Different metabolic rates for arachidonoyl molecular species of ethanolamine glycerophospholipids in rat brain

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Abstract The ethanolamine glycerophospholipids (EGP) contain most of the arachidonate (20:4, n-6) and adrenate (22:4, n-6), potential precursors of biologically potent prostaglandins and related compounds. Much better methods utilizing high performance liquid chromatography (HPLC) techniques are now available for the study of the molecular species of all three classes, namely diacyl, alkenylacyl (plasmalogen), and alkylacyl. Different molecular species may have different functions. This possibility was studied by examining the rates of incorporation of [^{3}H]arachidonic acid into the three major molecular species of each of the three classes of ethanolamine glycerophospholipids. After the intracerebral injection of [^{3}H]20:4 into rat brain, it was rapidly converted to 22:4(n-6). Of the total radioactivity, 10–20% was located in 22:4 in alkenylacyl and diacyl-GPE. In the alkylacyl-GPE, labeled 22:4 was preferentially incorporated and accounted for 50–60% of the total radioactivity. The primary arachidonoyl molecular species of alkenylacyl, alkylacyl, and diacyl-GPE were the 18:1–20:4, 16:0–20:4, and 18:0–20:4 species. The alkylacyl class contained almost equal proportions of these three molecular species. On the other hand, the 20:4 in alkenylacyl and diacyl classes was combined largely with 18:0 groups at the *sn*-1 position. In particular, the 18:0–20:4 species comprised about 80% of arachidonoyl molecular species of the diacyl class. In all three classes, the highest specific radioactivities were found in the 18:1–20:4 species, whereas the 18:0–20:4 species had the lowest specific radioactivity. Over the period 60 min–24 hr, the diacyl 18:0–20:4 and all three arachidonoyl molecular species of the alkenylacyl class increased in specific radioactivity more rapidly than the other arachidonoyl molecular species. — **Nakagawa, Y., and L. A. Horrocks.** Different metabolic rates for arachidonoyl molecular species of ethanolamine glycerophospholipids in rat brain. *J. Lipid Res.* 1986. **27:** 629–636.

Supplementary key words ether lipids • molecular species • arachidonic acid • adrenic acid • plasmalogen

In mammalian tissues, 20:4, the precursor of prostaglandins, is mainly esterified in glycerophospholipids. The metabolism of this highly polyunsaturated fatty acid in brain glycerophospholipids has been studied extensively by a number of investigators (1–4). Rat brain EGP are rich in the molecular species containing 20:4, but these glycerophospholipids incorporate much less radioactive 20:4 during initial time periods than do the choline glycerophospholipids (4).

Little is known about the mechanisms that control the distribution of 20:4 among glycerophospholipids. More exact knowledge of the metabolism of 20:4 in glycerophospholipids requires resolution of the complex mixture of various kinds of molecular species containing 20:4. The relative metabolic activities of the palmitoyl and stearoyl pairs of unsaturated choline and ethanolamine glycerophospholipids have been found to be quite different (5–7), but all of the major individual molecular species containing 20:4 were not separated in these studies. For the separation of individual molecular species of glycerophospholipids, two methods have been used: silver nitrate and reversed-phase thin-layer chromatography (8) or countercurrent distribution (9). Both procedures fail to separate mixtures of certain components, (e.g., 1-palmitoyl, 1-stearoyl, and 1-oleoyl-2-arachidonoyl glycerophospholipids) and also fail to resolve the ether classes from the diacyl class. The separation of these classes and their molecular species is important for analyses of the brain EGP, because the brain EGP is enriched in alkenylacyl compounds (10). Recently, we have developed a method for the separation of individual molecular species of alkenylacyl, alkylacyl, and diacyl-GPE using reverse-phase HPLC (11).

Prostaglandins are formed from free 20:4 in neural tissues (12). Very small concentrations of free 20:4 are found in normal rat brain (13), but stimulation can cause the release of 20:4 from glycerophospholipids (13, 14). The elongation product, 22:4 (n-6), can also be a substrate for the formation of prostaglandins (15). In neural tissues, the EGP, particularly the plasmalogens, contain the largest amounts of 20:4 and 22:4, and thus are likely sources of

Abbreviations: CGP, choline glycerophospholipids; EGP, ethanolamine glycerophospholipids; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; HPLC, high performance liquid chromatography. The fatty acids are identified by the number of carbon atoms and double bonds. Thus, 18:1 represents oleic acid which contains 18 carbon atoms and one double bond. The molecular species of glycerophospholipids are identified by the fatty chain at the *sn*-1 position (left-hand side) and the fatty acid at the *sn*-2 position (right-hand side) of the glycerol moiety. Thus 18:1–20:4 represents the 1-oleoyl,2-arachidonoyl molecular species of a glycerophospholipid.

these prostaglandin precursors (16). Mechanical damage of the spinal cord causes the degradation of ethanolamine plasmalogens: 16% degraded at 5 min without significant decreases in any other phospholipid class (17). Large quantities of 20:4 were released simultaneously.

