Phospholipids and acyl groups in subcellular fractions from human cerebral cortex

Grace Y. Sun

Laboratory of Neurochemistry, Cleveland Psychiatric Institute, Cleveland, Ohio 44109

Abstract Subcellular fractionation of human brain cortex obtained at autopsy yielded microsomal and synaptosome-rich fractions from the gray matter and microsomal and purified myelin fractions from the white matter. The phospholipids of myelin were high in plasmalogens, and the molar ratio of alkenyl acyl *sn*-glycero-3-phosphorylethanolamine to diacyl *sn*-glycero-3-phosphorylethanolamine was 4. The acyl groups of the myelin phosphoglycerides were enriched in monoenes (mainly 18:1 and 20:1) and a tetraene, 22:4(n − 6). The phospholipids in the synaptosome-rich fraction were high in diacyl *sn*-glycero-3-phosphorylcholine, and the molar ratio of the alkenyl acyl *sn*-glycero-3-phosphorylethanolamine to diacyl *sn*-glycero-3-phosphorylethanolamine was 0.88. The acyl groups of synaptosomal ethanolamine phosphoglycerides were rich in 22:6(n − 3) but contained a very low amount of 20:1. The lipid composition of microsomes from the gray matter was different from that of microsomes from the white matter but was nearly identical with that of the synaptosome-rich fraction. Except for a slightly lower proportion of alkenyl acyl *sn*-glycero-3-phosphorylethanolamine and sphingomyelin, the lipid composition of microsomes from the white matter was also similar to that of the myelin. There were also species-related differences between the brain lipid composition of human and subhuman primates and that of the rodents. Furthermore, the brain lipid composition in normal human subjects is rather constant and does not seem to be affected much by individual variations.

Supplementary key words phosphoglycerides · myelin · microsomes · synaptosome-rich fraction

Phospholipids and their nonpolar side chains not only are essential constituents of all cellular membranes but they can affect the functional activities of the membranes. In order to better understand the roles of these lipids, an investigation of the lipid composition in subcellular fractions is essential. The compositions of subcellular lipids in brains of a number of mammalian species have been reported (1–8). However, studies related to lipid composition in gray and white matter of human brain are lacking in detailed characterization of lipids in subcellular fractions (9–18). One of the difficulties in such an investigation with the human brain samples is the uncertainty of membrane integrity and composition in specimens obtained at autopsy. Isolated myelin and microsomes from human brain, however, have been demonstrated to be suitable for various types of chemical analyses (14, 15, 19). Recently, Fewster, Hirono, and Mead (20) have also shown that the ethanolamine phosphoglycerides in human myelin can be preserved for a considerable length of time without obvious degradation. In our laboratory, we have examined also the phospholipid and acyl group compositions of subcellular fractions from a piece of biopsy material obtained from human brain during craniotomy. Results are in good agreement with those obtained from the frozen autopsy samples.

It has been demonstrated that the lipid composition of the gray matter is strikingly different from that of the white matter. Nevertheless, information concerning the microsomal lipids from the gray matter and white matter has not been extensive, and results are often contradictory (1–8, 19). Correlation between the lipid composition of microsomes from the gray matter and white matter with the other subcellular fractions may yield useful information for subsequent metabolic studies related to lipid biosynthesis in brain as well as differences in lipid composition and metabolism in pathological brain samples. Thus, this study is an attempt to use human brain specimens from autopsy to characterize the phospholipids and acyl groups in subcellular fractions of gray matter and white matter.

MATERIALS AND METHODS

Source of material

Portions of the frontal lobes were obtained from autopsies of adult human brains 4–10 hr after death of the

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Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; GPC, *sn*-glycero-3-phosphorylcholine; GPE, *sn*-glycero-3-phosphorylethanolamine; EPG, ethanolamine phosphoglycerides; IPG, inositol phosphoglycerides; SPG, serine phosphoglycerides; PA, phosphatidic acids.

subjects. Brain samples were kindly supplied by Dr. P. Tang from St. Luke's Hospital, Cleveland, Ohio. For the present study, only brain samples with no obvious signs of neurological disease were collected. However, brain samples were collected regardless of sex and race from subjects ranging from 61 to 87 yr of age. Brain samples were frozen immediately after dissection and were stored in sealed plastic containers at −20°C until further chemical analysis.

