Metabolism of Chimyl Alcohol and Phosphatidyl Ethanolamine in the Rat Brain[1]

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ABSTRACT

Following intracerebral injection of ^{14}C-phosphatidylethanolamine and ^{3}H-chimyl alcohol into 18 day old rats, the ethanolamine phosphoglycerides were isolated and analyzed. The ^{14}C and ^{3}H activities in the dimethyl acetals derived from alkenyl acyl ethanolamine phosphoglycerides and in the glyceryl ethers derived from the alkyl acyl ethanolamine phosphoglycerides were measured. The absence of ^{14}C in the dimethyl acetals indicates that phosphatidyl ethanolamine is not transformed into phosphatidal ethanolamine under these circumstances. The increase with time of the ^{3}H content of the glyceryl ethers and dimethyl acetals indicates that chimyl alcohol was a precursor of both types of phospholipids.

INTRODUCTION

ALTHOUGH DERIVATIVES of alkyl and alkenyl glyceryl ethers have been shown to be widespread components of both neutral and phospholipids (2,3,16), the biogenesis of these ether linkages is still obscure. It has been considered possible that phospholipids containing these groupings may be formed by reduction of the corresponding diacyl compounds (7,17) and equally possible that the alkenyl derivatives could be formed by dehydrogenation of the alkyl ethers (18). However, the mechanism of formation of the latter is not understood nor can it be surmised whether the transformations of one class to another might take place as the glyceryl ethers or as the phospholipids.

Experiments in this laboratory (1) in which ^{14}C-labeled palmitaldehyde was injected directly into the brains of 18 day rats provided evidence that the aldehyde did not react directly with some glycerol derivative to give an alkenyl ether. Rather, it was first oxidized to palmitic acid, which was then incorporated into the ethanolamine phosphoglycerides. On the other hand, Thompson (15) has found that in the slug, *Arion ater,* the alkyl, acyl GPE[1] appears to be the precursor of the alkenyl, acyl GPE. Malins (8) has reached the same conclusion for the dogfish and Horrocks and Ansell (4) have suggested that the same pathway exists in the rat brain.

The present study represents an attempt to clarify the interrelationships among these compounds.

PROCEDURES

Materials

Uniformly-labeled ^{14}C phosphatidylethanolamine, obtained from Applied Science Laboratories, Inc., was purified by chromatography on a DEAE cellulose column before use. Chimyl alcohol, uniformly-labeled in the alkyl moieties with tritium was a gift from G. A. Thompson, Department of Biochemistry, University of Washington and was purified by thin-layer chromatography (see below) before use. All solvents were ACS reagent grade and were distilled prior to use. Ethanol (1%) was added to the chloroform as a preservative.

Methods

Treatment of Animals. Three groups of 18 day old male Sprague-Dawley rats were obtained from Berkeley Pacific Laboratories together with their lactating mothers. Rats of this age were used since the synthetic reactions leading to myelin formation are at a maximum at this time (9). Each rat was anesthetized lightly with ether and was given an intracerebral injection of 0.375 ml of an emulsion consisting of: 20 mg, 6 μc chimyl alcohol-^{3}H, 7.3 mg, 25 μc PE-^{14}C and tween 20 (36 mg/ml). Groups A, B and C were sacrificed after 4, 8 and 16 hr, respectively.

The animals were sacrificed by CO_2 asphyxiation and the brains removed and immediately frozen on dry ice. The pooled brains from each group were extracted with chloroform: methanol (2:1) under nitrogen as described by Rouser *et al.* (11). Evaporation of the extract in a rotary flash evaporator at 30C followed by drying in vacuo over KOH gave the total lipid.

[1] Abbreviations used; CA, Chimyl alcohol; GLC, gas liquid chromatography; GPE, glyceryl-3-phosphorylethanolamine; PE, phosphatidylethanolamine; TLC, thin-layer chromatography; TFA, trifluoroacetyl.

TABLE I
Radioactivity Recovered in Total Lipid Extract after Intercerebral Injection of ^{14}C PE and ^{3}H Chimyl Alcohol into 18-Day-Old Rat Brains

Group of Rats	A	B	C
Number of rats/group	5	5	4
Activity of ^{14}C PE inject/rat (cpm x 10^{-6})	1.36	1.36	1.36
Activity of ^{3}H chimyl alc inject/rat (cpm x 10^{-6})	0.34	0.34	0.40
Exposure time to substrates (hr)	4	8	16
Pooled weights of brains (g)	7.3	8.2	6.5
Extracted lipid (% wet wt of brain)	6.7	8.5	7.1
Activity in total lipids			
– ^{14}C (cpm x 10^{-6})	3.46	3.45	3.12
– ^{3}H (cpm x 10^{-6})	0.65	0.66	0.51
Per cent of injected activity recovered in total lipid			
– ^{14}C PE	51	50	50
– ^{3}H chimyl alc	38	32	32

