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Lipid Composition of Synaptic Plasma Membranes Isolated from Rat Brain by Zonal Centrifugation*

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ABSTRACT: A plasma membrane fraction derived from rat brain nerve endings or the synaptic plasma membrane fraction, isolated by zonal centrifugation, was analyzed for their lipid content and their composition of lipid classes and their aliphatic moieties. With the exception of glycolipids and sphingomyelin, the lipid classes of synaptic plasma membrane are very similar to whole brain. Phosphatidylcholine and phosphatidylethanolamine are the major lipids and make up 62% of the total membrane lipid. Cholesterol and ceramide make up the major neutral lipids and 21% of total brain lipid. The synaptic plasma membranes contain lower quantities of glyceryl ethers in phosphatidylethanolamine and phosphatidylcholine fractions than in whole brain. Certain distinctive features in fatty acid content were found; synaptic plasma membranes contained much less 18:1 acyl moieties in phosphatidylethanolamine and phosphatidylserine + phosphatidylinositol than found in whole brain, whereas most other acyl moieties with the exception of the longer chain unsaturated fatty acids are similar. The 22:6 acyl chains in synaptic plasma membrane account for approximately 32% of the total fatty acids in phosphatidylethanolamine and phosphatidylserine + phosphatidylinositol, but in whole brain the 22:6 fatty acids account for only about 26 and 20% of phosphatidylethanolamine and phosphatidylserine + phosphatidylinositol, respectively. The alk-1-enyl moieties in phosphatidylethanolamine are restricted to 16:0, 18:0, and 18:1 chain lengths in both synaptic plasma membrane and whole brain.

The components of neuronal membranes need to be known because elucidation of the structure and dynamic function of this membrane is dependent upon such knowledge. Specific lipids of membranes undoubtedly subserve specific functions; studies of model membranes and experiences with enzyme reconstitution illustrate the vital role and specificity of lipids in the functional properties of biomembranes. Tetrodotoxin, a molecule that specifically blocks changes in sodium conductance during nerve action potentials, interacts with and causes spreading of surface films made from nonpolar lipids extracted from squid axons, but polar lipids do not show such interactions (Villegas and Camejo, 1968; Camejo and Villegas, 1969). Phospholipids are essential for respiratory activity in mitochondria (Fleischer *et al.*, 1962), and the reconstitution of electron transport and oxidative phosphorylation from mitochondrial components has specific phospholipid requirements (Racker and Bruni, 1968). Structural protein preparations from mitochondria bind cardiolipin in larger quantities than other mitochondrial membrane lipids (Richardson *et al.*, 1964).

Certain phospholipids are effective in reactivating Na–K-ATPase activity (Tanaka and Strickland, 1965). Phosphatidylinositol has been found to have high affinity for calcium at physiological monovalent salt concentrations (Papahadjopoulos, 1968), and calcium is intimately involved in membrane bioelectric activity (Papahadjopoulos and Ohki, 1969). It is, therefore, appropriate to know the lipid composition of nerve membranes to evolve an understanding of their physiologic functioning.

The content, but no detailed class and fatty acid data, of various lipids in subcellular fractions of brain including SPM[1] has been reported by Lapetina and coworkers (1968). Whittaker (1966) has also reported previously unpublished data obtained by Sheltawg on certain lipid classes from synaptosome ghosts or SPM fractions. A detailed analysis of lipid classes from whole synaptosomes has been presented by Eichberg and coworkers (1964) and by Seminario and coworkers (1964). Kishimoto and coworkers (1969) have briefly

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[1] Abbreviations used are: SPM, synaptic plasma membrane; PA, phosphatidic acid; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine.

reported fatty acid data on certain brain subcellular fractions, including synaptosomes and partially characterized synaptosome subfractions; they found that these membranes were rich in longer chain fatty acids.

A complete analysis of the major lipid classes or the nature of the aliphatic moieties of each class in the plasma membranes of nerve cells has not been reported. In this paper we present analyses of the lipid classes and the composition of the acyl, alkyl, and alk-1-enyl moieties of each class in plasma membranes derived from a specialized portion of the neuron, the SPM. Similar analyses of the lipids from whole brain are also included for comparison. The SPM fraction we have analyzed consists primarily of plasma membranes derived from synaptosomes or nerve endings, and the major element consists of presynaptic and postsynaptic membranes and a synaptic thickening (Cotman *et al.*, 1968b).

