of polyunsaturated fatty acids. Synaptosomal plasma membranes were prepared from adult rat brain and characterized enzymatically and chemically as described elsewhere[27]. Synaptosomes were prepared by density gradient centrifugation on isotonic Ficoll–sucrose density gradients after extensive washing of the crude mitochondrial pellet to eliminate microsomal contamination. After osmotic shock of the synaptosomes, the plasma membranes were isolated by sucrose density gradient centrifugation. When necessary, the membranes were washed 5 times to eliminate sucrose.

Enzymatically, these membranes have been shown to be over 90 % pure plasma membrane, the major contaminating membrane being the outer mitochondrial membrane (5–10 %). Myelin, lysosomes, inner mitochondrial and microsomal membrane and soluble proteins are not present in significant amounts. Some of the lipid data (absence of cerebrosides and diphosphatidyl glycerol, (Table I)) confirm the purity of these membranes. It is more difficult to measure the amount of plasma membrane which could be derived from the glial cells of the central nervous system, but several estimates set this contamination at less than 10 %. Thus over 80 % of the protein in these preparations seems to be derived from the neuronal plasma membrane.

Isolation of lipids

Lipids were extracted according to the method described by Rouser[28] by addition of methanol to aqueous suspensions of membrane (10–15 mg protein in

5 ml), followed by the addition of the appropriate volume of chloroform to give a final extraction with 19 vol. chloroform–methanol (2:1 v/v). The precipitated protein was sedimented and extracted with 10 vol. of chloroform–methanol (1:2, v/v). The two extracts were combined and brought to a final chloroform–methanol ratio of 2:1 (v/v). The extracts were then partitioned with 0.2 vol. of 0.1 M KCl according to Lowden and Wolfe[29] and Suzuki[30].

The lower phase was evaporated to dryness under N_2 at 37 °C and the residue was taken up in a small volume of chloroform. Samples were taken for total lipid phosphorous by the method of Marinetti[31]. Other samples of the total lipids were applied to small silicic acid columns (5 cm × 1 cm). Cholesterol was eluted with choroform and estimated by the method of Idler and Baumann[32]. Estimations of total lipid phosphorus on the total lipid extract or after elution of the phospholipids from the silicic acid columns did not differ significantly. Galactolipids were determined by either analyzing lower phase hexose or by passing an aliquot of the total lipid extract through a silicic acid column. After eluting the neutral lipids with chloroform, glycolipids were eluted with acetone. Galactose was estimated by hydrolyzing an aliquot of the sample in 2 M HCl at 100 °C for 2 h. After hydrolysis the HCl was removed by lyophilization and the residue was placed on a column of Dowex 50-X4. Neutral sugars were eluted with water. After lyophilization the hexoses was estimated by the phenol–H_2SO_4 reaction.

The upper phases from the partition were combined, and evaporated to dryness. The residue was taken up in a small volume of water, transferred to dialysis tubing and dialyzed against water for 36 h with three changes. Aliquots were taken for analysis of total ganglioside *N*-acetylneuraminic acid by the method of Svennerholm[33]. The rest of the material was evaporated to dryness and dissolved in chloroform–methanol (2:1, v/v).

Thin-layer chromatography

For bidimensional chromatography of the phospholipids, thin layers (200 μm) of silica gel H were prepared with a Desaga apparatus. After activation an aliquot of the total lipid extract (containing 20–40 μg of lipid phosphorus) was applied as a small spot about 2 cm from the corner of the plate. The plate was developed in the first direction in chloroform–methanol–20% NH_3 (70:25:5, by vol.) and in the second direction with chloroform–methanol–acetone–acetic acid–water (140:25:35:20:9, by vol.; see ref. 34). The various lipid classes were identified using specific sprays or cochromatography with authentic standards. The lipids were routinely revealed with iodine. Each spot was circled, the iodine was allowed to evaporate and the silica gel of the area was removed using an aspirator made from a Pasteur pipette. The aspirator was converted to a small column, and the lipids were eluted with 10 ml of chloroform–methanol–acetic acid–water (50:50:10:10, by vol.) and finally methanol (2 ml). The solvent was evaporated and the residue was dissolved in 5 ml of chloroform–methanol (2:1, v/v) and partitioned with 1 ml of water. The chloroform phase was recovered and the aqueous phase was washed with chloroform (2 times 2 ml). The chloroform phases were combined, evaporated to dryness and phosphorus was determined as described above. Recovery of the applied phosphorus was 96–102%.