Subcellular fractionation

For each subcellular fractionation experiment, three pieces of brain tissue were selected from subjects of different ages. The gray matter was then carefully separated from the white matter. The gray matter and the white matter obtained from each brain sample were further divided into three portions weighing approximately 1 g each. Brain tissue was homogenized in 20 vol of 0.32 M sucrose containing 1 mM EDTA and 0.05 M Tris–HCl (pH 7.4). The procedure for subcellular fractionation was described previously (1) except that a discontinuous sucrose density gradient was used. Subcellular fractionation of the white matter yielded mostly myelin and a small white microsomal pellet. The amount of mitochondria from the white matter was too low for chemical analysis and was therefore discarded. Fractionation of the gray matter yielded a grayish microsomal pellet and a large synaptosome-rich fraction. Only a small amount of myelin was present in the gray matter and this fraction was not analyzed. All the myelin fractions were purified by osmotic shock, reflotation on 0.8 M sucrose, and further centrifugation in water to remove the sucrose. The synaptosome-rich fraction was suspended in 0.9% NaCl and resedimented. Typical portions of the isolated fractions were taken for examination of their ultrastructure by electron microscopy. The procedure for fixing and staining the pellets for electron microscopy has been described (21).

Analysis of lipids

The final membrane pellets obtained from subcellular fractionation were each suspended in 7 ml of water. Membrane lipids were extracted by adding to the suspension 35 ml of chloroform–methanol 2:1 (v/v) (22); this was mixed thoroughly two times and was kept overnight at 4°C. After phase separation, the organic phase was filtered and the membrane residue was repeatedly washed with several portions of chloroform–methanol 2:1 (v/v) (23). The organic phases were combined and then evaporated; the lipid residue was dissolved in chloroform and stored at 4°C for chemical analysis. This extraction procedure yielded 95–100% of the total extractable membrane lipids. Further extraction of the aqueous phase with chloroform–methanol 2:1 (v/v) yielded 0–5% of the phospholipids as observed by thin-layer chromatography.

A portion of the total lipid extract was applied to a Unisil silicic acid (Clarkson Chemical Co., Williamsport, Pa.) column for separation of the less polar lipids, galactolipids and phospholipids (24). The phospholipid fraction was further separated by separation–reaction–separation TLC on silica gel G plates impregnated with 0.01 M Na_2CO_3 (23). For the first dimension, the thin-layer plates were developed in a solvent system containing chloroform–methanol–15 N NH_4OH 65:25:4 (v/v/v). Exposure of the plates to HCl fumes after the first dimension quantitatively cleaved the alk-1-enyl groups from the respective phosphoglycerides. The plates were then developed in the second dimension using a solvent system of chloroform–methanol–acetone–acetic acid–water 75:15:30:15:7.5 (by vol). Lipid spots were visualized by exposing the thin-layer plates to iodine vapor. Spots containing the phospholipids were scraped from the thin-layer plates for phosphorus determination (25).

For analysis of the acyl groups, phospholipids were separated by two-dimensional TLC without exposure to HCl fumes. For the first dimension, the solvent system of chloroform–methanol–15 N NH_4OH 65:25:4 (by vol) was used. For the second dimension, thin-layer plates were developed in chloroform–methanol–0.05 M ammonium acetate 100:50:10 (by vol). The lipid spots were visualized by spraying the thin-layer plates with 2% 2′,7′-dichlorofluorescein in ethanol. The procedure for separation of the alkenyl acyl GPE and diacyl GPE for acyl group analysis has been described (26). The acyl groups of the phosphoglycerides were converted to their methyl esters by alkaline methanolysis (23), and analysis of methyl esters by GLC was according to conditions described previously (27). In later preparations, GLC analysis was also carried out with a Hewlett-Packard gas chromatograph model 5750 under similar conditions.