Methods

Animals and Membrane Isolation. Male Sprague–Dawley rats, 70 days or older, were fed *ad libitum* with Purina laboratory diet. Water was available at all times. The rats were stunned and decapitated, and the cerebral cortex was rapidly removed and placed in ice-cold 10% sucrose (w/w). Synaptic plasma membranes were isolated from homogenates as previously described (Cotman *et al.*, 1968a). This procedure combines differential and zonal centrifugation to yield a relatively homogeneous population of membranes derived from synaptosomes. An aliquot of homogenate was removed for analysis of whole brain lipids. Gradient fractions of synaptic plasma membranes at a density from about 1.12 to 1.14 sucrose (27.5–31.5% w/w) were pelleted and washed twice with cold distilled water to remove sucrose; the homogenates were also pelleted and washed twice with cold distilled water to remove residual sucrose. Protein in the various samples was measured by ultraviolet absorption and by the method of Lowry *et al.* (1951).

Extraction of Lipids. Lipids of whole brain homogenates and synaptosome membranes were extracted by the Bligh and Dyer (1959) technique. Completeness of the lipid extraction procedure was checked by measuring the quantity of fatty acids released after the extracted residue from one whole brain and from one batch of membranes saponified with 10% KOH in 75% aqueous ethanol. After acidification, the fatty acids were extracted and then quantitated by two independent procedures for lipid mass after resolution by thin-layer chromatography: photodensitometry (Privett *et al.*, 1965) and scintillation quenching (Snyder and Moehl, 1969). Both methods showed less than 0.5% fatty acids present in both tissue residues and indicated that the Bligh and Dyer extraction procedure was quantitative for removing lipids from rat brain. We also found that the extraction procedure removed all traces of sucrose from the cell preparations as demonstrated by the absence of sucrose in extracts chromatographed in the chromatographic systems described in the next section.

Identification and Quantitation of Components. The lipid extracts were resolved into neutral lipid classes by thin-layer chromatography on silica gel G in hexane–diethyl ether–acetic acid (80:20:1, v/v). Only free sterols were found in significant amounts in the neutral lipid fraction; they were resolved by thin-layer chromatography and quantitated by photodensitometry (Privett *et al.*, 1965) and liquid scintillation quenching (Snyder and Moehl, 1969).

The major classes of polar lipids were identified on silica gel HR with the use of phospholipid standards and two-dimensional thin-layer chromatography. All reference compounds were purchased from The Hormel Institute, Austin, Minnesota, and Supelco, Inc., Bellefonte, Pa. A solvent system of $CHCl_3$–MeOH–HAc–H_2O (50:25:8:4, v/v) was used for development in the first direction and (after the plates were dried in a vacuum desiccator for 1 hr) $CHCl_3$–MeOH–NH_4OH (65:35:8, v/v) was used for development in the second direction. This two-dimensional technique was capable of resolving PA, PE, PS, PI, PC, sphingomyelin, lysoPC, and sulfatides. The phosphorus content of PE, PS + PI, PC, sphingomyelin, and lysoPC was determined by the method of Rouser *et al.* (1966).

The identification of the ceramide and cerebroside was based on thin-layer chromatography in diethyl ether; cholesterol migrated at a higher R_F than the ceramide. After the plate was air dried for 1–2 min, it was chromatographed again, in the same direction; we used $CHCl_3$–MeOH (70:30, v/v) but stopped this development about 0.5 in. short of the first solvent front. This technique resolved cholesterol (R_F 0.9), ceramides (R_F 0.8), and cerebrosides (R_F 0.5) which were quantitated by photodensitometry (Privett *et al.*, 1965).

The PE, PS + PI, and PC lipid classes were purified for further analysis by preparative thin-layer chromatography in a single dimension on silica gel HRB with a solvent system of $CHCl_3$–MeOH–HAc–H_2O (50:25:8:4, v/v). The extraction of the lipid classes from the silica gel and the purity of each class were checked by methods previously reported (Snyder, 1969). Each phosphoglyceride class was then reduced with $LiAlH_4$ and the content of the alkyl and alk-1-enyl glyceryl ethers was determined by photodensitometry (Wood and Snyder, 1968).