For analysis of the fatty acids of each phospholipid class the total lipid extract

(5–10 mg) was applied as a band to a thin-layer plate. The plate was developed in choroform–methanol–acetic acid–water (100:50:12:6, by vol.). Narrow strips on each side of the thin-layer plate were exposed to iodine vapours and the areas corresponding to each phospholipid class were marked. The gel which had been exposed to iodine was removed and discarded. The areas of silica gel corresponding to ethanolamine phosphoglycerides, choline phosphoglycerides and sphingomyelin were scraped into test tubes and covered with the methylating reagent. Analysis of these lipids by bidimensional thin-layer chromatography, after they had been extracted from the silica gel, indicated that they were less than 1 % contaminated with other phospholipid classes. However, it was imperative to use no more than 10 mg of lipid on the original plate. Since serine phosphoglycerides and inositol monophosphoglycerides migrated together in this system, the area of silica gel corresponding to these lipids was removed and extracted with 20 ml chloroform–methanol (1:1, v/v) and then methanol (20 ml). The extract was applied as a band to a thin-layer plate which was developed in a solvent of chloroform–methanol–acetone–acetic acid–water (140:25:35:20:9, by vol.). This procedure provided pure serine phosphoglycerides. In some cases inositol monophosphoglycerides were slightly contaminated with serine phosphoglycerides were slightly contaminated with serine phosphoglycerides (5–10%). Such contamination was immediately evident in the fatty acid pattern and such samples were not used.

Chromatography of the neutral lipids was carried out using thin layers of silica gel G. Aliquots of the total lipid were applied as a spot to the plate which was then developed in a solvent of hexane–ethyl ether–acetic acid (80:20:2, by vol.). After development the plate was allowed to dry and sprayed with phosphomolybdic acid. The relative quantities of neutral lipids were determined by optical densitometry.

The gangliosides were analyzed using ascending chromatography[35] with chloroform–methanol–2.5 M NH_3 (60:35:8, by vol.) as solvent or by descending chromatography[36] using propanol–water (80:20, v/v) as a developing solvent. After drying, the plates were sprayed with the resorcinol–HCl reagent of Svennerholm[33] and heated at 120 °C for 20 min. An estimate of the proportions of sialic acid in each species of ganglioside was obtained by optical densitometry.

Gas–liquid chromatography

The fatty acids of the phospholipid classes were converted to their methyl esters by covering the silica gel scrapings, in pyrex test tubes, with 2 ml of 6 % H_2SO_4 in methanol (v/v). The tubes were sealed and heated at 90 °C for 5 h. In the case of sphingomyelin, the heating was continued for 12 h. After the tubes had cooled, water (5 ml) was added and the methyl esters were extracted with hexane (3 times 2 ml). The hexane extracts were combined, back-washed with water (2 ml), and evaporated to dryness at 37 °C. The residue was taken up in a small volume of hexane and analyzed by gas–liquid chromatography using a Varian Aerograph gas chromatograph Model 2001 (Varian Aerograph Inst. Co., Walnut Creek, Calif., U.S.A.). Glass columns (5 ft × 4 mm) were packed with 10 % EGGS on Gas-Chrom P. The columns were conditioned for 6 h at 225 °C before use. Operating conditions were: injector 210 °C, detector 225 °C, column 180 °C, and carrier gas (N_2) 25 ml/min. The instrument was calibrated with standard fatty acid mixtures supplied by Applied

Science Laboratories (State College, Pa., U.S.A.). Fatty acids were identified as described elsewhere[37] using known standards, semilogarithmic plots of the relative retention times and hydrogenation of the samples. In addition, the methyl esters of each class of unsaturated fatty acids were isolated by argentation thin-layer chromatography and analyzed by gas-liquid chromatography.