RESULTS

Ultrastructure

Subcellular fractionation of human brain tissue obtained from autopsy yielded homogeneous fractions with satisfactory purity. By separating the gray matter from the white matter before dispersion, possible contamination of the myelin fraction with neuronal membranes and the synaptosome-rich fraction with myelin fragments was largely eliminated. Electron microscopic examination of the purified myelin fraction showed typical multilaminar membrane structures with no obvious contamination by other types of brain membranes (Fig. 1). Myelin fragments isolated from the cerebral cortex had smaller internal diameters and contained fewer lamellae when compared with myelin obtained from the brainstem region. The synaptosome-rich fraction contained mostly pinched off nerve endings composed of bound mitochondria, vesicles, and synaptic contacts (Fig. 2). In this fraction, there might have been present a small amount of the extrasynaptosomal mi-

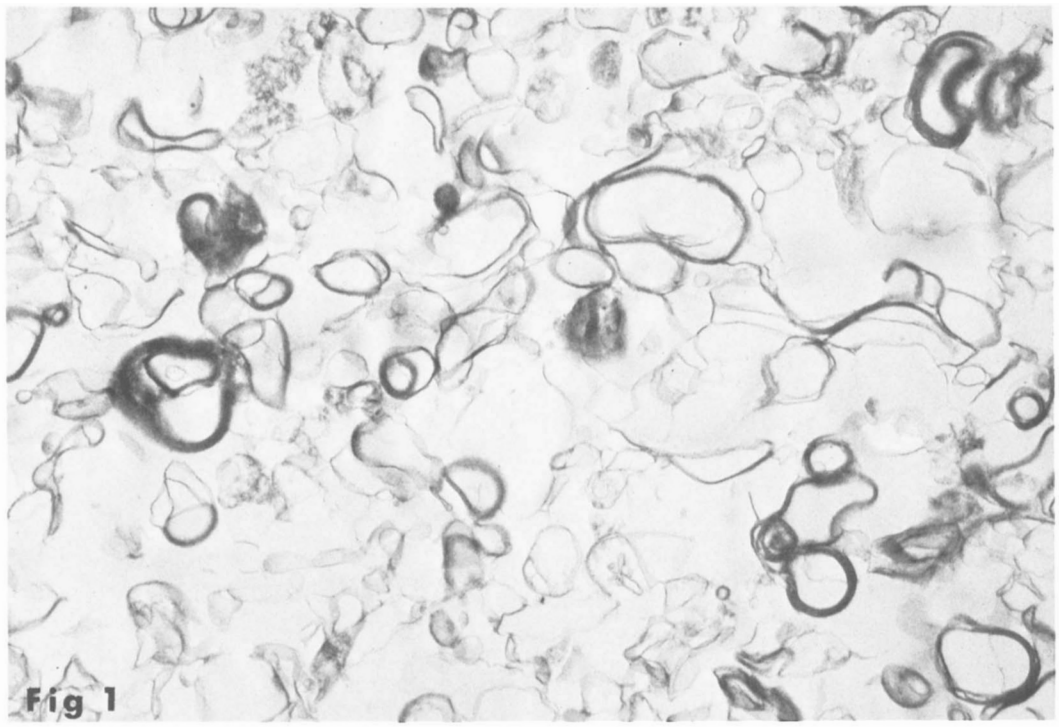

Fig. 1. Electron micrograph of the human myelin isolated from the subcortical white matter. Magnification × 21,600.