Preparative thin-layer chromatography was carried out so that the silica gel layers remained saturated by the chromatographic solvents during the visualization of areas with iodine vapor. This was accomplished by covering the thin-layer chromatography plate with a clean glass plate of equal dimensions immediately after removal from the chromatographic tank. The cover plate was shifted slightly so that the outer lanes (about 1 cm wide) of the standard and samples could be detected with the iodine vapor. The outer lanes were then marked with a pencil so that the phospholipid bands resolved from the samples could be scraped into beakers containing appropriate solvents for elution; the lipid classes isolated by this technique were used for subsequent analyses. Possible loss of polyunsaturated fatty acids in the phospholipids due to preparative thin-layer chromatography under these conditions was carefully checked with PE isolated from brains of female rats (Charles River strain). This PE preparation was purified two more times by preparative thin-layer chromatography, and the percentage of 18:0, 20:4, and 22:6 fatty acids in each preparation was determined by gas–liquid partition chromatography; none of the samples exposed to iodine vapor were used for analysis. We found that the fatty acid composition was essentially unaltered by the preparative thin-layer chromatography technique outlined above. Values of the fatty acids before and the values after two preparative thin-layer chromatography runs (in parentheses) were as

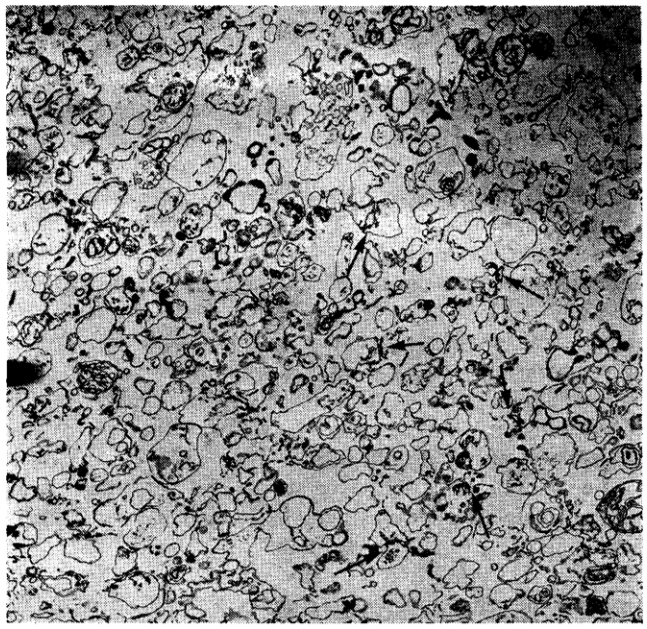

FIGURE 1: Synaptic plasma membrane fraction (×16,000). A number of the membrane elements show prominent synaptic thickenings (arrows).

TABLE I: Lipid Classes Present in Whole Brains and Synaptic Plasma Membranes in Rats.

	SPM[a] (%)	Whole Brain[b] (%)
Cholesterol	18.6 ± 1.2	19.4 ± 1.3
Ceramide	1.6 ± 0.3	1.5 ± 0.35
Glycolipid fraction[c]	0.9 ± 0.15	9.6 ± 0.5
PE	28.2 ± 1.3	30.5 ± 0.7
PS + PI	11.8 ± 0.6	8.7 ± 0.9
PC	33.9 ± 0.6	28.8 ± 0.7
Sphingomyelin	3.0 ± 0.3	1.4 ± 0.4
LysoPC	1.0 ± 0.7	Trace

[a] Average of five separate samples. [b] Average of three separate samples. [c] Migrated at same thin-layer chromatography R_F as a cerebroside standard. The cholesterol, ceramide, and cerebrosides are expressed as direct weight percentage of total weight of lipids; and the phospholipids, based on phosphorus content, are expressed as percentage of total phosphorus of the remaining weight percentage. The percentage P recovered of the total P applied to the thin-layer chromatography plate for analysis of the phospholipids was 98.8 ± 2.5% on the basis of five individual runs.

follows: 18:0, 19.3% (19.6, 19.2%); 20:4, 11.7% (11.9, 11.6%), and 22:6, 18.4% (18.8, 18.3%).