RESULTS

The values obtained for the lipid class composition of the three membrane fractions (E, F and G) which have been shown previously to be enriched 7–14-fold in plasma membrane markers such as $(Na^+ + K^+)$-ATPase[27], are given in Table I. Neither free fatty acids nor neutral glycerides were significant components since they made up only 1–2 % of the total lipids. The amount of cholesterol (0.158–0.202 mg per mg protein) is considerably lower than the values which have been reported for myelin[10–13] as well as other plasma membranes[10,13], but are similar to values reported by Cotman *et al.*[26] for synaptic plasma membranes prepared by zonal centrifugation. In agreement with previous analyses, only trace amounts of lower phase hexose were found in these membranes and cerebrosides could not be detected on the thin-layer plates (Fig. 1) when a system was used that readily resolved them from the phospholipids. Other investigators have also found very low amounts of neutral glycolipids in synaptosomes[10,22], in synaptic membranes[26] and in isolated neuronal perikarya[20,22,38], and astrocytes[20,22]. However the gangliosides (0.076–0.125 mg per mg protein) make up a significant proportion of the lipids. They are most enriched in Fraction F, which correlates with the distribution of enzyme markers for plasma membranes[27]. The phospholipids (0.714–0.928 mg per mg protein) represent approx. 75 % of the lipids. The molar ratio of cholesterol to phospholipids (0.436–0.486), which is in excellent agreement with that reported by Cotman *et al.*[26], is much lower than that observed for myelin[10–13] or for some other synaptosomal plasma membrane preparations[10,13], but is between the values recently reported for isolated neuronal perikarya[21,22,38] from various animals. The lipid/protein ratio is high

TABLE I

LIPID CLASS COMPOSITION OF SYNAPTOSOMAL PLASMA MEMBRANE FRACTIONS

Values are expressed in mg per mg protein (determined by method of Lowry). Quantities of phospholipid were obtained by multiplying the value of phospholipid phosphorus by 25 while gangliosides were determined by multiplying the value of sialic acid by 2.8 (average of 2.2 molecules of sialic acid per molecule of ganglioside with a molecular weight of 1860). E, F and G correspond to membranes obtained from the interphase of sucrose densities of 0.4–0.6, 0.6–0.8, 0.8–1.0 molar sucrose.

Lipid class (*number of experiments*)	*Membrane fractions*		
	E	*F*	*G*
Free fatty acids and triglycerides (1)	0.010	0.015	0.020
Cholesterol (4)	0.202 ± 0.018	0.158 ± 0.025	0.178 ± 0.026
Phospholipid (4)	0.928 ± 0.015	0.714 ± 0.013	0.734 ± 0.088
Ganglioside (4)	0.092 ± 0.014	0.125 ± 0.013	0.076 ± 0.006
Galactolipid (2)	<0.005	<0.005	<0.005
Molar ratio of cholesterol/phospholipid	0.436	0.444	0.486

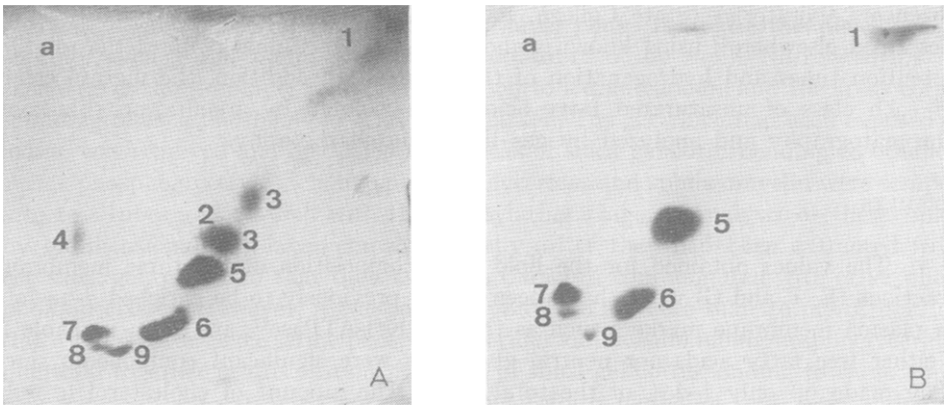

Fig. 1. Two dimensional thin-layer chromatogram of total lipid extracts from: (A) total rat brain; (B) rat synaptosomal plasma membranes. The figures refer to neutral lipids (1), diphosphatidylglycerol (2), cerebrosides (3), phosphatidic acid (4), ethanolamine phosphoglycerides (5), choline phosphoglycerides (6), serine phosphoglycerides (7), inositol monophosphoglycerides (8), sphingomyelin (9), lysophosphatidylcholine (10). Diphosphatidylglycerol and cerebrosides containing hydroxy fatty acids overlap. Lipids were revealed with iodine vapours.

