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Turnover Rates of the Molecular Species of Ethanolamine Plasmalogen of Rat Brain

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Abstract: 1,2-Diradyl-3-acetylglycerols prepared from 1-*O*-alk-1'-enyl-2-acylglycero-3-phosphoethanolamine (alkenylacyl-GPE, ethanolamine plasmalogen) and 1-alkyl-2-acylglycero-3-phosphoethanolamine (alkylacyl-GPE) of rat brain at 18 days of age were subfractionated into six species by $AgNO_3$-impregnated TLC. The percent compositions of subfractions were compared with that of 1,2-diacylglycero-3-phosphoethanolamine (diacyl-GPE). The incorporation rate of [1-^{3}H]glycerol into each molecular species was also estimated to examine the turnover rate and selective synthesis of molecular species of ethanolamine phosphoglycerides (EPG). Among the molecular species of EPG, a major proportion contained polyunsaturated fatty chains, and the sum of tetraene-, pentaene-, and hexaene-containing species was >65% in common with three classes of EPG. It was possible to calculate the turnover time, synthesis rate, and synthesis rate constant of ethanolamine plasmalogen in myelinating rat brain by the equation of Zilversmit et al. since the time-dependent change of specific activity and the distribution of molecular species indicated that each molecular species of alkenylacyl-GPE is synthesized from the corresponding species of alkylacyl-GPE. The observed turnover time of ethanolamine plasmalogen was about 5 h. The observed turnover times of the various molecular species were of the order: tetraene ≥ hexaene > pentaene ≥ monoene ≥ diene. The synthesis rate constants of each molecular species, in the formation of alkenylacyl-GPE from alkylacyl-GPE, were of the order: hexaene > tetraene > pentaene > diene ≥ monoene. This result indicated that the hexaene species is preferentially synthesized from alkylacyl-GPE among molecular species of ethanolamine plasmalogen in the desaturation of alkylacyl-GPE. **Key Words:** Ethanolamine plasmalogen—Molecular species—Turnover rate—Rat brain—1-Alkyl-2-acylglycerophosphoethanolamine desaturase. **Masuzawa Y. et al.** Turnover rates of the molecular species of ethanolamine plasmalogen of rat brain. *J. Neurochem.* **42,** 961–968 (1984).

Ethanolamine plasmalogens are found at high levels in the nervous tissue of various mammals. The plasmalogens differ from corresponding diacyl phospholipids in the composition (Sun and Horrocks, 1969; Etzrodt and Debuch, 1970; Waku and Nakazawa, 1979) and the turnover (Corbin and Sun, 1978; Waku and Nakazawa, 1979) of their acyl groups. Several investigators have revealed that the distribution of the molecular species of ether-linked lipids can be regulated distinctly from diacylglycerolipids by enzymes that are nevertheless analogous in action to the enzymes responsible for diacylglycerolipid synthesis (summarized in Lands and Crawford, 1976; Holub and Kuksis, 1978). The microsomal acyltransferases, such as 1-alkylglycerophosphate acyltransferase (2.3.1.63; Fleming and Hajra, 1977) or 1-alkenylglycerophosphocholine acyltransferase (2.3.1.25; Waku and Lands, 1968), had a quite different acyl-CoA selectivity than those shown for 1-acyl analogues. Further, the enzymes

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Abbreviations used: EPG, Ethanolamine phosphoglycerides; GPC, Glycerol-3-phosphocholine; GPE, Glycerol-3-phosphoethanolamine. All locants of glycerol are *sn*. Double bond number for molecular species of plasmalogen does not include the alk-1'-enyl bond. Fatty chains are designated by number of carbon atoms:number of double bonds. The term molecular species in this text indicates the subfractions separated by $AgNO_3$ TLC according to the degree of unsaturation.

that are involved in the synthesis routes peculiar to ether-linked phospholipids can also govern the species composition of ether-linked phospholipids (summarized in Wykle and Snyder, 1976; Bishop and Hajra, 1981).