Determination of Chain-Length Distribution in Acyl, Alk-1-enyl, and Alkyl Groups of PE, PS, and PC. The $LiAlH_4$ reduction products were separated and recovered by preparative thin-layer chromatography as previously described (Snyder, 1969). The alcohols, derived from the fatty acids, were acetylated by heating them for 1 hr at 100° in the presence of 2 ml of acetic anhydride and 0.5 ml of pyridine (Snyder, 1969). The alk-1-enyl glyceryl ethers from the PC were hydrolyzed to aldehydes (Anderson *et al.*, 1969) by scraping the entire thin-layer chromatography band directly into 1 ml of diethyl ether, and then by adding 1 ml of concentrated HCl; after being shaken for 1–2 min, the mixture was extracted with hexane–diethyl ether (1:1, v/v). The aldehydes were kept in CS_2 and analyzed as intact molecules by gas–liquid partition chromatography (Wood and Harlow, 1969). The alkyl glyceryl ethers of the PC from the whole brain lipids were converted to isopropylidene derivatives (Wood, 1967) for gas–liquid partition chromatography analyses.

A dual flame Victoreen gas chromatograph (Model 4000), fitted with 6 ft × 1/8 in. columns packed with 10% EGSS-X on 100–120 mesh Gas-Chrom P, was used for all analyses. The carrier gas flow (He) was 30–35 ml/min, and the injector and detector temperatures were 270 and 280° for all analyses. Fatty aldehydes derived from the alk-1-enyl linked lipids, fatty alcohol acetates derived from fatty acids, and isopropylidene derivatives of *O*-alkylglycerols were analyzed at column temperatures of 175, 190, and 200°, respectively. The gas–liquid partition chromatography peaks were identified by retention times compared with standards. In addition, the alcohol acetates of the fatty acids were hydrogenated in the presence of Adams catalyst and analyzed again by gas–liquid partition chromatography. The analyses after hydrogenation served to substantiate the identification of certain peaks (*i.e.*, 22:A, 22:B, and 22:C are likely 22:4, 22:5, and 22:5.

Results

The synaptic plasma membrane preparations used in this investigation have been previously characterized by their high ATPase and extremely low cytochrome oxidase and actimycin-insensitive NADH oxidase activities (Cotman *et al.*, 1968a). Enzymatic and morphologic analyses indicate that contamination due to mitochondria, endoplasmic reticulum, and myelin is about 10%. Furthermore, the electrophoretic pattern of insoluble proteins and their amino acid content in SPM is unlike that found for mitochondria, myelin, or microsomes (Cotman *et al.*, 1968b). Figure 1, a representative electron micrograph, illustrates the morphology and distinctive membrane character of the SPM fraction used for lipid analyses. The membrane vesicles are about the same size as synaptosomes and some display a synaptic thickening and postsynaptic element. The ratio of lipid to protein, calculated to be 0.89 ± 0.17, indicates that the SPM is about two times richer in lipids than plasma membranes isolated from rat liver (Pfleger *et al.*, 1968) by the same technique.

Phospholipids. The major phospholipids, PE and PC, found in SPM account for 62% of the total membrane lipids (Table I). The PC (34%) is only slightly higher than PE (28%). PI and PS, unresolved in the chromatographic system used, make up approximately 12% of the total membrane lipids; two-dimensional chromatography (Figure 2) indicated that PS comprises the major component of this mixed fraction, but quantitative determinations were not carried out. Sphingomyelin accounts for only 3% of the membrane lipids and lysophosphatidylcholine about 1%. With the exception of