TABLE II

PERCENT DISTRIBUTION OF SIALIC ACID IN GANGLIOSIDES OF SYNAPTOSOMAL PLASMA MEMBRANES

Nomenclature as defined by Svennerholm[39]. Sample A was determined on the present rats, Sample B was taken from analyses by Suzuki[40].

Ganglioside type	*Membrane fraction* *			*Total brain*	
	E	*F*	*G*	*A*	*B*
GQ_1	7.2	7.2	5.3	6.8	8.1
GT_{1b}	29.3	26.8	28.1	26.4	29.0
GD_{1b}	21.4	18.4	18.1	19.8	18.5
GD_{1a}	34.4	36.1	36.7	36.0	34.0
GM_1	7.8	12.3	11.9	10.9	9.1
GM_2					1.2

* As defined in Table I.

(0.98–1.22) and is significantly higher than that reported by Cotman *et al.*[26] due to their neglect of the gangliosides.

The distribution of sialic acid in the various types of gangliosides is shown in Table II. The analysis of total rat brain gangliosides is comparable to other reports[40,41]. The results show that there is no extensive difference in the ganglioside species in the three membrane fractions. All of these are very similar to total rat brain gangliosides, even though the monosialoganglioside GM_1 has been claimed[20,21] to be in myelin. More recently gangliosides have been claimed to be present in astrocytes[21], but the molecular species were not reported. Analyses of neurons and glial cells from rabbit cerebral cortex indicated there was no difference in ganglioside distribution in the two cells[22].

The similarity in lipid composition and ganglioside distribution between the three membrane fractions (Tables I and II), is also obvious in the phospholipid class

distribution (Table III). The major phospholipids in each membrane were choline (40.4–41.6%), ethanolamine (33.1–35.3%) and serine (13.2–14.5%) phosphoglycerides. Inositol monophosphoglycerides (3.4–4.3%) and sphingomyelin (4.6–6.2%) were present in much lower amounts while there was very little lysophosphatidylcholine (0.5–1.0%). Diphosphatidylglycerol was not found in Fractions E and F but occasionally trace amounts were present in Fraction G. The phospholipid composition of these membranes distinguishes them from all other brain subcellular fractions. They differ from microsomes[10] in containing a lower percentage of sphingomyelin and a higher percentage of ethanolamine phosphoglycerides.

TABLE III

PHOSPHOLIPID COMPOSITION OF SYNAPTOSOMAL PLASMA MEMBRANE FRACTIONS

Results represent analyses of four membrane preparations ± S.D. Values are expressed as percent recovered phosphorus. Recovery of phosphorus was 96–102%.

Phospholipid	*Membrane fractions* *		
	E	*F*	*G*
Diphosphatidylglycerol	—	—	0.7 ± 0.2
Phosphatidic acid	0.3 ± 0.1	0.6 ± 0.2	0.6 ± 0.4
Ethanolamine phosphoglycerides	35.3 ± 1.6	34.2 ± 1.6	33.1 ± 1.5
Choline phosphoglycerides	40.3 ± 2.4	41.6 ± 2.2	40.4 ± 1.8
Serine phosphoglycerides	14.3 ± 0.7	13.2 ± 0.9	14.5 ± 0.8
Inositol monophosphoglycerides	4.4 ± 0.5	3.5 ± 0.2	3.4 ± 0.2
Sphingomyelin	4.6 ± 0.5	5.1 ± 0.4	6.2 ± 0.6
Lysophosphatidylcholine	0.5 ± 0.3	1.0 ± 0.4	0.7 ± 0.4
Unknown	0.4 ± 0.2	0.8 ± 0.4	—

* As defined in Table I.

In view of the similarity between the three membrane fractions shown in the previous two tables, as well as the enzymatic similarity previously demonstrated[27], the fatty acid composition of the phospholipids is reported only for Fraction F (Table IV). This fraction was enzymatically the most pure. The values for this fraction are representative of Fractions E and G. Choline phosphoglycerides have a very specific fatty acid composition since over 50% of the fatty acids was palmitic acid. Taken together palmitic and oleic acids accounted for 80% of the fatty acids. This is in agreement with the results of Cotman *et al.*[26] and Kishimoto *et al.*[14]. The latter also suggested that dipalmitoyl lecithin could be a major molecular species. This molecular species has been detected[42] in large quantities in total lecithin from ox brain. In contrast to the other phosphatides, the choline phosphoglycerides contain very little arachidonic (4.3%) or docosahexaenoic (2.7%) acid.