Separation of the Lipid Components. The total lipids were fractionated on a DEAE cellulose column as described by Rouser et al. (11,12). The eluting solvents and components of each eluate were as follows: chloroform-methanol (9:1) — cholesterol, ceramide, cerebroside, choline phosphoglycerides and sphingomyelin (front fraction); chloroform-methanol (7:3) — ethanolamine phosphoglycerides; methanol — water-soluble non-lipid material; glacial acetic acid — free fatty acids, serine phosphoglycerides, gangliosides; methanol to wash out the acetic acid; chloroform-methanol: 28% ammonium hydroxide (4:1:-0.2) — inositol phosphoglycerides and other acidic lipids. The eluates from the column were monitored continuously by TLC using the solvent system chloroform-methanol-28% ammonium hydroxide (50:49:1) and standards obtained from Applied Science Laboratories.

The fatty acid and aldehyde moieties of the ethanolamine phosphoglycerides were obtained by subjecting the isolated fraction to methanolysis according to the method of Morrison and Smith (10). The resulting mixtures of methyl esters and dimethyl acetals were analyzed directly by GLC or the dimethyl acetals were isolated by saponification of the mixture with 5% methanolic KOH at 60C for 1 hr. The dimethyl acetals were extracted into petroleum ether (br 30-60C) and the aqueous phase was acidified and the fatty acids extracted with petroleum ether and re-esterified with diazomethane. The conditions for GLC and subsequent collection of the methyl esters and dimethyl acetals were as described previously (1).

The ethanolamine phosphoglyceride fraction was analyzed for glyceryl ether components by the acetolysis procedure recommended by Thompson (14). The glyceryl ethers thus obtained were converted to the TFA derivatives by the method of Wood and Snyder (19) and were analyzed by GLC on an ethylene glycol succinate column. The identity of the glyceryl ethers was checked by TLC with the solvent system petroleum ether-ether-glacial acetic acid (40:60:1).

The front fraction isolated from the DEAE-cellulose column was analyzed for saturated and α,β-unsaturated glyceryl ethers as follows: The fraction was first subjected to TLC with the system chloroform-methanol (98:2). A dividing line was drawn below the cholesterol band and the rest of the plate was developed with chloroform-methanol (95.5). Bands corresponding to (a) choline phosphoglycerides, sphingomyelins, cerebroside, (b) monoglyceride ceramide, glyceryl ether, (c) cholesterol, (d) diglyceride, (e) aldehyde, cholesterol esters, triglycerides were separated, scraped off the plate and extracted with appropriate solvents. The several components were subjected to methanolysis and analyzed for dimethyl acetals as described. The glyceryl ethers were freed by refluxing the neutral lipids with 2 N ethanolic KOH (14) and were identified by GLC of their TFA derivatives.

Counting was performed in a Packard Tri-Card Scintillation Spectrometer with samples dissolved in Toluene containing 5 g PPO and 0.3 g dimethyl POPOP per liter. Efficiencies were 15% for ^{3}H and 62% for ^{14}C.

RESULTS

Each rat received approximately 1.6 μc ^{14}C-PE and 0.4 μc ^{3}H-chimyl alcohol intracerebrally. The weights and radioactivities of the brain lipids of the 3 groups of rats are shown in Table I. It is evident that label from both substrates was incorporated into the brain lipids of the rats. The weights and radioactivities of the chromatographically separated lipid fractions are recorded in Table II. As expected, there were no significant differences in the per cent composition of the brain lipids of the different groups. A major portion of the ^{14}C activity (25-35%) appears in the front fraction, which contains cholesterol, ceramide, cerebroside, choline phosphoglycerides, sphingomyelin and lyso phosphatidyl choline. In addition, there seem to be no significant differences in distribution of ^{14}C (from PE) with time. The ^{3}H (from chimyl alcohol), on the other hand, appears to decrease

TABLE II

Weights and Radioactivity of Lipids in Rat Brains after Injection of ^{14}C Phosphatidyl Ethanolamine and ^{3}H-Chimyl Alcohol

Total lipid chromatographed	Group A (2 hr)			Group B (4 hr)			Group C (16 hr)		
	Wt. 487.4	^{14}C-PE $3.5x10^6$	^{3}H-CA $0.65x10^6$	Wt. 695.3	^{14}C-PE $3.5x10^6$	^{3}H-CA $0.66x10^6$	Wt. 462	^{14}C-PE $3.1x10^6$	^{3}H-CA $0.51x10^6$
Per cent distribution[a]									
Front fraction	50.4	31.5	78.1	55.6	26.9	74.9	47.9	35.2	66.9
Phosphatidyl ethanolamine fraction	20.9	26.8	9.6	19.3	33.9	14.0	21.3	26.8	18.4
Non-lipid fraction	8.9	2.7	1.2	7.8	2.7	1.00	9.4	2.1	1.4
Phosphatidyl serine fraction	10.8	15.4	5.2	11.2	14.2	4.2	12.4	12.5	4.8
Inositide fraction	9.1	26.0	7.0	6.3	22.4	6.1	9.0	23.3	8.6
Per cent recovery	99.0	82.0	108.0	93.2	86.0	98.4	97.5	76.1	106.0