The differences in catabolism of the diacyl and plasmalogen types of the EGP suggest different functions for them. Because the alkylacyl type is a metabolic precursor of the alkenylacyl type (10), the study of the metabolism of 20:4 molecular species of the three types may help to clarify their function. In this study, we have investigated the heterogeneity of the metabolism of individual arachidonoyl molecular species of EGP in rat brain.

MATERIALS AND METHODS

[5,6,8,9,11,12,14,15-^{3}H]Arachidonic acid [20:4(n-6)] (100 Ci/mmol) was purchased from New England Nuclear (Boston, MA). HPLC-grade organic solvents were purchased from EM Science (Gibbstown, NJ) except for methyl-*t*-butyl ether which was from Burdick and Jackson Labs (Muskegon, MI). Distilled water was purified using a Milli-Q system plus an Organex-Q cartridge (Millipore Co., Bedford, MA). Any remaining organic materials were removed with an Organic-pure water purifier (Barnstead, Boston, MA). HPLC was done with two solvent-delivering systems (Model 100, Altex Scientific Co., Berkeley, CA) and microprocessor (Model 420, Altex). A model LC-75 detector (Perkin-Elmer, Norwalk, CT) was operated at 205 nm. The column block heater was from Jones Chromatography Co. (Columbus, OH). Fatty acid methyl esters were analyzed by gas-liquid chromatography (Model GC-8A, Shimadzu Co., Kyoto, Japan) equipped with a glass column (5.4 m × 2.6 mm ID) packed with 10% Alltech CS 10 on 100/120 mesh Chromosorb W (Alltech Associates, Deerfield, IL). Integration for the quantitation of fatty acid methyl esters was performed with software and an A/D 760 interface from Nelson Analytical (Cupertino, CA) on an HP-85 desk top computer (Hewlett-Packard, Palo Alto, CA). [^{3}H]Labeled samples were counted in an LS 7000 liquid scintillation counter (Beckman Instruments, Berkeley, CA) after the addition of Aquasol-2 scintillation cocktail (New England Nuclear).

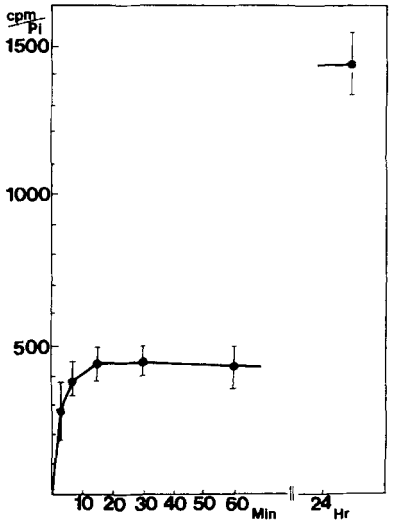

Fig. 1. Specific radioactivity of EGP of rat brain as a function of time after intracerebral injection of [^{3}H]20:4. Each point represents the mean value of three different rat brain samples.

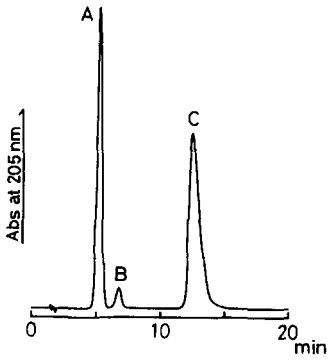

Fig. 2. Separation of alkenylacyl (A), alkylacyl (B), and diacyl (C) compounds by normal-phase HPLC on a μPorasil column. The solvent system used was cyclopentane–hexane–methyl-*t*-butyl ether–acetic acid 73:24:3:0.8 (by vol) at a flow rate of 2 ml/min. Other details are described in Materials and Methods.

Rats weighing approximately 250 g (Sprague-Dawley) were used for the experiments. [^{3}H]20:4 was purified by thin-layer chromatography before use. After evaporation of the organic solvent, the labeled 20:4 was complexed with bovine serum albumin (0.3 mM) in saline. Each rat was injected intracerebrally with 10 μl (12.5 μCi) of 20:4 solution. They were decapitated in groups of three at 3, 6, 15, 30, and 60 min, and 24 hr after injection. Immediately following dissection, each rat brain was dispersed in 20 volumes of ice-cold saline by homogenator (Polytron, Brinkman Instruments, Westbury, NY). Total lipids were extracted by the method of Bligh and Dyer (18). EGP was separated and purified by diethylaminoethyl cellulose (DEAE-cellulose) and subsequent silicic acid column chromatography (19). The purity of the EGP was confirmed by obtaining a single spot on thin-layer chromatography. 1-Acyl, 1-O-alkyl-, and 1-O-alkenyl-2-acyl-3-acetyl glycerols were prepared from the EGP as described previously (19). Purified EGP was dissolved in diethyl ether and 0.1 M Tris–HCl buffer (pH 7.4). Phospholipase C (1–3 mg, EC 3.1.4.3, *Bacillus thuringiensis*) was added to the incubation mixture. After stirring at 37°C for 6 hr, the diethyl ether layer was removed and spotted on a silica gel plate for checking complete hydrolysis of EGP. The 1,2-diradylglycerols (radyl can be acyl, alkenyl, or alkyl) were acetylated with 0.5 ml of acetic anhydride and 0.1 ml of pyridine at 37°C for 3 hr. After evaporation, the residue was dissolved in HPLC-grade hexane for HPLC