Inositol monophosphoglycerides also have very few major fatty acids. Stearic (38.4%) and arachidonic (36.9%) acids were the major components but very little docosahexaenoic acid (4.7%) was present in this phosphoglyceride. Whole bovine inositol brain mono-, di-, and triphosphoglycerides have somewhat similar fatty acid compositions[43]. Serine phosphoglycerides, in contrast, contained stearic (48.6%) and docosahexaenoic (34.1%) acids as the major components. This composition is quite different to that of white matter[44] and myelin[16], but is relatively close to that reported for grey matter serine phosphoglycerides[45].

TABLE IV

FATTY ACID COMPOSITION OF PHOSPHOLIPIDS OF SYNAPTOSOMAL PLASMA MEMBRANES

Fatty acids are identified by total number of carbon atoms and double bonds. Identification of 22:4 (*n*-6) and 22:5 (*n*-6) was based on the equivalent chain length values reported by Jamieson and Reid[69]. On argentation chromatography they migrated as trienoic and tetraenoic fatty acids. However the identification given agrees with studies of the fatty acids in human brain phospholipids[70]. Numbers in parenthesis represent number of membrane preparations analyzed. Deviation from the mean was less than 5% for major fatty acids and less than 10% for minor components. Dimethyl acetal derivatives formed from alk-1-enyl acyl phosphatides during transmethylation. They are expressed as a percentage of the total fatty acids.

Fatty acid	*Phospholipid*				
	Choline phospho-glycerides (4)	*Inositol monophospho-glycerides (2)*	*Serine phospho-glycerides (2)*	*Ethanolamine phospho-glycerides* (4)*	*Sphingo-myelin (2)*
16:0	57.2	10.0	0.5	5.9	5.2
18:0	11.8	38.4	48.6	29.3	87.5
18:1	22.1	8.5	7.6	7.8	0.1
18:2 (*n*-6)	0.6	0.3	—	—	—
20:0	—	—	—	—	1.9
20:1	0.7	—	—	0.9	—
20:2	0.4	—	—	—	—
20:4 (*n*-6)	4.3	36.9	2.0	14.6	—
20:5 (*n*-3)	0.3	1.3	—	—	—
22:0	—	—	—	—	0.7
22:1	—	—	—	—	0.7
22:4 (*n*-6)	0.2	—	3.9	7.3	—
22:5 (*n*-6)	—	—	3.2	1.7	—
22:6 (*n*-3)	2.7	4.7	34.1	32.4	—
23:0	—	—	—	—	0.2
23:1	—	—	—	—	0.1
24:0	—	—	—	—	1.3
24:1	—	—	—	—	0.9
16 dimethyl acetal derivatives	—	—	—	5.4	—
18 dimethyl acetal derivatives	—	—	—	12.1	—

* Containing alk-1-enyl acyl and diacyl species.

The fatty acid composition of the ethanolamine phosphoglycerides was slightly more complex. There were three major fatty acids; stearic (29.3%), arachidonic (14.6%) and docosahexaenoic (32.4%) acids. The dimethyl acetal derivatives of 16- and 18-carbon aldehydes were estimated to be 17.5% when compared to the fatty acids. This corresponds to a plasmalogen content of approx. 30% in the ethanolamine phosphoglycerides. A similar value was found by Cotman *et al.*[26] using more elaborate analytical techniques. The plasmalogens make up a smaller proportion of the total ethanolamine phosphoglycerides than in myelin[10–13]. The fatty acid composition of the ethanolamine phosphoglycerides is again quite different from that of myelin[10–13], but is relatively close to that of human grey matter[44].

The fatty acid composition of the sphingomyelin is striking in that stearic acid accounted for 87.5% of the fatty acids. The long chain fatty acids, characteristic of sphingomyelins from myelin, were almost completely absent. A similar finding was reported by Kishimoto *et al.*[14].