The desaturation of alkylacyl-GPE to form ethanolamine plasmalogen (1-alk-1'-enyl-2-acyl-glycero-3-phosphoethanolamine, alkenylacyl-GPE) is one of the reactions specific to ether-linked phospholipids (Wykle et al., 1972; Horrocks and Radminska-Pyrek, 1972; Paltauf and Holasek, 1973). Although Wykle and Schremmer (1979) reported that the alkyl desaturase (1-alkyl-2-acylglycerophosphoethanolamine desaturase, 1.14.99.19) of sarcoma cells did not exhibit specificity for the 2–~20-carbon acyl chain at position 2 *in vitro*, it remains to be explored whether alkylacyl-GPE containing a 22-carbon acyl chain is selectively desaturated. In addition, the alkyl desaturase may exhibit different selectivity *in vivo*, since substrate accessibility to the enzyme seems to play an important role in the desaturase activity in enzymic studies (Wykle and Schremmer, 1979). Previously (Waku and Nakazawa, 1978, 1979) we had estimated the incorporation rate of the radioactive glycerol into molecular species of alkylacyl- and alkenylacyl-GPE of Ehrlich ascites tumor cells and found a rapid turnover of hexaene species of alkylacyl-GPE. In this paper, we report on the analysis of the molecular species composition of ether-linked ethanolamine phospholipids of rat brain in comparison with diacyl compounds, and have calculated the turnover time and synthesis rate constant for each of the species of ethanolamine plasmalogen, to discover how each molecular species of ether-linked phospholipids turns over in developing brain, and how the composition of the molecular species is governed under physiological conditions, especially in the desaturation of alkylacyl-GPE.

MATERIALS AND METHODS

All chemicals were of reagent grade and solvents were distilled before use. [1-^{3}H]glycerol (2.5 Ci/mmol) was purchased from Amersham International (Amersham, U.K.). Wistar rats were obtained from Sankyo Laboratory Service (Tokyo, Japan) and maintained on normal rat food (CD-5, Nippon Kure-a, Tokyo). Male 18-day-old rats, before weaning, were given intracerebral injections of 70 μl of Ringer's balanced salt solution (buffered to pH 7.4 with 50 m*M* Tris-HCl) containing 200 μCi/ml of [^{3}H]glycerol. After a selected period, groups of two to four rats were decapitated and the brains were quickly removed.

The brains were immediately homogenized in ice-cold saline and the lipids were extracted as described by Bligh and Dyer (1959). Ethanolamine phosphoglycerides (EPG) were fractionated by DEAE and silicic acid column chromatography as described previously (Waku et al., 1974). Throughout the fractionation procedure, a small amount of butylated hydroxytoluene was added to the solvents. The EPG fraction was hydrolyzed by phospholipase C (*B. cereus*) and acetylated as described previously (Waku et al., 1974). 1-Radyl-2-acyl-3-acetylglycerols prepared by this treatment were separated into 1-alkenyl-2-acyl-3-acetylglycerols, 1-alkyl-2-acyl-3-acetylglycerols, and 1,2-diacyl-3-acetylglycerols by TLC, according to Renkonen and Luukkonen (1976). The individual spots were visualized under ultraviolet light by spraying with primuline, a nondestructive fluorescent reagent. The three types of diradylacetylglycerol were eluted from silica gel with chloroform-methanol-water (1:2:0.8) and extracted as described by Bligh and Dyer (1959). The purity of each fraction was confirmed by TLC. For further analysis, in terms of the content of molecular species, each diradylacetylglycerol was fractionated by 20% $AgNO_3$-impregnated TLC, and separated species were eluted from silica gel as described previously (Waku and Nakazawa, 1978). A part of the lipid fraction was used for the measurement of the radioactivity and the remaining sample was used for the analysis of the fatty chains. The recovery of lipids and radioactivity from the plate was >90%.

Fatty acyl moieties of separated molecular species were transmethylated with sodium methoxide and the quantities were estimated by GLC using methyl heptadecanoate as an internal standard. A 2-m column containing 15% diethylenesuccinate on chromosorb W was run at 195°C with nitrogen as a carrier gas in a Hitachi 163 gas-liquid chromatograph. The distribution of *O*-alkenyl chains was determined as described previously (Waku et al., 1974). In brief, aldehydes were liberated by treatment of 1-*O*-alkenyl-2-acyl-3-acetylglycerol with acetic acid containing 1% $HgCl_2$ and hydrogenated with $LiAlH_4$. The resulting alcohols were acetylated and subjected to GLC using a column packed with 20% ethylene glycol succinate on Gas-Chrom P. To determine the distribution of *O*-alkyl chains, trimethylsilyl derivatives of 1-alkylglycerol were prepared from 1-alkyl-2-acyl-3-acetylglycerol and GLC was performed by using a column packed with 5% Apiezon L on Chromosorb W (Waku and Nakazawa, 1978).