TABLE 1. Relative amounts of alkenylacyl, alkylacyl, and diacyl classes in rat brain EGP and the composition of the fatty acids at the *sn*-2 position

		Composition[a]									
		16:0	18:0	18:1	18:2	20:1	20:3	20:4	22:4	22:5	22:6
		%									
Alkenylacyl	(45.6 ± 1.8)[b]	2.9 ± 0.3	2.2 ± 0.3	30.6 ± 1.7	0.3 ± 0.1	7.8 ± 0.6	1.0 ± 0.2	16.4 ± 0.6	11.6 ± 0.4	0.6 ± 0.1	26.7 ± 1.5
Alkylacyl	(5.6 ± 0.9)[b]	7.9 ± 2.3	n.d.	29.9 ± 0.9	0.5 ± 0.1	8.8 ± 0.3	0.9 ± 0.2	13.6 ± 1.3	14.5 ± 1.0	0.6 ± 0.2	23.2 ± 2.4
Diacyl[c]	(48.9 ± 1.3)[b]	3.1 ± 2.0	n.d.	14.6 ± 3.6	1.6 ± 0.2	2.8 ± 0.4	0.6 ± 0.2	25.4 ± 0.6	6.4 ± 0.6	1.4 ± 0.4	45.6 ± 0.6

[a] Values are expressed as the mean percentage ± SD (n = 6) obtained from six different rats.
[b] The values in parentheses show the relative percentage of each subclass of the EGP.
[c] The composition of fatty acids at the *sn*-2 position of diacyl compounds was determined according to previous methods (16). EPG was hydrolyzed by snake venom phospholipase A_2 (*Naja naja atra*). The resulting lysoglycerophospholipid was purified by thin-layer chromatography and was transmethylated to determine the fatty acid composition at the *sn*-1 position of diacyl-GPE. The fatty acid composition at the *sn*-2 position of diacyl-GPE was calculated from analyses of the total fatty acids and the fatty acids at the *sn*-1 position.

separation of alkenylacyl, alkylacyl, and diacyl classes according to the methods of our previous paper (11). The acetylated diradylglycerols were resolved into three classes by normal-phase HPLC on a 3.9 mm × 30 cm μPorasil column (Waters Associates Inc., Milford, MA). The solvent system was cyclopentane–hexane–methyl-*t*-butyl ether–acetic acid 73:24:3:0.03 (by vol) pumped at a flow rate of 2 ml/min at 36°C. After the collection of alkenylacyl, alkylacyl, and diacyl fractions, the arachidonoyl molecular species of each class were separated by reverse-phase HPLC on a 4.6 mm × cm Zorbax ODS column (DuPont Co., Wilmington, DE) (11). For the separation of arachidonoyl molecular species of alkenylacyl and alkylacyl compounds, the solvent system was acetonitrile–2-propanol–methyl-*t*-butyl ether–water 63:28:7:2 (by vol). The flow rate was 1 ml/min and the column temperature was 33°C. Detection of each peak was by absorption at 205 nm. Fractions from the column were collected for the quantitation of each peak by gas–liquid chromatography analysis. After transmethylation with 0.5 M NaOH in methanol for 30 min at room temperature, the quantities of alkenylacyl, alkylacyl, and diacyl classes and their molecular species were determined by gas–liquid chromatography of the fatty acid methyl esters; 17:0 methyl ester was used as the internal standard. HPLC on a Zorbax ODS column (4.6 mm × 15 cm) was also used for the separation of fatty acid methyl esters according to the method of Aveldaño, VanRollins, and Horrocks (20). Fatty acid methyl esters from alkenylacyl, alkylacyl, and diacyl compounds dissolved in acetonitrile were injected, then eluted with 100% acetonitrile. The flow rate was 0.6 ml/min and the column temperature was 28°C. Fractions