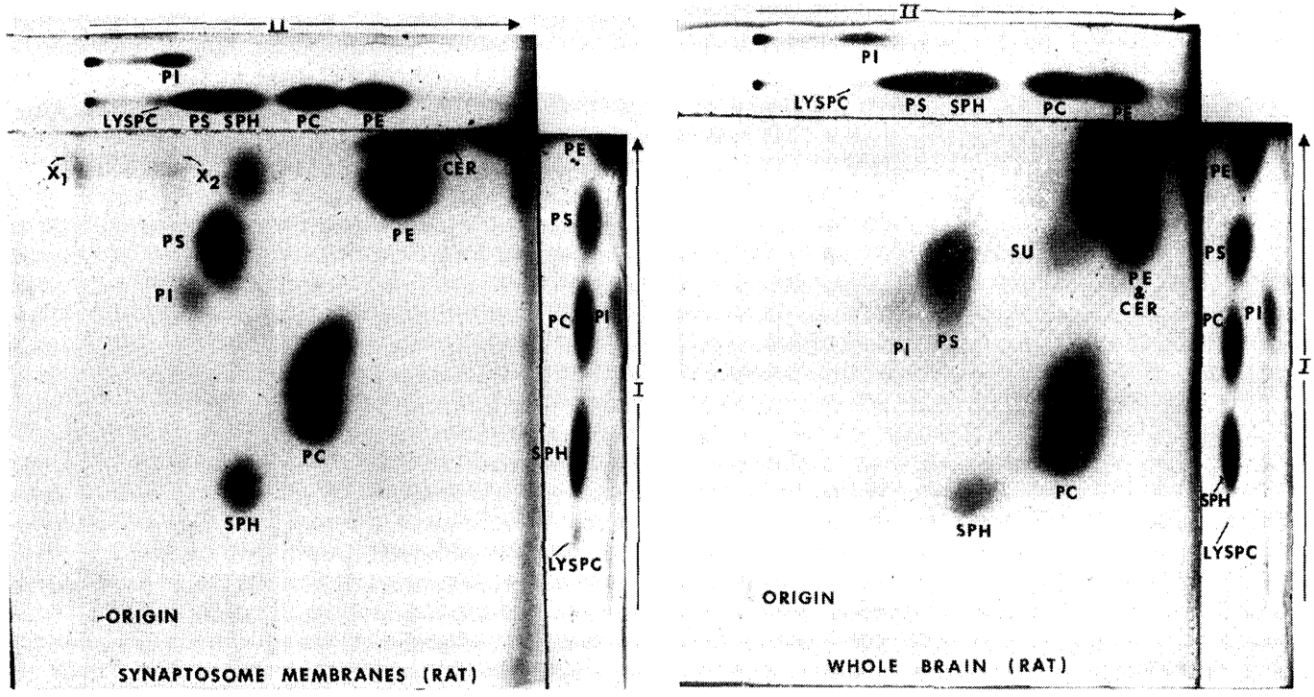

FIGURE 2: Two-dimensional thin-layer chromatograms of total lipids from whole brain (*left*) and synaptic plasma membranes (*right*). The abbreviations refer to the following lipid classes: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; SPH, sphingomyelin; LYSPC, lysophosphatidylcholine; CER, ceramide; SU, sulfatides; X_1 and X_2 are unidentified lipids. The solvent system for direction I was $CHCl_3$–MeOH–HAc–H_2O (50:25:8:4, v/v) and the solvent system for direction II was CH_2Cl_2–MeOH–NH_4OH (65:35:8, v/v). The 250-μ adsorbent layer was silica gel HR.

TABLE II: Quantity of Glyceryl Ethers in PE and PC Lipid Fractions of Whole Brains and SPM of Rats.[a]

	Alk-1-enyl Glyceryl Ether		Alkyl Glyceryl Ether	
	SPM	Whole Brain	SPM	Whole Brain
PE	13.9 (14.2–13.6)	19.4 (20.1–18.8)	1.4 (1.6–1.3)	2.60 (1.95–3.25)
PC	0.08 (Trace–0.17)	0.42 (0.62–0.23)	0.17 (Trace–0.35)	1.50 (1.65–1.34)

[a] Expressed as weight percentage of each class based on the total alkyl and alk-1-enyl glyceryl ethers liberated by $LiAlH_4$ reduction (multiply each value by 2 for an approximation of percentage of intact phosphoglyceride).

the glycolipids ($\simeq$1%) and sphingomyelin ($\simeq$3%), the lipid class composition of the SPM was very similar to whole brain. Two minor unidentified lipids in SPM were detected by two-dimensional thin-layer chromatography (Figure 2). The small quantities present did not permit further analyses; it is not known whether these unidentified lipids might be characteristic of only SPM.