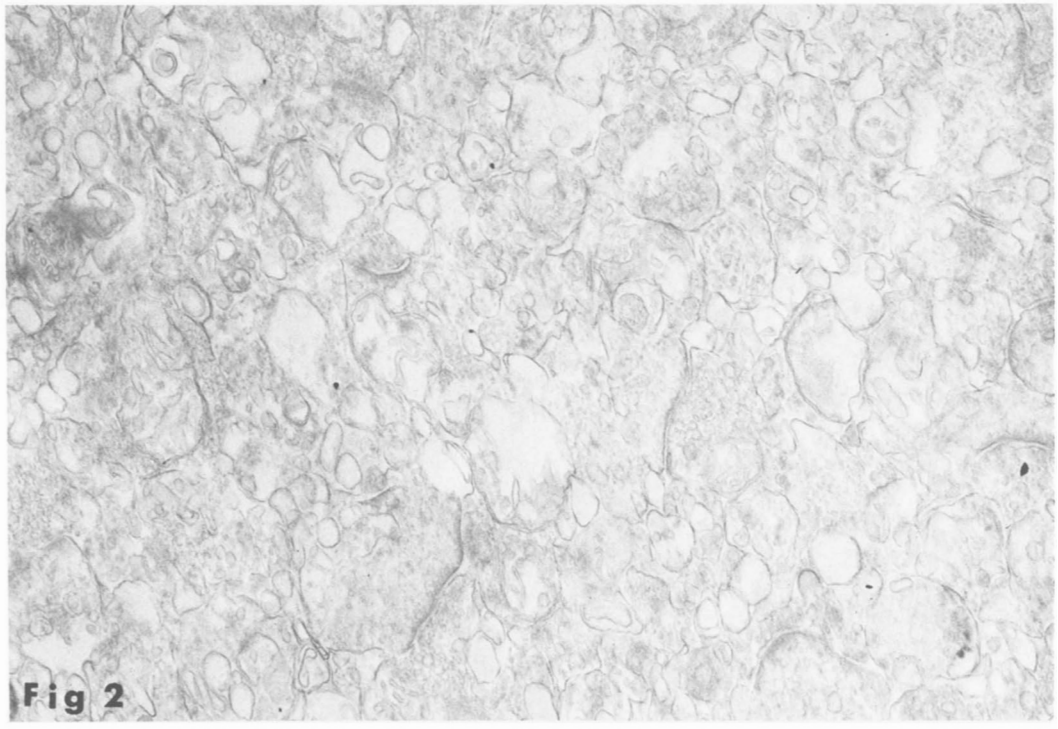

Fig. 2. Electron micrograph of the synaptosome-rich fraction isolated from the cortical gray matter. Magnification × 21,600.

tochondria, but a contamination by the myelin fragments was not seen. The electron microscopic images of the microsomes isolated from the gray matter and white matter were quite different (Fig. 3). The microsomal membranes were vesicular in nature. However, the microsomes from white matter were less well defined and contained some "small myelin" fragments that could have accounted for approximately 10–15% of the total membrane population.