DISCUSSION

From the lipid analyses it can be seen that these membranes are characterized by a relatively high lipid/protein ratio (1.0) and contain three major types of lipids: cholesterol, phospholipid, and ganglioside. While this membrane is considerably richer in lipid than plasma membranes from liver[46–48] it does not contain as much lipid as myelin[10–13]. In Tables V and VI the present data has been compared to analyses of synaptosomal plasma membranes by other workers. Taking account of the probable presence of gangliosides in the membranes of Cotman *et al.*[26], their data are very similar to ours. However, there are significant differences when our data is compared to that of Whittaker[49] and Lapetina *et al.*[13] who found higher levels of cholesterol and cerebrosides, but lower levels of gangliosides. If ganglioside is a marker for plasma membrane, our preparation is considerably purer than the latter preparations, which is in accord with enzymatic data[27]. Again in Table VI the phospholipid composition of this preparation is very similar to that reported by Cotman *et al.*[26] but rather different from that reported by Whittaker[49].

The synaptosomal plasma membrane differs from most other plasma membranes. Erythrocyte ghosts[50], and plasma membranes from platelets[51], liver[46–48] and

TABLE V

COMPARISON OF LIPID COMPOSITION OF VARIOUS SYNAPTOSOMAL PLASMA MEMBRANE PREPARATIONS

Results (mg per mg protein) of other authors were recalculated for uniformity of presentation. Gangliosides were not determined by Cotman *et al.*[26]. The results of Whittaker[49] were not recalculable in the same form from the presented data (except for ganglioside); the values were given as cholesterol/phospholipid molar ratio (0.72) and cerebroside/phospholipid molar ratio (0.05).

Lipid class	*Preparation*			
	Present analysis	*Cotman et al.*[26]	*Lapetina et al.*[13]	*Whittaker*[49]
Cholesterol	0.158	0.165	0.267	Not calculable
Cerebroside	<0.005	0.007	0.134	Not calculable
Phospholipid	0.714	0.704	0.666	Not calculable
Ganglioside	0.125	Not determined	0.045	0.040

TABLE VI

PHOSPHOLIPID COMPOSITION OF VARIOUS SYNAPTOSOMAL PLASMA MEMBRANE PREPARATIONS

Phospholipid	*Preparation*		
	Present analysis	*Cotman et al.*[26]	*Whittaker*[49]
Phosphatidic acid	0.3	—	6
Ethanolamine phosphoglycerides	34.2	36.2	30
Choline phosphoglycerides	41.6	43.5	24
Serine phosphoglycerides	13.2	15.2	3
Inositol monophosphoglycerides	3.5		1
Sphingomyelin	5.1	3.7	6
Lysophosphatidylcholine	1.0	1.0	Not determined
Alkyl ether	Not determined	Not determined	4

L cells[52] all have higher cholesterol/phospholipid molar ratios and contain much greater amounts of sphingomyelin. In general, other plasma membranes seem to be characterized by a low (< 1 % total lipid) content of glycolipids, but in the case of the synaptosomal plasma membranes, there are very high levels of ganglioside. The intestinal microvillar plasma membrane has however been reported to contain a large amount of neutral glycolipid[53].

The most striking feature of these membranes is perhaps their fatty acid composition. All the phospholipids examined have distinct specific fatty acid patterns. The distributions do not correspond to the free fatty acid pool of the brain[54], nor to the fatty acid composition of brain neutral glycerides[55]. In addition, the fatty acid composition of the ethanolamine phosphoglycerides and serine phosphoglycerides is very different from the composition of these lipids in plasma membranes from other tissues[46–52]. In general these phosphoglycerides in other tissues contain much lower levels of docosahexaenoic acid and correspondingly higher levels of monoenoic, dienoic and tetraenoic fatty acids.

On the basis of the fatty acid compositions of inositol monophosphoglycerides, in this preparation, in total brain[8], myelin[16] and synaptosomes[22] it is probable that similar populations of inositol phosphoglycerides exist in the various compartments of the central nervous system. This does not appear to be the case for the other phospholipids. Choline phosphoglycerides in synaptosomes[22] and in the synaptosomal plasma membrane have fatty acid compositions which are similar to that of total brain lecithin before myelination but differ from that of total adult brain lecithin[55]. Thus it is probable that the glial cells present in immature brain contain choline phosphoglycerides similar in composition to the neuronal phospholipids. When glial cell metabolism is diverted to the production of myelin, it is not known whether the fatty acid composition of the cell itself changes. However Fewster and Mead[56] have shown that the fatty acid composition of lecithin from isolated oligodendrocytes is similar to that of myelin, and both are different from the synaptosomal plasma membrane. The fatty acid composition of rabbit synaptosomal and glial choline phosphoglycerides have however been reported to be similar[22].