Ether-linked lipids are also present in substantial quantities in the SPM of rat brains. Ether-linked phospholipids are not easily resolved from the major diacyl phospholipid classes by chromatography (Renkonen, 1968); quantitative measurements were therefore based on analysis of alkyl and alk-1-enyl glyceryl ethers liberated from the phosphatides after $LiAlH_4$ reduction. The quantities of alkyl and alk-1-enyl glyceryl ethers in specific classes of phospholipids (PE and PC) were compared to those present in these classes of phospholipids found in whole brain (Table II). The SPM contained considerably lower quantities of glyceryl ethers in the PE and PC fractions than whole brain.

Phosphorus-Free Lipids. Approximately 21% of total membrane lipids occurred in the neutral lipid fraction as cholesterol and ceramide (Table I). The molar ratio of cholesterol to polar lipid is about 0.5. This is lower than that reported for cholesterol to phospholipid ratios for SPM by Lapetina *et al.* (1968) and Whittaker (1966) and for plasma membranes of liver (Pfleger *et al.*, 1968). Glycolipids accounted for only 0.9% of the total lipids of SPM; the glycolipid had an R_F identical with that of cerebrosides used as a standard although the sugar component was not identified.

Aliphatic Moieties of Individual Lipid Classes. The composition of the acyl moieties of the PE, PS + PI, and PC fractions and the composition of the alkyl and alk-1-enyl moieties of PE isolated from the whole brain and from synaptic

TABLE III: Composition of Acyl Chains (weight per cent) in Phospholipids of Synaptic Membranes and Whole Brain of Rats.

Designation of Side Chain	Acyl Moieties					
	Synaptosomes PE	Whole Brain PE	Synaptosomes PS + PI	Whole Brain PS + PI	Synaptosomes PC	Whole Brain PC
16:0	7.5	5.8	3.6	3.5	50.7	39.5
16:1	0.3	0.3	0.3	0.3	1.0	1.2
17:0	0.4	0.1	0.2	0.1	0.2	0.6
18:0	23.7	19.4	40.8	36.2	12.6	14.0
18:1	6.6	19.6	7.2	19.7	24.2	28.8
18:2	0.2	0.5	Trace	0.2	0.6	0.9
20:0	Trace	0.2	Trace	0.2	Trace	0.4
18:3	0.4	4.3	Trace	2.4	1.0	1.9
20:3	0.3	0.6	Trace	0.9	0.1	0.3
20:4	18.0	15.2	10.0	12.3	5.6	6.8
22:A[a]	8.4	7.2	3.8	3.6	0.8	0.4
22:B[a]	1.5	1.0	2.0	1.1	Trace	0.5
22:C[a]	Trace	Trace	Trace	Trace	Trace	Trace
22:6	32.9	25.7	32.2	19.6	3.4	4.6

[a] A, B, and C for the 22 carbon chain are thought to contain four, five, and five double bonds, on the basis of retention time and gas–liquid chromatography analysis of identical sample after hydrogenation. The values represent mean values of two separate brain preparations; the maximum range in variation observed between duplicate samples of the acyl moieties was 2%.

plasma membranes are given in Tables III and IV. The synaptic membranes contain much less 18:1 acyl moieties in the PE and PS + PI fractions than that found in whole brain, whereas the 18:1 acyl moieties in PC are approximately the same for the membranes and the whole brain. Furthermore, the low content of 18:1 chains in PE of SPM is also reflected by the alk-1-enyl moieties. The 18:0 acyl moieties are quite similar for each class of lipids in the membranes and whole brains, but the amount of 18:0 alk-1-enyl moieties of PE is somewhat higher in the membranes when compared with whole brain. The 16:0 acyl moieties are restricted primarily to PC in both the whole brain and membranes, the latter being about 10% higher. The alk-1-enyl moieties in the PE fraction of both membranes and whole brains consist almost exclusively of 16:0, 18:0, and 18:1 chains; longer carbon chains and higher degrees of unsaturation in alk-1-enyl linkage with glycerol are absent.