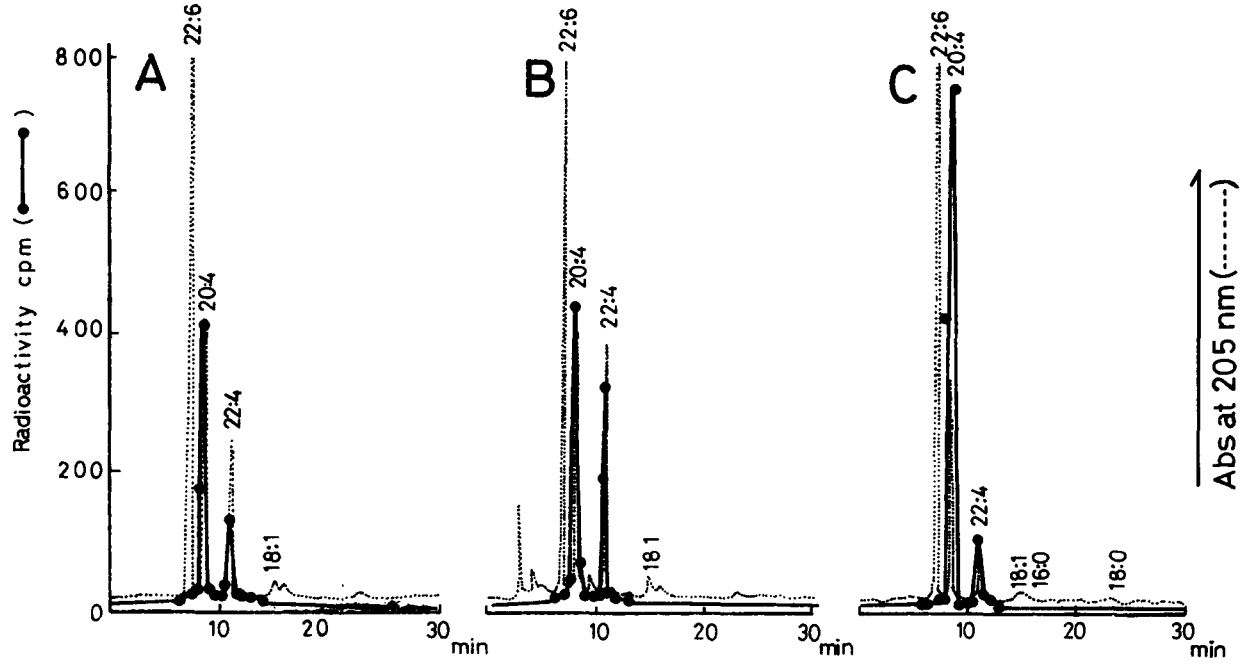

Fig. 3. Distribution of radioactivity among the fatty acid methyl esters from alkenylacyl (A), alkylacyl (B), and diacyl (C) GPE of rat brain 1 hr after intracerebral injection of [^{3}H]20:4. Fatty acid methyl esters prepared by alkaline methanolysis of alkenylacyl, alkylacyl, and diacyl-GPE were separated by HPLC on a Zorbax ODS column using 100% acetonitrile. The flow rate was 0.6 ml/min. Fatty acid methyl esters were detected by absorbance at 205 nm (- - - - -) and radioactivity was measured by liquid scintillation counter after collection of 0.4-ml fractions of effluent (● — ●).

of 0.4 ml were collected and the radioactivity was counted in a scintillation counter after the addition of 5 ml of liquid scintillation cocktail. Specific radioactivities were calculated from these results.

RESULTS

Each animal was given an intracerebral injection of [^{3}H]20:4 complexed with bovine serum albumin and was killed at 3, 6, 15, 30, and 60 min, and 24 hr. The incorporation of [^{3}H]20:4 into EGP increased rapidly in the first 15 min after injection and then increased gradually thereafter (**Fig. 1**). The specific radioactivity at 24 hr was approximately 3 times higher than that at 60 min. The 1,2-diradyl-3-acetylglycerols were separated into alkenylacyl, alkylacyl, and diacyl classes with normal-phase HPLC on a μPorasil column (**Fig. 2**). The alkenylacyl and diacyl classes accounted for 45.6 and 48.9% of the total EGP of rat brain (**Table 1**). Recoveries were essentially complete for HPLC with an average of 99.5% as determined by gas–liquid chromatography. The compositions of the fatty acids at the 2-position of the alkenylacyl, alkylacyl, and diacyl-GPE are shown in Table 1. The fatty acid composition of the diacyl-GPE was quite different from those of the ether-linked classes. The predominant fatty acids in the diacyl class were 20:4 and 22:6, comprising more than 75% of the total fatty acids at the *sn*-2 position. On the other hand, the amounts of such polyunsaturated fatty acids, except for 22:4, in the alkenylacyl and alkylacyl classes were relatively small when compared with the diacyl class. Ether compounds contained considerable amounts of 16:0 and 18:1 at the *sn*-2 position. Fatty acid methyl esters from the alkenylacyl, alkylacyl, and diacyl compounds were separated with reverse-phase HPLC for determination of the distribution of ^{3}H. **Fig. 3** shows the chromatograms of fatty acid methyl esters from the alkenylacyl, alkylacyl, and diacyl classes and the distribution of radioactivity among fatty acids labeled with [^{3}H]20:4 for 1 hr. The ^{3}H was found exclusively in 20:4 and 22:4, the elongation product from 20:4. No significant radioactivity was found in other fatty acid methyl esters. The percentage of ^{3}H in the 20:4 was 80–90% of the total radioactivity in the alkenylacyl and diacyl classes

TABLE 2. Distribution of total ^{3}H in arachidonate

Time	Alkenylacyl	Alkylacyl	Diacyl
		%	
3 min	81.6 ± 2.6	42.2 ± 5.5	90.6 ± 1.4
30 min	78.8 ± 4.1	47.8 ± 9.2	85.3 ± 1.1
60 min	82.6 ± 4.4	48.5 ± 8.5	87.3 ± 3.6
24 hr	80.4 ± 1.7	44.6 ± 3.8	86.5 ± 2.2

All values are given as the mean ± SD and are derived from three separate experiments.