The radioactivity was measured in a Packard 3320 scintillation counter, using the scintillation fluid described in a previous paper (Waku and Nakazawa, 1978).

To determine the pool size of EPG, the total lipid extracted from rat brain was subjected to two-dimensional TLC using the solvent system described previously (Masuzawa et al., 1973). EPG, choline phosphoglycerides, serine phosphoglycerides, inositol phosphoglycerides, sphingomyelin, cardiolipin, and phosphatidic acid were fractionated and the phosphorus of each fraction was determined by the method of Rouser et al. (1966). The pool size of each molecular species of EPG was determined from the phosphorus content of ethanolamine phospholipid and the quantity of the fatty acyl moiety of each molecular species.

From the time-dependent change of specific radioactivity of each molecular species of alkylacyl-GPE and alkenylcyl-GPE, the turnover time, synthesis rate, and synthesis rate constant of brain alkenylacyl-GPE were calculated by the equation of Zilversmit et al. (1942).

RESULTS

The acyl composition of molecular species of EPG of the rat brain at 18 days of age (the stage of

myelination; Wells and Dittmer, 1967) was determined. Each type of 1,2-diradyl-3-acetylglycerol, diacyl-, alkylacyl-, alkenylacylglycerol, derived from ethanolamine phospholipid, was separated into six bands by $AgNO_3$-impregnated TLC. The fatty acid compositions of the separated bands are shown in Table 1, parts a–c. The percentages of alkyl or alkenyl chains in each band of ether-linked lipids are shown in Table 2. By calculation of average number of double bonds (not including alk-1′-enyl bond) per molecule, bands 1–6 can be designated as saturated, monoene, diene, tetraene, pentaene, and hexaene, respectively. EPG of rat brain contained a high amount of polyunsaturated species and the sum of tetraene, pentaene, and hexaene species accounted for 88% of diacyl-GPE, 65% of alkylacyl-GPE, and 86% of alkenylacyl-GPE. The mol % of each species of alkenylacyl-GPE was similar to that of diacyl-GPE in spite of the difference of synthesis pathway. Also, the proportion of molecular species of alkenylacyl-GPE was similar to that of alkylacyl-GPE, which was previously considered to be the precursor of alkenylacyl-GPE (Wykle et al., 1972; Horrocks and Radminska-Pyrek, 1972; Paltauf and Holasek, 1973), apart from the following differences. Alkenylacyl-GPE contained more of the hexaene species than did alkylacyl-GPE. In addition, although arachidonic acid was the predominant fatty acid in tetraene species of alkenylacyl-GPE, docosatetraenoic acid was more abundant than arachidonic acid in the corresponding species of alkylacyl-GPE. The relative composition of each fatty chain at the first position was essentially the same between the corresponding molecular species of alkenylacyl- and alkylacyl-GPE (Table 2).

TABLE 1. *Fatty acid composition of molecular species of ethanolamine phosphoglycerides in the developing rat brain*

a. Diacylglycerophosphoethanolamine

Band	16:0	16:1	18:0	18:1	18:2	20:1	20:3	20:4	20:5	22:4	22:5	22:6	Total (mol %[a])	Average number of double bonds[b]
					(nmol/100 nmol of fatty acids)									
1	0.4	tr[c]	0.2	tr									0.7 ± 0.2	0.3
2	1.4	0.1	2.3	3.7		0.2							7.7 ± 1.0	1.0
3	0.1	tr	0.3	2.9	0.2	0.2							3.6 ± 0.4	1.9
4	1.6		18.0	0.4			0.6	15.5	0.3	1.0			37.2 ± 1.0	3.7
5	0.4	tr	2.2	1.2		tr		1.6	tr	1.1	0.7	0.4	7.7 ± 1.0	4.7
6	4.0		15.5	1.8	0.1	tr		0.9	0.2	0.9	0.8	18.8	43.1 ± 1.1	5.9
Total	7.9	0.1	38.5	9.9	0.3	0.5	0.6	18.0	0.5	3.0	1.5	19.3	100	