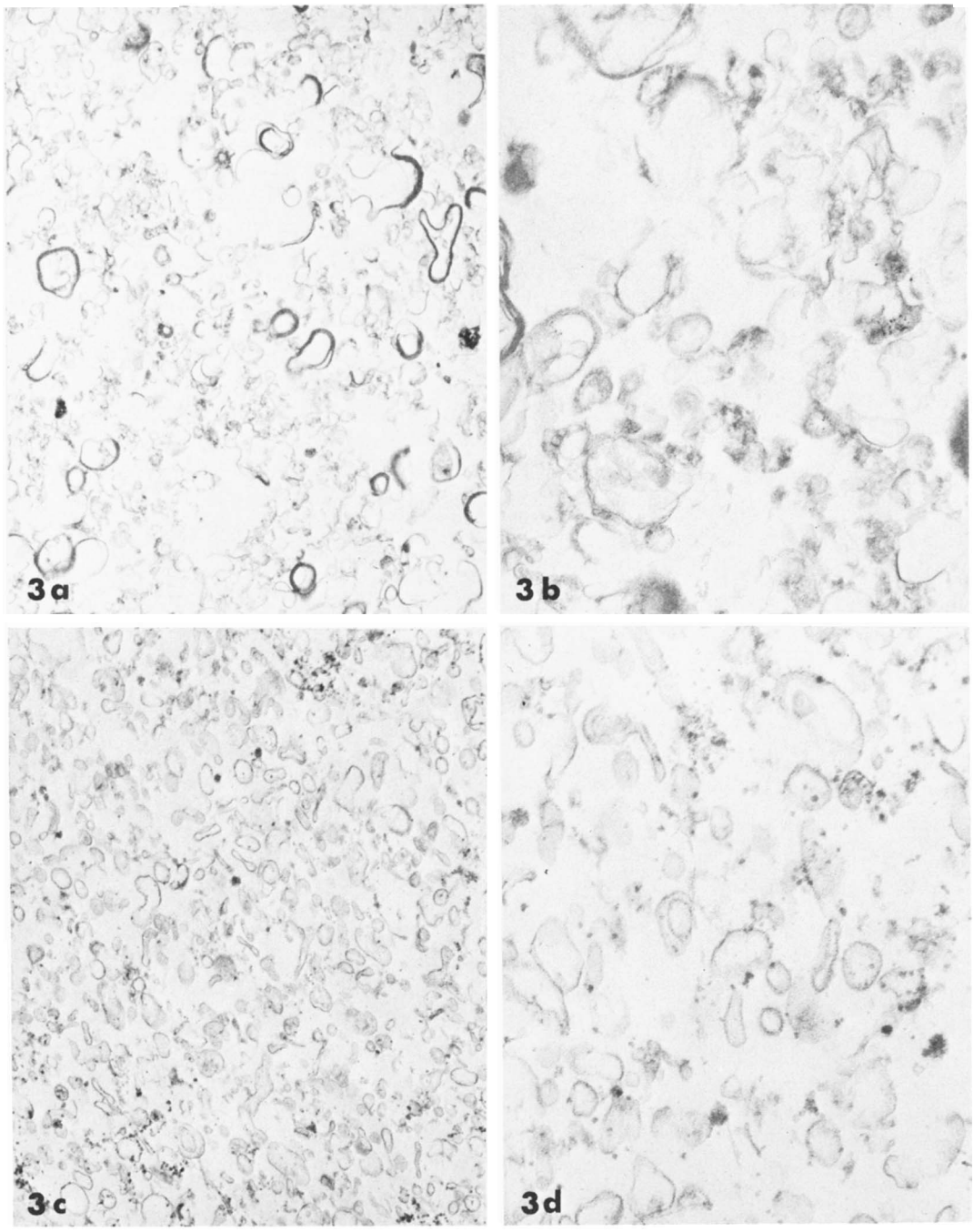

Fig. 3. Electron micrographs of the microsomal fractions from human cerebral cortex. *a*, Microsomes from the subcortical white matter × 21,600; *b*, microsomes from the white matter at higher magnification, × 60,000; *c*, microsomes from the gray matter × 21,600; *d*, microsomes from the gray matter at higher magnification, × 60,000.

Composition of phospholipids

The distribution of phospholipids among the subcellular fractions of human brain is shown in Table 1. The phospholipids of the subcellular fractions from gray matter were rich in diacyl GPC and diacyl GPE. Also, the molar ratios of alkenyl acyl GPE to diacyl GPE in these fractions were less than 1. The phospholipids in subcellular fractions from the white matter contained higher proportions of sphingomyelin and alkenyl acyl GPE than phospholipids in the gray matter. Also, the ratios of alkenyl acyl GPE to diacyl GPE in these fractions were higher than 1. In the gray matter, the distribution of phospholipids in the microsomes and in the synaptosome-rich fraction was almost identical. In the white matter, the phospholipid profile was also similar for the microsomes and the purified myelin except for a lower alkenyl acyl GPE to diacyl GPE ratio and a lower proportion of sphingomyelin in

TABLE 1. Phospholipid compositions of subcellular fractions from human brain cortex

	Myelin (5)[a]	Microsomes		Synaptosome-rich Fraction (15)
		White Matter (10)	Gray Matter (10)	
	% of total lipid phosphorus ± SEM			
SPG	15.6 ± 0.53	18.1 ± 0.73	11.3 ± 0.60	10.6 ± 0.52
IPG	2.8 ± 0.09	4.4 ± 0.23	5.0 ± 0.15	4.2 ± 0.17
Sphingomyelin	12.3 ± 0.80	9.4 ± 0.65	5.6 ± 0.22	5.0 ± 0.25
Diacyl GPC	26.4 ± 0.19	27.8 ± 0.42	37.4 ± 0.50	37.1 ± 0.52
Alkenyl acyl GPE	32.5 ± 1.00	27.9 ± 0.88	17.3 ± 0.25	17.9 ± 0.27
Diacyl GPE	8.3 ± 0.48	10.3 ± 0.36	21.1 ± 0.40	21.9 ± 0.47
Others (PA + CL)[b]	0.9 ± 0.22	2.4 ± 0.21	1.8 ± 0.16	4.1 ± 0.15

Results for myelin were obtained from analyses of material from subjects whose ages were 61–67 yr. Results for the other subcellular fractions were obtained from analyses of material from subjects whose ages were 61–87 yr. Phospholipids were separated by TLC as described in the text. Phosphorus content was determined in individual lipid areas from the thin-layer plates.