In particular the ethanolamine and serine phosphoglycerides of the synaptosomal plasma membrane differ in their fatty acid compositions from those of gray and white matter, myelin[45], and rabbit brain synaptosomes[22]. The polyunsaturated fatty acids (arachidonic and docosahexaenoic acid) found in the synaptosomal plasma membrane ethanolamine and serine phosphoglycerides are almost completely absent from myelin and white matter, and from glial cells isolated from the corpus collosum (oligodendrocytes)[56]. Other investigations[22,57] have indicated that neurons and glial cells derived from the cortex both contain large amounts of polyunsaturated fatty acids in ethanolamine and serine phosphoglycerides. However the problem of contamination of glial cell fractions with synaptosomes has not been completely resolved.

The fatty acid composition of the sphingomyelins is particularly interesting. The fatty acids in the synaptosomal plasma membrane sphingomyelins are almost exclusively stearic acid while the long chain fatty acids present in large amounts in the sphingomyelins from white matter, myelin[45] and isolated oligodendrocytes[56] are almost absent. In this case the results of Pomazanskaya *et al.*[57] are difficult to explain, since the sphingomyelin of their astrocytes has a fatty acid composition similar to that of the synaptosomal plasma membrane, whereas the fatty acid com-

position of the sphingomyelin of their neurons differs markedly from that of the synaptosomal plasma membrane.

The high degree of unsaturation in the ethanolamine and serine phosphoglycerides of synaptosomal plasma membranes poses questions concerning their functional significance and biosynthesis. Although docosahexaenoic acid is present in phosphatides of other tissues it usually never exceeds 10 % of the total fatty acids of a given phospholipid under normal dietary conditions. However, ethanolamine and serine phosphoglycerides from synaptosomal plasma membrane and retinal rod outer segments[58], both excitable membranes, contain over 30 % docosahexaenoic acid. It has been suggested[59] that the polyunsaturated phosphatides may have an important role in the excitability of the membranes. The large quantities of polyunsaturated acids are not an exclusive property of the synaptosomal plasma membrane since other neuronal intracellular membranes also contain this type of fatty acid in the same phosphoglycerides[14,60]. This is not an argument against a functional role of these highly unsaturated phospholipids, since the presence of polyunsaturated phospholipids in the other subcellular membranes could be a passive reflection of a fatty acid metabolism or phospholipid synthesis, which is specialized to provide phosphatides which have a specific function to fulfil. The polyunsaturated fatty acids are obviously of importance since in essential fatty acid deficient animals, the arachidonic and docosahexaenoic acids of brain phospholipids are replaced by 20:3, 22:3 and 22:5 fatty acids which can be synthesized from palmitoleic and oleic acids[61].

The other problem posed is that of the biosynthesis of these highly unsaturated species. In the liver *de novo* synthesis of ethanolamine and choline phosphoglycerides mainly produces species containing saturated fatty acids and monoenoic or dienoic fatty acids[62]. Subsequently polyunsaturated fatty acids may be introduced into the 2-position by the deacylation–reacylation cycle described by Lands and Hart[63]. Saturated fatty acids may be introduced into phospholipids, containing docosahexaenoic acid, by acylation of the unsaturated 2-monoacyl phosphatide[64]. In the case of the synapse, phospholipids may be synthesized locally[65] or reach the synapse by axoplasmic flow[66], possibly in the form of lipoprotein complexes[67]. That the Lands cycle may occur in the nerve-ending is suggested by the different half lives of the glycerol, phosphate, and fatty acid moieties of synaptic phosphoglycerides[65,68]. Since the half life of the fatty acids is much longer than those of glycerol or phosphate, the above mechanisms may enable the neuron to conserve the highly unsaturated fatty acids which must be derived from dietary essential fatty acids.

ACKNOWLEDGEMENTS

This work was in part supported by a grant from the Institut National de la Santé et de la Recherche Médicale (contract 71-11698). We are grateful to Dr L. Sarlième for the analysis of gangliosides by descending thin-layer chromatography and to Miss O. Lallemand for skilful technical assistance.

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