[a] All values are expressed as a per cent of the total weight of radioactivity obtained from the column (see under methods for description of chromatographic separation).

in the front fraction and increase in the ethanolamine phosphoglycerides during the times studied, indicating possible incorporation of chimyl alcohol into PE or closely related compounds. When this fraction was separated, following methanolysis, into methyl esters, dimethyl acetals and TFA derivatives of chimyl alcohol, the results shown in Table III were obtained. It was found that ethanolamine phosphoglycerides of both alkyl ether and alkenyl ether types contained ^{3}H from the chimyl alcohol, but had incorporated no ^{14}C from the injected PE.

The front fraction from group B was separated into its components by TLC, as described above, giving the distribution of weight and radioactivity shown in Table IV. When these fractions (other than cholesterol) were subjected to methanolysis to obtain the methyl esters and acetals or to hydrolysis (14) to obtain the glyceryl ethers, it was found that the ^{14}C activity of all fractions was in the methyl esters. Most of the ^{3}H activity of the phospholipid fraction was also in the methyl esters but in the fraction migrating with monoglyceride, the ^{3}H activity was associated entirely with the glyceryl ethers. The diglyceride fraction, following hydrolysis, had 12% of the ^{3}H activity in the methyl esters and 50-60% in the glyceryl ethers. The distribution of activity in the triglyceride fraction was about the same.

TABLE III

Relative Specific Activities[a] of the Methyl Esters, Dimethyl Acetals and TFA Derivatives from Rat Brains after Injection of PE and Chimyl Alcohol

	Group A (4 hr)		Group B (8 hr)		Group C (16 hr)	
	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H
Methyl esters						
16:0	41.8	....	27.5	....	28.4	....
18:0	4.2	....	1.6	....	1.2	....
18:1	14.2	....	10.4	....	9.8	....
Dimethyl acetals						
16:0	....	2.8	....	3.0	....	10.4
18:0	....	....	....	....	....	....
18:1	....	....	....	....	....	....
TFA glyceryl ethers						
16:0	....	30.7	....	38.5	....	65.2
18:0	....	....	....	....	....	....
18:1	....	....	....	....	....	....

[a] Average from three determinations. Relative specific activities taken as ratios of cpm in a collected fraction to the peak area of the fraction.

TABLE IV

Percent Distribution of Activity in the Front Fraction from DEAE Cellulose Separation of Lipids from Brains of Group B Rats

Fraction	Wt	^{3}H	^{14}C
Phospholipid phosphatidyl choline sphingomyelin cerebroside	55.9	13.2	69.0
Monoglyceride Fraction (containing glyceryl ethers)	8.5	50.9	11.5
Cholesterol	21.3	3.2	2.3
Diglyceride Fraction (containing glyceryl ethers)	5.5	16.3	3.5
Triglyceride	8.5	16.2	13.5

DISCUSSION

The previous study (1) revealed that palmitaldehyde is not incorporated directly into the alkenyl ether linkage of phosphatidal ethanolamine and indicated that this type of compound arises by reduction of an acyl ester linkage. It was not possible from those results, however, to state at what stage such a reduction might take place.

The present experiments indicate that it does not take place in the phospholipid stage since there was no incorporation of ^{14}C from the injected PE into the dimethyl acetals or glyceryl ethers of the extracted ethanol phosphoglyceride fraction. Therefore, any reduction of the acyl group to the alkenyl must take place as the diglyceride or phosphatidic acid. Kiyasu and Kennedy (6) have shown that such a diglyceride (α-alkenyl-β-acyl-) could be converted to ethanolamine plasmalogen by a particulate fraction from rat liver.

On the other hand, the present results also provide evidence that the glyceryl ethers may serve not only as precursors of the alkyl-acyl phosphoglycerides but also of the alkenyl acyl compounds since ^{3}H from the injected chimyl alcohol does appear in both the dimethyl acetals and the glyceryl ethers derived from this fraction. Thus, an oxidation of the ether linkage of the type proposed by Thompson (15) is implicated. Such an oxidation has been found in part by Tietz et al. (18) although the product was not the alkenyl ether. The evidence available at the present time supports both a reductive and an oxidative origin for the alkenyl ether linkage of the phosphatidal ethanolamines but does not decide between them. It is possible that both pathways exist in the rat brain. The stage at which such reactions take place is also open to question although the present evidence supports the idea that it is at the "diglyceride" stage. These questions await the results of additional experiments.

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