[a] The numbers in parentheses represent the number of analyses.

[b] PA, phosphatidic acid; CL, cardiolipin.

the microsomes. Furthermore, the variation in phospholipid composition of these brain membranes among individuals was rather small.

Composition of the acyl groups from phosphoglycerides

A characteristic acyl group profile was associated with each major phosphoglyceride in the white matter and in the gray matter. In the myelin fraction, monoenes (mainly 18:1 and 20:1) constituted 45% of the acyl groups of diacyl GPE and more than 60% of the alkenyl acyl GPE (Table 2). The myelin ethanolamine phosphoglycerides also contained a high proportion of 22:4(n − 6). The acyl groups in the myelin diacyl GPC were composed mainly of 16:0, 18:0, and 18:1. A small amount of 24:4 (n − 6) was also present in the myelin ethanolamine phosphoglycerides and was mainly associated with the alkenyl acyl GPE.

The acyl groups of ethanolamine phosphoglycerides in the synaptosome-rich fraction were characterized by a high proportion of 22:6(n − 3) and a lower proportion of the monoenes (Table 3). In fact, 20:1 was barely detectable in the diacyl GPE. The acyl groups in synaptosomal diacyl GPC were also composed mainly of 16:0, 18:0, and 18:1, but the proportions were different from those in the myelin fraction.

Microsomes from the gray matter had a very different acyl group profile compared with the microsomes from white matter (Table 4). The microsomal ethanolamine phosphoglycerides from the gray matter contained 31.1% 22:6(n − 3) compared with 9.7% in EPG from the white matter microsomes. The ethanolamine phosphoglycerides from the gray matter microsomes contained only trace amounts of 20:1, whereas EPG from the white matter contained 7.1%. On the other hand, the acyl group profiles of the microsomal phosphoglycerides from gray matter were nearly identical with those of the synaptosome-rich fraction. With the exception of a lower proportion of the monoenes and a higher proportion of the 22:6 (n − 3), the acyl group profiles of microsomal phosphoglycerides from the white matter were also similar to those of the myelin.

TABLE 2. Acyl group compositions of major phosphoglycerides from the myelin fraction of human brain subcortical white matter

Acyl Groups	Total EPG (5)[a]	Alkenyl Acyl GPE (3)	Diacyl GPE (3)	Diacyl GPC (5)	SPG + IPG (5)
	% (by wt)				
16:0[b]	2.9	2.7	5.7	31.1	1.3
16:1	0.6			2.8	
18:0	5.8		19.3	12.4	40.1
18:1	42.2	50.0	37.3	48.6	39.9
18:2				0.8	
20:1	8.8	11.2	7.6	1.5	4.5
20:2[c]	1.1	1.2	0.8		0.8
20:3(n − 6)	0.7	0.7	0.5		0.9
20:4(n − 6)	8.6	7.5	9.2	2.0	4.0
22:3[c]	2.1	2.3	1.2		1.4
22:4(n − 6)	20.9	19.5	10.6	1.1	3.4
22:6(n − 3)	5.7	1.9	6.8		2.1
24:4(n − 6)	1.3	0.9			1.9

Material from subjects 61–67 yr of age was used.

[a] Number of analyses.

[b] Number of carbon atoms: number of double bonds.

[c] Position of double bonds was not identified.

TABLE 3. Acyl group compositions of major phosphoglycerides from the synaptosome-rich fraction of human brain gray matter

Acyl Groups	Total EPG (10)[a]	Alkenyl Acyl GPE (6)	Diacyl GPE (6)	Diacyl GPC (10)	SPG + IPG (10)
	% (by weight)[b]				
16:0[c]	5.0	2.5	6.3	45.9	3.0
16:1				2.6	
18:0	26.0	3.7	36.5	11.0	43.2
18:1	11.4	9.3	10.0	30.9	12.7
18:2	0.7		1.9	0.8	
20:1	0.7	2.0	0.7	0.6	
20:3 (n − 6)	0.6		1.4	0.6	
20:4 (n − 6)	13.0	20.4	11.3	4.7	10.8
22:4 (n − 6)	11.6	20.7	7.0	1.0	5.1
22:5 (n − 6)	1.4	1.3	1.7		1.6
22:6 (n − 3)	29.6	36.9	22.7	2.8	22.7

[a] Number of analyses.