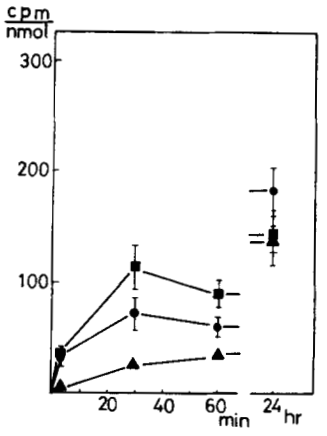

Fig. 4. Specific radioactivity of alkenylacyl (▲), alkylacyl (■), and diacyl (●) GPE of rat brain after injection of [^{3}H]20:4. The radioactivity of 20:4 was determined after the separation of 20:4 from total fatty acids with reverse-phase HPLC as described in Fig. 3. The specific radioactivity is expressed as cpm [^{3}H]20:4 divided by the nmoles of 20:4 in each class of EGP.

at all incubation times, but 50–60% of the total radioactivity in the alkylacyl class was in 22:4 with the remainder in 20:4 (**Table 2**).

The specific radioactivities of 20:4 in the alkenylacyl, alkylacyl, and diacyl classes were calculated (**Fig. 4**). The incorporation rate of [^{3}H]20:4 into the alkylacyl and diacyl classes increased rapidly in the initial 30 min, then gradually increased up to 24 hr. The radioactivity incorporated into the alkenylacyl class increased slowly during the first 60 min. At 24 hr, the specific radioactivity of the alkenylacyl class had increased to a greater extent than that of the alkylacyl and diacyl classes and at that time was not significantly different from them.

The arachidonoyl molecular species were separated from the purified alkenylacyl, alkylacyl, and diacyl compounds with reverse-phase HPLC (**Fig. 5**). The 18:0–20:4, 16:0–20:4, and 18:1–20:4 species were resolved. The content of each arachidonoyl molecular species in the alkenylacyl, alkylacyl, and diacyl classes was determined by gas–liquid chromatography (**Table 3**). All three classes of EGP contained the three kinds of arachidonoyl molecular species, although the distribution of the molecular species was clearly different in each class. The alkylacyl class contained almost equal proportions of 18:0–, 16:0–, and 18:1–20:4 species; however, in the alkenylacyl and diacyl classes, 20:4 was preferentially coupled with a 18:0 fatty chain. The 18:0–20:4 species comprised about 80% of the total arachidonoyl molecular species in the diacyl class.

Fig. 6 shows the incorporation rate of [^{3}H]20:4 into the 18:0–20:4, 16:0–20:4, and 18:1–20:4 species of the three classes of EGP. In all classes, the highest specific radioactivity was found in the 18:1–20:4 species, while the 18:0–20:4 species had the lowest specific radioactivity at all incubation periods. The specific radioactivity of the diacyl 18:1–20:4 species was almost 7 times higher than