b. Alkylacylglycerophosphoethanolamine

Band	16:0	16:1	18:0	18:1	18:2	20:1	20:3	20:4	20:5	22:4	22:5	22:6	Total (mol %[a])	Average number of double bonds[b]
					(nmol/100 nmol of fatty acids)									
1	1.0		0.4	0.1									1.5 ± 0.8	0.1
2	3.0	0.3	0.5	13.5		4.1							21.4 ± 2.0	1.1
3	0.3	0.3	0.1	8.2	0.3	2.6							11.8 ± 1.2	1.7
4							1.4	12.8	0.9	15.2			30.3 ± 2.2	3.9
5								2.6	0.3	5.1	1.0		9.0 ± 1.7	4.7
6								0.3		0.5	0.8	24.1	25.7 ± 1.2	6.0
Total	4.3	0.6	1.0	21.8	0.3	6.7	1.4	15.7	1.2	20.8	1.8	24.1	100	

c. Alkenylacylglycerophosphoethanolamine

Band	16:0	16:1	18:0	18:1	18:2	20:1	20:3	20:4	20:5	22:4	22:5	22:6	Total (mol %[a])	Average number of double bonds[b]
					(nmol/100 nmol of fatty acids)									
1	0.5	tr	0.1	tr									0.7 ± 0.4	0.1
2	0.9	0.2	0.1	7.5		1.3							10.0 ± 0.7	1.1
3	tr	tr	tr	2.7	0.1	0.5							3.5 ± 0.3	1.7
4							1.5	18.9	0.6	13.4			34.3 ± 1.9	4.0
5							0.1	3.5		2.8	1.6	0.3	8.2 ± 1.2	4.6
6								1.1	tr	1.0	1.4	39.8	43.4 ± 2.0	6.0
Total	1.5	0.2	0.2	10.2	0.1	1.9	1.6	23.4	0.6	17.3	3.0	40.0	100	

Three types of 1-*O*-radyl-2-acyl-3-acetylglycerol from ethanolamine phosphoglycerides were isolated as described in Materials and Methods. About 0.1 μmol of both diacyl and alkenylacyl compounds, e.g., 1,000 nmol and 500 nmol of fatty esters, respectively, and about 0.1 μmol of alkylacyl compound, e.g., 100 nmol of fatty esters, were further separated into six subfractions with differing degrees of unsaturation by $AgNO_3$-impregnated TLC. After transmethylation by sodium methoxide, the fatty acid composition of each subfraction was determined by GLC. Values stated are the averages from three separate experiments.

[a] Values are the means ± SEM of three separate experiments by fatty acid analysis.

[b] $\frac{\text{Total number of double bonds}}{\text{nmol of glycerides}}$ are calculated from fatty chain composition shown in this table and in Table 2.

[c] Trace amounts (<0.05 nmol).

TABLE 2. *Percentage compositions of alkyl and alkenyl chain of the molecular species of alkylacyl-GPE and alkenylacyl-GPE in 18-day-old rat brain*

	Alkyl chain (%)			Alkenyl chain (%)		
Band	16:0	18:0	18:1	16:0	18:0	18:1
1	27.2	69.5	3.2	32.5	60.6	6.8
2	26.0	42.7	31.5	34.4	46.5	19.2
3	12.7	13.1	74.1	11.8	10.9	77.3
4	48.8	48.4	2.8	43.4	53.7	2.9
5	23.9	15.8	60.4	25.9	31.6	42.5
6	47.2	38.6	14.2	39.5	48.0	12.4

The alkyl alcohols of each band of 1-*O*-alkyl-2-acyl-3-acetylglycerol from alkylacyl-GPE were analyzed in the form of trimethylsilyl derivatives, by GLC, as described in text. The fatty alcohols derived from each band of 1-*O*-alkenyl-2-acyl-3-acetylglycerol, from ethanolamine plasmalogen, were also analyzed as acetate esters. Values are the averages of three separate experiments.

Figure 1 shows the incorporation rate of [1-^{3}H]-glycerol into diacyl-, alkylacyl-, and alkenylacyl-GPE for the brains of 18-day-old rats, as a function of time. The specific radioactivities of diacyl- and alkylacyl-GPE were much higher than that of alkenylacyl-GPE and reached a maximum at 30 min after the injection of [1-^{3}H]glycerol. However, the specific radioactivity of alkenylacyl-GPE increased very slowly and continued to increase over 1 h. The specific activity curves of both ether-linked phospholipids almost coincided with that of the precursor-product relationship (Zilversmit et al., 1942).