[b] The deviation of individual values from the mean did not differ by more than 5%. Subjects ranged in age from 61 to 87 yr.

[c] Number of carbon atoms: number of double bonds.

TABLE 4. Acyl group compositions of major phosphoglycerides from the microsomal fractions of human brain cortex

Acyl Groups	Gray Matter			White Matter		
	Total EPG	Diacyl GPC	SPG + IPG	Total EPG	Diacyl GPC	SPG + IPG
	% (by wt)					
16:0	4.6	46.6	3.1	3.7	36.6	2.1
18:0	24.6	11.7	42.2	9.5	12.2	40.7
18:1	11.6	30.8	9.2	37.7	43.2	32.9
18:2	0.7	0.8	0.8		1.6	
20:1	0.8	0.7		7.1	1.4	3.8
20:3(n − 6)	0.7	0.5	0.8			
20:4(n − 6)	13.0	4.2	13.5	9.8	2.3	7.0
22:3[a]				1.7		
22:4(n − 6)	12.2	1.3	6.1	18.9	2.6	6.1
22:5(n − 6)	1.2		0.9			
22:6(n − 3)	31.1	2.9	23.1	9.7		5.7

Six analyses of each fraction were done.
[a] Position of double bonds was not identified.

Comparison of acyl group composition from subcellular membranes obtained from biopsy and autopsy samples

A piece of brain cortical tissue was obtained from a 36-yr-old subject during surgery. This tissue was fractionated in the same manner as the autopsy specimens. As shown in Table 5, the composition of the acyl groups of ethanolamine phosphoglycerides in the myelin and synaptosome-rich fractions from the biopsy brain material was similar to that of the autopsy material. Since ethanolamine phosphoglycerides in the synaptosome-rich fraction contain a high proportion of the polyunsaturated acyl groups, the results further indicate that the polyunsaturated acyl groups in brain are apparently not affected by the postmortem conditions.

DISCUSSION

Subcellular fractionation of human brain tissue obtained from autopsy yielded homogeneous fractions that were suitable for determination of the lipid composition. The acyl group compositions of membrane fractions from autopsy specimens were nearly the same as those of fresh tissue. The synaptosome-rich membranes are generally characterized by a high level of polyunsaturated acyl groups. With the autopsy samples, we have not observed a decrease in these acyl groups. The morphological features of membranes obtained from postmortem material are not as well defined as those obtained from the fresh brain material; however, examination of these membrane fractions by electron microscopy indicated no evidence of dense body formation as reported by Swanson, Harvey, and Stahl (28) with the guinea pig brains. Evidently, these morphological changes occur only under extreme postmortem conditions, which do not pertain to the present preparations. The ultrastructure of subcellular membranes from the human brain samples is in fact similar to those obtained from fresh brain material from the primates (26).

The microsomes obtained from the cortical gray matter differ in ultrastructural appearance from those obtained from the subcortical white matter (29). Microsomes from the gray matter are not contaminated by myelin fragments, but the presence of a small amount of synaptosomal plasma membranes cannot be eliminated. The microsomes from human subcortical white matter are similar to those previously isolated from the bovine white matter (19). However, a small amount of myelin fragments is present.

Since postmortem changes in enzymic activities have been known to occur with frozen tissue samples, the purity of the subcellular fractions should not be evaluated by assaying the activities of marker enzymes. However, except for some contamination of myelin in the microsomal fraction obtained from the gray matter, other subcellular fractions isolated by the present procedure were pure as judged by their ultrastructural appearance and results of the chemical analysis. Analysis of the phospholipids and

TABLE 5. Acyl group compositions of ethanolamine phosphoglycerides from subcellular fractions of human brain tissues: comparison between tissues obtained from autopsy and biopsy