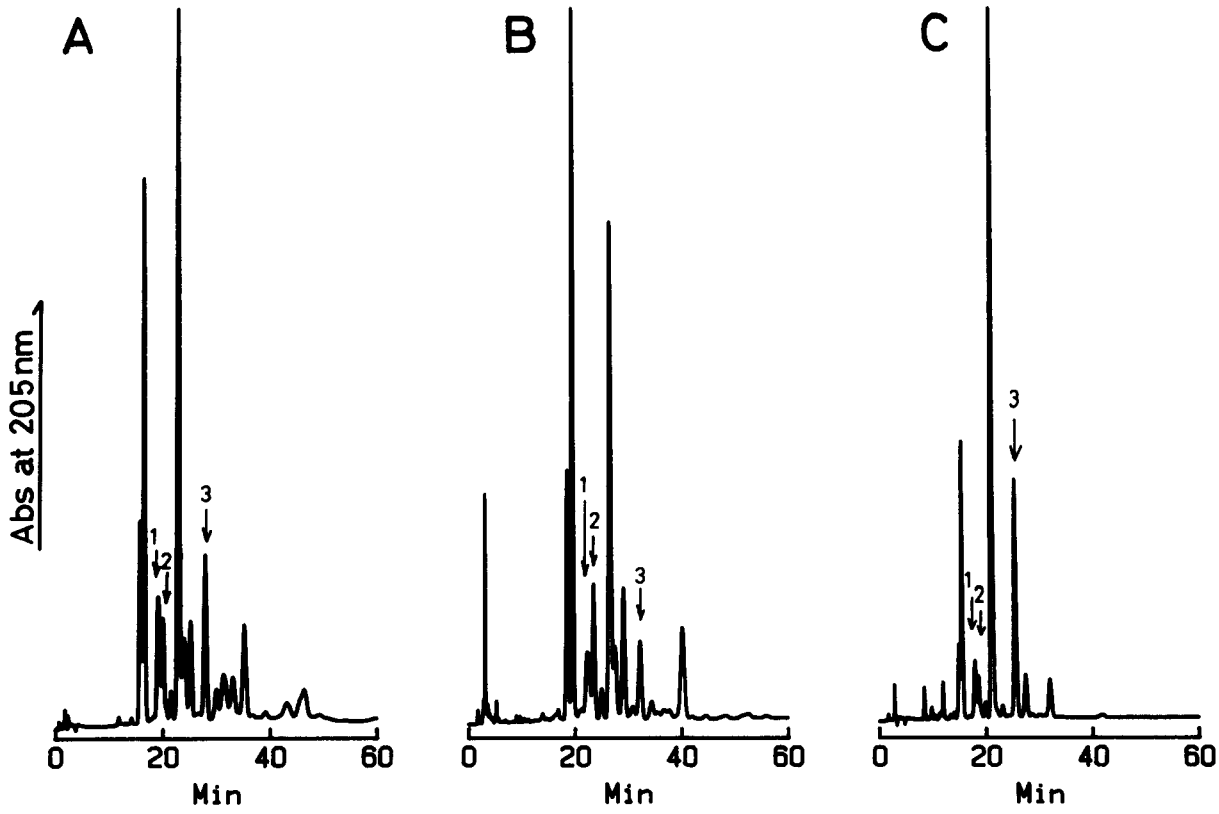

Fig. 5. Separation of molecular species of alkenylacyl (A), alkylacyl (B), and diacyl (C) GPE with reverse-phase HPLC. The solvent system was acetonitrile–2-propanol–methyl-*t*-butyl ether–water 63:28:7:2 (by vol) for the separation of alkenylacyl and alkylacyl compounds and 72:18:8:2 for the separation of diacyl compounds. The flow rate was 1 ml/min. Arachidonoyl molecular species were resolved into the 18:1–20:4 (No. 1), 16:0–20:4 (No. 2), and 18:0–20:4 (No. 3) species which correspond with peaks 4, 5, and 12 in Fig. 2 of ref. 11. The other peaks are other unsaturated molecular species. The highest peak heights correspond to several molecular species containing 22:6 which have a very high molar absorbance value at 205 nm. Although some other molecular species may have overlapped with those containing arachidonate, there was no interference with the determination of the specific radioactivity of the arachidonate within the collected fractions.

that of the 18:0–20:4 species which accounts for the largest proportion of the arachidonoyl molecular species in the diacyl class. It is interesting to note that the specific radioactivity of the 18:0–20:4 species of the diacyl class was similar to that of the 18:1–20:4 and 16:0–20:4 species of the alkenylacyl class, although the specific radioactivity of the total alkenylacyl class was much lower than that of the total diacyl class. Over the period 60 min to 24 hr, the specific radioactivity of all three arachidonoyl molecular species increased equally by 5–8 times in the alkenylacyl class and by 2 times in the alkylacyl class. In the diacyl class, the 18:1–20:4 and 16:0–20:4 species increased twofold in specific radioactivity over the same time interval, but the specific radioactivity of the 18:0–20:4 species at 24 hr was sixfold greater than that at 60-min labeling.

New HPLC methods were very useful for the separation of alkenylacyl, alkylacyl, and diacyl classes and also their molecular species. Trewhella and Collins (5) used $AgNO_3$ thin-layer chromatography and countercurrent distribution to separate the individual molecular species of choline and ethanolamine glycerophospholipids. Their method is effective for the separation of 1-palmitoyl, 1-stearoyl, and 1-oleoyl-2-arachidonyl species, but it has several disadvantages: *1*) it is difficult to completely resolve each arachidonoyl molecular species; *2*) the ether compounds are not separated from the diacyl compounds; *3*) a relatively long analytical time is required for each sample; *4*) a large amount of sample (more than 5 μmol) is required for analysis. The present HPLC procedure can be used to separate small amounts of sample (less than 0.1 μmol) into molecular species of the alkenylacyl, alkylacyl, and diacyl-GPE easily within 1 hr. The main limitation of this method is that derivatization of glycerophospholipids by hydrolysis with phospholipase C and acetylation with acetic anhydride is required before the individual molecular species can be resolved.