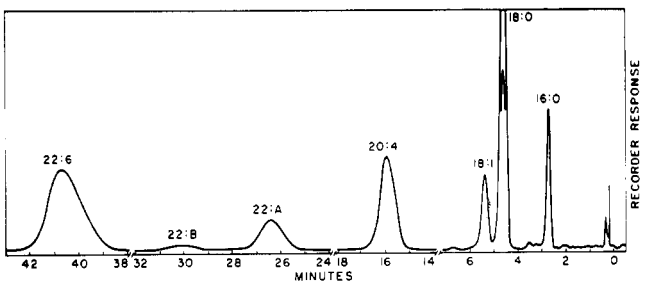

FIGURE 3: Gas–liquid partition chromatogram of methyl esters of fatty acids derived from phosphatidylethanolamine of synaptic plasma membranes. The numbers that identify each peak refer to chain length : number of double bonds.

The fatty acid composition of isolated phospholipids also shows that SPM contains a somewhat greater portion of highly unsaturated fatty acids than whole brain. The 22:6 fatty acids account for better than 32% of the total fatty acids in the PE (Figure 3) and PS + PI fractions of SPM, but in whole brain the 22:6 fatty acids account for only about 26 and 20% of PE and PS + PI, respectively (Table III).

TABLE IV: Composition of Alkyl and Alk-1-enyl Glyceryl Ether Side Chains.

Designation of Side Chain	Alk-1-enyl Moieties[a]		Alkyl Moieties[b]	
	Synaptosomes (PE)	Whole Brain (PE)	Whole Brain (PC)	Whole Brain (PE)
16:0	29.8	22.6	52.5	37.0
16:1			1.5	0.5
17:0	1.0	1.0	0.8	0.8
18:0	54.2	41.2	10.9	29.7
18:1	13.5	34.8	32.8	32.0
18:2 + 19:0	0.6	0.2	1.5	Trace
20:0	0.4	0.2	Trace	Trace
18:3 + 20:1	0.5	0.2	Trace	Trace

[a] The values represent mean values of two separate brain preparations. The average variation between duplicate samples for the three major chain lengths was 3.1%. [b] The values represent an analysis on single samples of brain preparations.

Discussion

The most striking feature of our analyses is the high proportion of longer chain fatty acids with a high degree of unsaturation in the SPM fraction. These fatty acids are primarily acyl moieties of PE and PS + PI. PC, a major phospholipid in SPM, contains only a small amount of long-chain fatty acids. Unsaturated fatty acids of long-chain length are lower in total brain homogenates than in SPM, and lipids in myelin also contain reduced quantities of long-chain fatty acids (G. Rouser, 1969, personal communication; Kishimoto *et al.*, 1969). These data indicate that the longer chain unsaturated fatty acids are at least one distinguishing feature of synaptic plasma membranes, since the longer chain unsaturated fatty acids are not prominent in phospholipids from liver cell plasma membranes (Pfleger *et al.*, 1968). Plasma membranes of erythrocytes (Ways and Hanahan, 1964) also contain lower quantities of longer-chain unsaturated fatty acids than brain SPM. Approximately 60% of the fatty acids of PE in the SPM consist of 20:4 and 22:4–22:6 fatty acids, whereas the PE of the erythrocyte membranes from rat contains less than 44% of 20:4 and 22:4–22:6 fatty acids (Ways and Hanahan, 1964). Kishimoto and coworkers (1969) report the presence of high quantities of 20:4, 22:4, and 22:6 fatty acids in PE and PS of synaptosome (54% of total fatty acids), synaptic vesicle (54%), and terminal "ghost" (44%) fractions. The terminal "ghost" fraction would correspond most closely to our SPM fraction. It is probable, though, that on the basis of the sucrose density at which Kishimoto and coworkers collect their terminal "ghost" fraction that the fraction is heavily contaminated with mitochondria so is not directly comparable to ours.