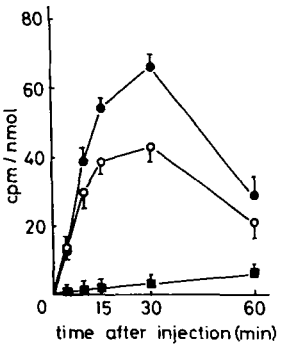

FIG. 1. Incorporation of [1-^{3}H]glycerol into the three types of ethanolamine phospholipid of rat brain. [1-^{3}H]Glycerol (14 μCi) was injected intracerebrally into the brains of 18-day-old rats. After selected time periods, the brains were quickly removed and the lipid fractions extracted. 1-Radyl-2-acyl-3-acetylglycerol was prepared from the ethanolamine phospholipid fraction and subjected to TLC. The specific activities (cpm/nmol of glycerol moiety) of diacyl (●—●), alkylacyl (○—○), and alkenylacyl (■—■) compounds were then estimated. The quantity of glycerol was calculated from the results of quantitative fatty acid analysis by methanolysis with sodium methoxide using methyl heptadecanoate as an internal standard. Error bar indicates ± SEM of three experiments.

Figure 2a shows the time-dependent change of specific radioactivities of molecular species of diacyl-GPE. The curves for hexaenes and monoenes were similar and the specific activities were much higher than for dienes and tetraenes. The specific activity of tetraene species was the lowest although it gradually increased during the experimental period.

The time-dependent change of the specific radioactivity of each of the molecular species of both types of ether-linked EPG was also estimated and the results are shown in Figs. 2b and c. The order of specific radioactivity at 5 min was: diene > monoene > hexaene > pentaene > tetraene (the data for pentaene species are not shown). This order of specific radioactivity was the same for both alkylacyl-GPE and alkenylacyl-GPE. In alkylacyl-GPE, the curves of monoene and diene species rose rapidly, and reached a maximum at 15 min. However, the tetraene species showed behavior similar to that of the corresponding species of diacyl compounds, the specific activity being low, and the time-dependent increase slow. The time-dependent change of each molecular species of alkenylacyl-GPE reflected that of the corresponding species of alkylacyl-GPE.

From the above data, it is possible to estimate the turnover time of each molecular species of alkenylacyl-GPE, according to Zilversmit's equation (Zilversmit et al., 1942). Further, synthesis rates and synthesis rate constants can be calculated from the observed turnover time and tissue content of alkenylacyl-GPE and alkylacyl-GPE. The results are shown in Table 3. The changes of specific activities at earlier times (5, 10 min) were used for the calculation to exclude the effect of reutilization of radioactive precursors and of influx of radioactivity from the other pathways, other than *de novo*, as much as possible. The turnover time of total ethanolamine plasmalogen was found to be approximately 5 h. The calculated turnover times of molecular species of alkenylacyl-GPE were of the order: tetraene ≥ hexaene > pentaene ≥ monoene ≥ diene. The differences between the synthesis rates among molecular species was a reasonable reflection of the pool size of the particular species. The synthesis rate constant of each species showed the following order: hexaene > tetraene > pentaene > diene ≥ monoene.

DISCUSSION

The acyl chain compositions of brain ethanolamine phospholipids, including ether-linked lipids, have already been determined by several authors (Sun and Horrocks, 1969; Etzrodt and Debuch, 1970; Fleming and Hajra, 1977). Further, Crawford and Wells (1979) have analyzed the composition of the molecular species of diacyl-GPE in rat brain.