Reverse-phase HPLC is also useful for determination of the distribution of radioactivity among the fatty acids of glycerophospholipids. After intracerebral injection, [^{3}H]20:4 was rapidly converted into 22:4. Even at 3 min after injection of [^{3}H]20:4, about 10–20% of the total radioactivity was found in the 22:4 fraction from alkenylacyl and diacyl-GPE. The distribution of ^{3}H between the 20:4 and 22:4 fractions was quite different for each class. A high proportion of the ^{3}H (50–60%) was found in the 22:4 fraction from alkylacyl-GPE. This result indicates

TABLE 3. Relative proportion of arachidonoyl molecular species in alkenylacyl, alkylacyl, and diacyl-GPE from rat brain

	Alkenylacyl	Alkylacyl	Diacyl
		%	
16:0-20:4	27.5 ± 2.5	38.3 ± 2.1	9.0 ± 0.9
18:0-20:4	46.4 ± 1.9	32.3 ± 2.1	79.7 ± 2.3
18:1-20:4	26.0 ± 0.7	28.4 ± 2.1	11.3 ± 1.5

All values are given as the means ± SD and are derived from six experiments. The relative proportion of each molecular species was determined by gas-liquid chromatography.

that the alkylacyl-GPE preferentially incorporates 22:4 that had been elongated from 20:4. A similar preferential incorporation of 22:4 into alkylacyl-GPE was also found for Ehrlich ascites tumor cells and embryonic chick ventricular cells (21, 22). When [^{14}C]22:4 and [^{3}H]20:4 were incubated in vitro with Ehrlich ascites tumor cells or embryonic chick ventricular cells, labeled 22:4 was incorporated specifically into alkylacyl-GPE rather than into diacyl-GPE, while the incorporation rate of labeled 20:4 into alkylacyl-GPE was rather smaller than that of diacyl-GPE. A possible explanation for the specific incorporation of 22:4 into alkylacyl-GPE is that an ethanolamine phosphotransferase (EC 2.7.8.1) has a unique selectivity for the molecular species of alkylacylglycerol containing 22:4 (21). Many previous studies have shown that 1-radyl-glycerophospholipid acyltransferase has a strong specificity for 20:4 CoA, not for 22:4 CoA (23-26), and also that 1-alkyl-glycero-*sn*-3-phosphate acyltransferase in rat brain microsomes has almost the same specificity for 20:4 and 22:4 (27). The rapid conversion of 20:4 into 22:4 shows that it is necessary to examine the distribution of radioactivity among the fatty acids of glycerophospholipids in order to determine accurately the specific radioactivity of the [^{3}H]20:4.

The specific radioactivities of alkenylacyl, alkylacyl, and diacyl subclasses were still increasing in the EGP at 24 hr after the intracerebral injection. Because most of the free [^{3}H]20:4 was rapidly acylated and little radioactivity was found in the free fatty acid fraction at 1 hr after injection (data not shown), the increased radioactivity in the EGP at longer labeling periods must come from other lipid fractions such as CGP. Recently, the direct transfer of 20:4 from diacyl-GPC to ether-linked EGP has been observed in macrophages (28, 29) and platelets (30, 31). Sugiura et al. (28, 29) prelabeled macrophages with [^{3}H]20:4, removed the free [^{3}H]20:4 from the medium, and then observed a gradual decrease of radioactivity from the diacyl-GPC and increase in the alkenylacyl-GPE. The total radioactivity in the CGP and EGP was constant throughout the incubation (28). In the present study, the specific radioactivity of the CGP at 60 min was half of that at 30 min (data not shown). This suggests that the transfer of 20:4 from diacyl-GPC to EGP also occurs in brain tissue.

The HPLC separation allowed the assay of nine different arachidonoyl molecular species from the EGP of rat brain. The incorporation rates of [^{3}H]20:4 into the 18:0-