Acyl Groups	Myelin		Synaptosome-rich Fraction	
	Biopsy[a]	Autopsy[b]	Biopsy[a]	Autopsy[b]
	% (by wt)			
16:0	3.3	2.9	4.5	5.0
18:0	6.8	5.8	22.1	26.0
18:1	38.8	42.2	14.8	11.4
18:2	0.6		1.6	0.7
20:1	6.9	8.8	1.7	0.7
20:3(n − 6)	1.1	0.7	1.0	0.6
20:4(n − 6)	13.1	8.6	13.4	13.0
22:3[c]	1.0	2.1		
22:4(n − 6)	19.8	20.9	13.9	11.0
22:5(n − 6)	0.3		1.4	1.4
22:6(n − 3)	6.5	5.7	25.8	29.6
24:4(n − 6)	0.9	1.3		

[a] Biopsy sample was obtained from a 36-yr-old subject with no apparent neurological abnormality.
[b] Results from autopsy samples are mean percentages (5–10 subjects, 61–87 yr of age).
[c] Position of double bonds was not identified.

acyl groups from myelin and synaptosome-rich fractions indicated characteristic profiles. The results are in good agreement with results reported previously for both myelin (9, 10, 14) and human brain white matter (11, 12). The lipid profiles for the human myelin are also similar to those of the myelin isolated from the squirrel monkeys (26, 30) and rhesus monkeys (31). However, species-related differences are evident. In comparison with the rodents, myelin from the human and subhuman primates has a higher level of sphingomyelin, a higher molar ratio of alkenyl acyl GPE to diacyl GPE, and, among the acyl groups, a higher proportion of 22:4(n − 6) and a lower proportion of 20:1 (1, 3, 7). Furthermore, the acyl group composition of the phosphoglycerides from the human myelin is more complex in nature and there are higher proportions of the minor components.

The high content of 22:6(n − 3) acyl groups in the phosphoglycerides of the synaptosome-rich fraction may have important implications to the functional aspects of the neurons (32). The phospholipid and acyl group compositions of the synaptosome-rich fraction are in good agreement with those obtained previously for the rat (3) and for human gray matter (11, 12). Since there is no major difference in lipid composition among the subsynaptic components (33), our results are also in good agreement with data reported for the synaptosomal plasma membranes from rat (33, 34) and squirrel monkey (26). Thus, the slight difference in acyl group composition between the synaptosomes from human and the synaptosomal plasma membranes from the rat (3, 33, 34) is most probably due to a species-related difference. Results here also indicated that the 20:1 acyl group, which is an important component of the myelin and microsomes from the white matter, is found only as a trace component in the membranes of the gray matter. Thus, the presence of the 20:1 acyl group in the synaptosomal diacyl GPE can be used as a chemical marker to detect contamination due to white matter membranes.

Most previous studies on microsomal lipids have used brain tissue containing variable proportions of gray and white matter. Thus, results reported previously are not comparable with the present data. We have demonstrated that microsomes from the gray matter and the white matter differed not only in their ultrastructure but also in chemical composition. However, microsomes from the gray matter have lipid profiles similar to the synaptosome-rich fraction, and those from the white matter are similar to the myelin. Since the gray matter is made up largely of neuronal cells and the white matter of oligodendroglial cells, microsomes from different cell types may have different structures and functions. In our results, although the microsomes from white matter had a slightly higher proportion of 22:6(n − 3) and a lower ratio of alkenyl acyl GPE to diacyl GPE than that of the myelin, such differences do not indicate that in the same cell type microsomes would have a characteristic lipid composition different from other types of cellular membranes. However, it is possible that in the white matter there may be present other cell types besides the oligodendroglial type. Thus, the microsomes are obtained from a mixed cell population. Another possibility is that besides myelin there are present in the oligodendroglial cells other organelles such as mitochondria, which may have a characteristic lipid composition different from that of the microsomes (13). These membrane lipids are in constant exchange with those of the microsomes (35).

It has been demonstrated that the lipid composition of subcellular brain membranes from rodents may be altered depending on the intake of dietary fatty acids (36). However, results from the present study indicated no obvious increase in 20:3(n − 9) in brain lipids among the individual subjects. In fact, the brain lipid profiles obtained from the individuals were surprisingly constant and exhibited no obvious sex-related difference. There are, however, acyl group differences in brain during development and perhaps during maturation and senescence (11, 31, 37). The consistency in membrane lipid composition among different subjects further demonstrates that information yielded by the present method may be useful for comparison with results obtained from pathological brain tissues and for subsequent metabolic studies.

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