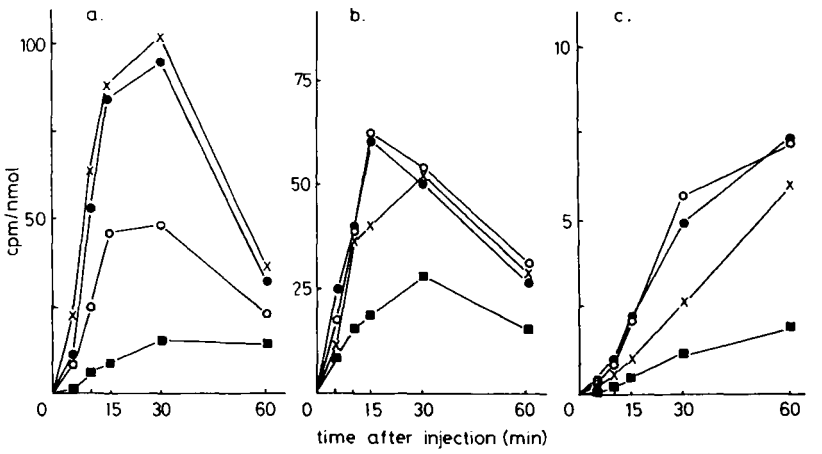

FIG. 2. The specific activities of molecular species of the three types of 1-radyl-2-acyl-3-acetylglycerol derived from ethanolamine phospholipids of 18-day-old rat brains as a function of time. **a:** Diacyl compound. **b:** Alkylacyl compound. **c:** Alkenylacyl compound. Each molecular species was separated by $AgNO_3$-TLC as described previously (Waku and Nakazawa, 1978, 1979). The specific radioactivity (cpm/nmol of glycerol moiety) was estimated as described in the legend to Fig. 1 and each point is the mean of two (30 and 60 min) or three experiments (5, 10, and 15 min). (●—●), Monoene species; (○—○), diene species; (■—■), tetraene species; (X—X), hexaene species.

However, there have been no reports on the composition of molecular species of ether-linked ethanolamine phospholipids in nervous tissues. As expected from the analysis of acyl group composition in previous papers (Sun and Horrocks, 1969; Etzrodt and Debuch, 1970; Fleming and Hajra, 1977), EPG of rat brain contained a high amount of polyunsaturated species in common with both diacyl and ether-linked phospholipids (Table 1).

Figure 2 indicates that a synthesis route other than *de novo*, probably a deacylation-reacylation mechanism (Hill et al., 1968), must contribute to the formation of tetraene species. Deacylation-reacylation, or another route differing from *de novo*, may be partly involved in the formation of diene species, since the time-dependent curve of this species was located between those of the other species. The time-dependent change of each molecular species of alkenylacyl-GPE reflected that of the corresponding species of alkylacyl-GPE. This was not clearly observed in our earlier studies using Ehrlich ascites cells (Waku and Nakazawa, 1978, 1979). Although Debuch et al. (1971) and Gunawan and Debuch (1977) proposed the possibility that desaturation of 1-alkyl-GPE is the first step for plasmalogen biosynthesis, our kinetic data and the distribution of molecular species indicate that each molecular species of alkenylacyl-GPE is mainly synthesized from the corresponding molecular species of alkylacyl-GPE. In addition, if most of alkenylacyl-GPE is synthesized by the acylation system, it is difficult to explain the rapid incorporation of labeled glycerol into monoene species of alkenylacyl-GPE and the slow incorporation into tetraene species, since every previous report relating 1-radyl-2-lyso-glycerophospholipid acyltransferase activity (2.3.1.23) indicated that 18:1-coenzyme A is esterified much less effectively than 20:4-coenzyme A (Waku and

TABLE 3. *The turnover time, synthesis rate, and synthesis rate constant of each of the molecular species of ethanolamine plasmalogen synthesized* de novo *in 18-day old-rat brain*

Molecular species	Pool size of EPG (μmol/g brain)		Turnover time (min)	Synthesis rate (nmol/g/min)	Synthesis rate constant (min^{-1})
	Alkenylacyl-	Alkylacyl-			
Monoene (01)[a] + (10)	0.78	0.19	250 [170]	3.1 [4.6]	0.016 [0.024]
Diene (11)	0.37	0.10	210 [190]	1.8 [1.9]	0.018 [0.019]
Tetraene (04)	3.8	0.26	360 [440]	10 [8.6]	0.040 [0.033]
Pentaene (14) + (05)	0.78	0.08	270 [340]	2.9 [2.3]	0.036 [0.029]
Hexaene (06)	4.4	0.22	310 [440]	14 [10]	0.064 [0.045]
Total	10.2	0.87	320 [390]	32 [26]	0.037 [0.029]

The pool size of each molecular species was determined from the phosphorus content of the ethanolamine phosphoglyceride fraction separated by two-dimensional TLC and quantity of fatty acyl moiety of each species of ethanolamine plasmalogen. Turnover rates, synthesis rates, and synthesis rate constants were calculated according to Zilversmit et al. (1942). The time-dependent change of specific radioactivities of each molecular species of alkylacyl- and alkenylacyl-GPE from 5 min to 10 min were used for the calculations. The figures in brackets are the values calculated using the time-dependent change from 10 to 15 min.