The presence of longer chain unsaturated fatty acids in SPM probably has important consequences on the physical arrangement of lipids and proteins in these plasma membranes. The degree of unsaturation would be expected to impart steric restrictions and to promote weak hydrogen-bonding interactions. The lack of physical studies on model membranes with these highly unsaturated lipids is probably related to the technical problems of their availability, their ease in autoxidation, and because they have not previously been described as a natural component of excitable membranes. Certain permeability properties of membranes appear to be affected by the degree of unsaturation in fatty acids. Finkelstein and Cass (1968) report that water permeability through artificial membranes made from phosphatidylcholines containing saturated fatty acids is low compared with that of phosphatidyl cholines containing partially unsaturated fatty acids. Furthermore, a requirement for unsaturated fatty acids is necessary for the enzymatic synthesis of lipopolysaccharides in bacterial cell walls. Rothfield and coworkers (Rothfield and Pearlman, 1966; Weiser and Rothfield, 1968) have demonstrated that the enzymatic transfer of sugars to an acceptor lipopolysaccharide does not occur unless certain phospholipids containing unsaturated fatty acids are present. The bacterial cell wall contains a high proportion of unsaturated fatty acids, and Weiser and Rothfield (1968) suggest that these lipids may be necessary to orient and align the lipopolysaccharides to enable it to bind to the soluble transferase enzyme that participates in sugar transferase reactions. Perhaps the unsaturated fatty acids may have a similar function for ganglioside biosynthesis in plasma membranes of brain.

It is not yet known how significant the long-chain unsaturated fatty acids might be in SPM since these fatty acids bound in erythrocyte membranes can be dramatically altered by diet (van Deenen, 1966) and are significantly different in various species (Walker and Kummerow, 1964; de Gier *et al.*, 1966). Furthermore, the content of long-chain polyunsaturated fatty acids varies with age in brain (Rouser *et al.*, 1968) even though the lipid classes of brain are relatively constant from animal to animal (Rouser and Yamamoto, 1968).

In many respects, the lipid class composition of SPM is somewhat similar to plasma membranes of liver and erythrocytes. However, liver plasma membranes contain less PE and more sphingomyelin (Pfleger *et al.*, 1968) and erythrocyte membranes also contain substantially more sphingomyelin than SPM (Rouser and Yamamoto, 1968). Estimates of phospholipids in squid axons show PE and PC to be nearly equal in quantity and to be the major phospholipids (Villegas and Camejo, 1968), as is true for SPM. Whittaker (1966) described a somewhat similar phospholipid class composition for SPM from guinea pigs; PE and PC, about equal in quantity, represented the major phospholipids (54% of total) although phosphatidic acid and also sphingomyelin are reported in significant amounts.

The neutral lipid fraction from SPM consists almost entirely of free sterols, whereas liver membranes contain small but significant amounts of triglycerides and free fatty acids (Skipski *et al.*, 1965; Pfleger *et al.*, 1968). Myelin is quite atypical since it contains large quantities of cholesterol and sphingomyelin but relatively little PE (Eichberg *et al.*, 1964). The only other specific membrane system from brain that has been studied originated from nuclei. In nuclei, 50% of the phospholipid is PC and 25% is PE (Rouser and Yamamoto, 1968); sphingomyelin and PS + PI account for most of the remaining phospholipids in nuclei.

The ether-linked lipids, classes of lipids that occur to the highest extent (as diacyl glyceryl ethers) in neoplasms, are found as phosphatides in a variety of membranes from different normal sources. In phospholipids, the ethers exist primarily in the alk-1-enyl form as PE. Plasma membranes of liver are enriched in the ether-linked lipids when compared to whole liver, whereas they are found in lower quantities in SPM when compared with whole brain.

The narrow range in chain lengths (16:0, 18:0, and 18:1) of both the alkyl and alk-1-enyl moieties and their linkage in only the 1 position of glycerol were found for both brain preparations. These data are typical of those found in other mammalian cells (Snyder, 1969); however, substantially more of the 16:0 chains in PE are in ether linkage than ester linkage. Although the biosynthesis of alkyl glyceryl ethers has only recently been elucidated (Snyder *et al.*, 1969), the function of ether-linked phospholipids in biomembranes is still unknown. We have previously proposed that the ether linkage may be important to the stability and orientation of lipid–protein configurations in biomembranes (Pfleger *et al.*, 1968).

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