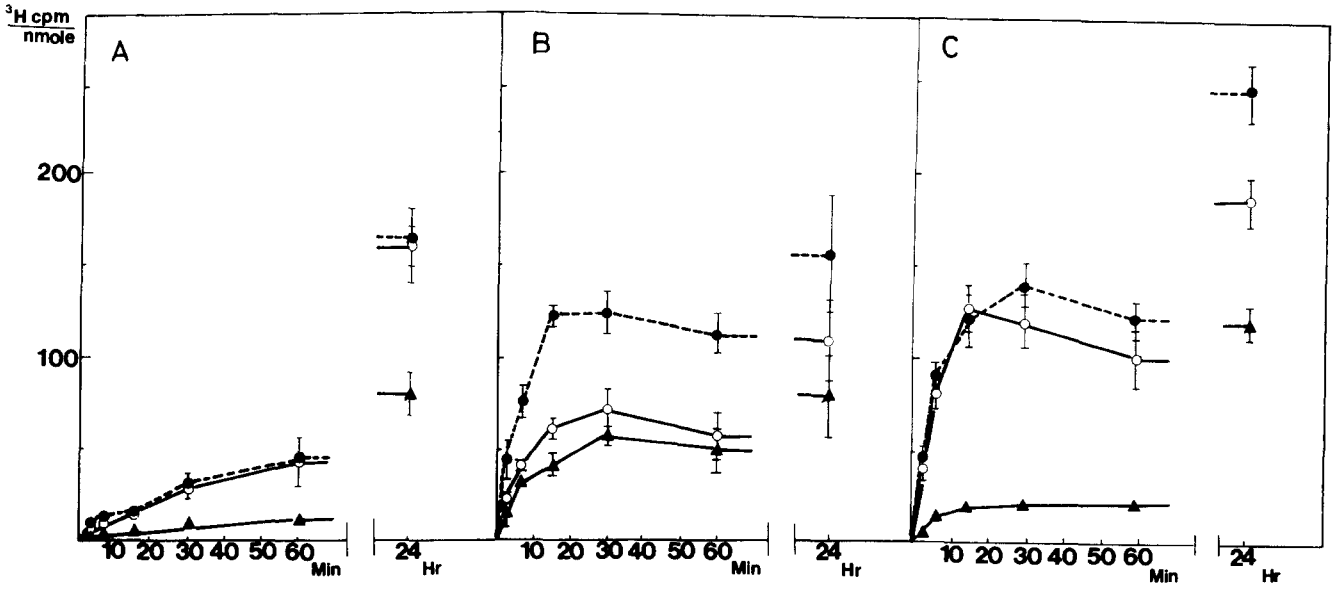

Fig. 6. Specific radioactivities of arachidonoyl molecular species of alkenylacyl (A), alkylacyl (B), and diacyl (C) GPE. The 18:1-20:4 (●), 16:0-20:4 (○), and 18:0-20:4 (▲) species of each class of EGP were separated with reverse-phase HPLC. Each molecular species was collected for the quantitation and determination of radioactivity.

20:4, 16:0–20:4, and 18:1–20:4 species of alkenylacyl, alkylacyl, and diacyl-GPE were quite different. The 18:0–20:4 species had the slowest incorporation rate of [^{3}H]20:4 into arachidonoyl molecular species of all three classes. This was also found for arachidonoyl molecular species of EGP from rat skeletal muscle (7). Shamgar and Collins (7) determined the specific radioactivity of individual molecular species after intraperitoneal injection of [^{32}P]-orthophosphate. Molecular species of EGP containing 18:2 and 22:6 have the fastest turnover and 18:0–20:4 species have the slowest. The relative specific radioactivity of the 18:0–20:4 species of skeletal muscle EGP was almost 3 times less than that of a mixture of the 16:0–20:4 and 18:1–20:4 species.

The exact mechanism for regulating the uptake of 20:4 into individual molecular species is not yet understood. Studies in vitro and in vivo indicate that 20:4 enters into glycerophospholipids predominately by deacylation and reacylation reactions (23–26, 32). The present results suggest that the acyltransferase must have a different specificity towards 1-alkenyl, 1-alkyl, and 1-acyl-GPE having different fatty chains at the sn-1 position. The selectivity for the 1-oleoyl species seems to be greater than that for the corresponding 1-stearoyl homologues. Holub (33) reported different selectivities of phosphocholinetransferase and phosphoethanolaminetransferase towards 1-palmitoyl and 1-stearoyl homologues of various unsaturated 1,2-diacylglycerols. A marked preference of the phosphocholinetransferase for the 1-palmitoyl over the 1-stearoyl homologues was observed with 2-oleoyl, 2-linoleoyl, 2-arachidonoyl, and 2-docosahexaenoyl diacylglycerols. For phosphoethanolaminetransferase, a selectivity towards 1-stearoyl-2-arachidonoyl and 1-stearoyl-2-oleoyl diacylglycerol over their 1-palmitoyl homologues was indicated. Between 60 min and 24 hr, the specific radioactivity of arachidonoyl molecular species of diacyl-GPE gradually increased. The specific radioactivity of the 18:1–20:4 and 16:0–20:4 species doubled over this time interval, but the specific radioactivity of the 18:0–20:4 species at 24 hr was more than sixfold greater than that at 60 min. The slower apparent turnover of the 18:0–20:4 species may cause the greater accumulation of [^{3}H]20:4 in the 18:0–20:4 species of diacyl-GPE than in the 18:1–20:4 and 16:0–20:4 species at 24 hr. This may explain the greater abundance of 18:0–20:4 species in diacyl-GPE. The different rates of turnover of molecular species may be of considerable importance in maintaining the characteristic compositions of membrane glycerophospholipids.

A functional role for the 18:1–20:4 and 16:0–20:4 molecular species is suggested because they had a much faster apparent metabolism of arachidonate than did the 18:0–20:4 species. A source of eicosanoid precursors for receptor-linked signal transduction is one possible function. Further studies of the metabolism of the arachidonate-containing molecular species of all glycerophospholipids are indicated and feasible.

We wish to acknowledge the excellent technical assistance of Mr. H. W. Harder. This research was supported in part by NIH research grant NS-08291.

Manuscript received 9 January 1985.

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