[a] Figures in parentheses indicate the predominant species in individual fractions. Respective numbers in parentheses indicate the number of double bonds of the fatty chains at the first and second positions of the glycerol moiety.

Lands, 1968; Okuyama et al., 1975; Waku and Nakazawa, 1977).

In the above calculation, it was assumed that influx of [1-^{3}H]glycerol into alkenylacyl-GPE is only from alkylacyl-GPE, and that each of the molecular species of alkenylacyl-GPE is synthesized only from the corresponding species of alkylacyl-GPE. The latter assumption may not necessarily hold, since a part of ethanolamine plasmalogen can also be formed by interconversion reactions (Mozzi et al., 1982) or acylation of lyso compound (Debuch et al., 1971; Natarajan and Sastry, 1974). However, the synthesis rate or synthesis rate constant of each molecular species is considered to reflect the actual value, since the specific activity of alkylacyl-GPE is much higher than that of alkenyacyl-GPE, shortly after injection (Wise and Elwyn, 1965). The synthesis rate constant for the hexaene species was 3.8-fold that of the diene species. This result suggests that the hexaene species is preferentially synthesized from alkylacyl-GPE rather than from the other four species. The preferential synthesis of the hexaene species of alkenylacyl-GPE, in the desaturation of alkylacyl-GPE, may explain the difference of the composition of this species, between alkylacyl-GPE and alkenylacyl-GPE (Table 1).

In brain, the half-life of ethanolamine plasmalogen has been calculated by estimating the efflux rate of radioactivities from the ethanolamine plasmalogen (Horrocks et al., 1975; Miller et al., 1977). The turnover time calculated in this study (turnover time about 5 h) was much less than that found in a previous study (half life 4–20 days in microsomes, 11–34 days in myelin; Miller et al., 1977). A similar discrepancy is found between the data for the turnover time in liver (Omura et al., 1967; Åkesson et al., 1970) and lung (Tierney et al., 1967; Moriya and Kanoh, 1974). This discrepancy may be explained as follows: (1) The equation used to calculate the half-life using the efflux rate of radioactivities is applicable when the influx of radioactivities from the precursors is negligible. However, a recycling of the injected label will occur after long periods (Miller et al., 1977). Therefore the observed half-life estimated by using graphs of specific activities against time would be much longer than the real value. (2) There may be several pools that have different turnover times (Horrocks et al., 1975). Dhopeshwarkar and Mead (1973) have proposed that separate compartments may exist in brain and that one of these has a very rapid turnover. If this is the case, the turnover time calculated in this study may apply to a specific part of brain turning over very rapidly. The rapid and slower turnover pools may be associated with different cell types or specific membranes as suggested previously (Freysz et al., 1969; Cohen and Bernsohn, 1973; Horrocks et al., 1975).

Our results reported here indicated that the hexaene species of ethanolamine plasmalogen, at least a small pool of this ether-linked lipid in brain, is preferentially formed in the desaturation of alkylacyl-GPE. Selective synthesis of hexaene species may also occur in the formation of 1-alkyl-2-acylglycerophosphate (Fleming and Hajra, 1977; Giusto and Bazan, 1979) or alkylacyl-GPE (Kanoh and Ohno, 1975; Holub, 1978; Masuzawa et al., 1982). In a preliminary experiment, where the incorporation rate of [^{3}H]1-alkyl-GPE into the alkylacyl-GPE of Ehrlich ascites cells was estimated, docosahexaenoic acid appeared to be an effective substrate for the acylation of 1-alkyl-GPE as well as arachidonic acid (K. Waku and Y. Masuzawa, unpublished). If all this is the case, in addition to the results in this report, one can conclude that all synthesis pathways tend to form hexaene species of ethanolamine plasmalogen. Although the function of docosahexaenoic acid is still not understood (Tinoco et al., 1979), this polyunsaturated fatty acid may be required during the development of the CNS, as previously suggested (Sun et al., 1975; Tinoco et al., 1979; Mekkena and Campagnini, 1979). In addition, docosahexaenoic acid is a potent inhibitor of biosynthesis of prostaglandins (Lands et al., 1973). The high content and the preferential formation of hexaene species in brain may relate to such properties.

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