ON THE SUBSTRATE SPECIFICITY OF ENOL ETHER FORMATION IN RAT BRAIN. METABOLISM OF O-ALKYL ETHANEDIOL PHOSPHORYL-ETHANOLAMINE*

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Received July 28, 1975

SUMMARY

1-O-[1'-^{14}C]Hexadecyl ethanediol 2-phosphorylethanolamine was administered to myelinating rat brain in order to establish general patterns of diol phospholipid metabolism and the specific substrate requirements of 1-O-alkyl 2-acyl sn-glycero-3-phosphorylethanolamine desaturase. It was shown that alkyl ethanediol PE was slowly metabolized by oxidative ether bond cleavage. Dehydrogenation to diol plasmalogen did not occur, thus providing further evidence for the high degree of substrate specificity of the desaturase present in brain.

Much current interest is focussed on plasmalogen biosynthesis in mammalian cells, particularly on the mechanism of vinyl ether formation. Dehydrogenation of 1-O-alkyl 2-acyl sn-glycero-3-phosphorylethanolamine to the alk-1-enyl analogue is catalyzed by a desaturase which apparently uses the microsomal electron transport system (2-6). In view of the results of recent *in vivo* (7-10) and *in vitro* (4) experiments on the substrate requirements of this ether desaturase, it was important to determine whether the enzyme also catalyzes the direct conversion of 1-O-alkyl ethanediol 2-phosphorylethanolamine to the corresponding diol plasmalogen.

*"Naturally occurring diol lipids. XIII." For the preceding paper in this series, see reference 1. Abbreviations: PE, lipids containing phosphorylethanolamine; PC, lipids containing phosphorylcholine; TLC, thin-layer chromatography; GLC, gas-liquid chromatography.

Diol plasmalogens (11) and other ether lipids containing a short-chain diol backbone (12-15) occur in mammalian tissues (11-13) and in certain marine invertebrates (14,15). We have recently found that 1-O-hexadecyl ethanediol intracerebrally administered to myelinating rat brain is rapidly metabolized, but not incorporated into diol phospholipids (1). Therefore, 1-O-[1'-^{14}C]hexadecyl ethanediol 2-phosphorylethanolamine was chemically synthesized and used as substrate in the present study.

MATERIALS AND METHODS

1-O-[1'-^{14}C]Hexadecyl ethanediol was prepared from [1-^{14}C] palmitic acid (57 mCi/mmole, Dhom Products, North Hollywood, Calif.) (1). 1-O-[1'-^{14}C]Hexadecyl ethanediol 2-phosphorylethanolamine was synthesized (85% yield) by condensation of 1-O-[1'-^{14}C] hexadecyl ethanediol (0.1 mCi) with phthalimidoethyldichlorophosphate (16) in pyridine at 0°C, followed by hydrolysis of the chloride and removal of the phthaloyl protective group with hydrazine in boiling ethanol**. The radiopurity of alkyl ethanediol PE was better than 99% as judged by TLC (chloroform-methanol-water, 55:35:6, v/v/v). O-Alkyl ethanediol (17) and O-alk-1-enyl ethanediol (18) used as standards were prepared according to established procedures. Hexadecanal was synthesized from hexadecyl methanesulfonate (19) by dimethylsulfoxide oxidation (20).

1-O-[1'-^{14}C]Hexadecyl ethanediol 2-phosphorylethanolamine was emulsified with sodium choleate (10 mg/ml) and was injected into the brains (10 µl/brain) of 18-day-old male albino rats (Sprague-Dawley strain, Dan Rolfsmeyer Co., Madison, Wis.) (21). Rats were killed by decapitation in groups of five after 3 and 24 hr. Lipids were extracted with 20 parts of chloroform-methanol, 2:1 (v/v), and nonlipid contaminants were removed by silicic acid treatment (22). Lipids were fractionated by chromatography (TLC) on layers of Silica Gel H (Merck), 0.5 mm thick, in tanks lined with filter paper. Degradations and derivatizations of lipids by $LiAlH_4$ reduction (18), methanolysis (21), enol ether cleavage (1), and acetylation (18) were done as previously described. Phospholipase C and D (General Biochemicals, Chagrin Falls, Ohio) hydrolyses were done according to established procedures (11,22,23).

GLC of alkyl acetates was done on a Victoreen 4000 instrument equipped with flame ionization and thermoconductivity detectors. The aluminum column, 180 cm x 0.4 cm, packed with ethylene glycol succinate (10% EGSS-X) on Gas-Chrom P, 100-120 mesh (Applied Science Laboratories, State College, Penn.), was operated at 200°C. GLC fractions were collected in glass tubes permitting recoveries of better than 80%. Radioactivities were determined with a Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.) in Aquasol (New England

**Madson, T. H. and Baumann, W. J., unpublished results.

Nuclear, Boston, Mass.) or toluene solutions of Permablend (Packard).

RESULTS AND DISCUSSION

1-O-[1'-^{14}C]Hexadecyl ethanediol 2-phosphorylethanolamine was administered intracerebrally to myelinating rat brain. The amounts of radioactivity administered and the amounts of label recovered in the lipid extract 3 and 24 hr after injection of alkyl ethanediol PE are listed in Table I. Thin-layer chromatography

[Table I here]

(chloroform-methanol-water, 65:35:8, v/v/v) of the total brain lipids revealed that after both time periods essentially all of the radioactivity (> 99%) was associated with the polar lipids (Table I), and particularly with the glycerol PC fraction, which has the same migration rate in TLC as alkyl ethanediol PE. Only

TABLE I. Metabolism of 1-O-[1'-^{14}C]hexadecyl ethanediol 2-phosphorylethanolamine in rat brain

	Time	
	3 hr	24 hr
Radioactivity administered (cpm x 10^{-5} per brain)	7.51	7.51
Radioactivity recovered with lipid extract (cpm x 10^{-5} per brain)	5.70	4.86
Distribution of radioactivity (%)		
Neutral lipids	0.6	0.9*
Polar lipids (excluding precursor)	4.7	21.0
Alkyl diol PE (precursor)**	94.7	78.1

*Methanolysis of the neutral lipids showed that more than 75% of the radioactivity was associated with fatty acids. The alkyl ethanediol fraction was unlabeled.

**Determined as O-alkyl ethanediol after $LiAlH_4$ reduction. Formation of alkyl ethanediol from phospholipid-bound O-alkyl glycolic acid (1) was excluded, because methanolysis of the total lipids of the 24 hr experiment afforded "normal" fatty acids only.

in the 24 hr experiment could substantial radioactivity (approx. 10%) also be attributed to the glycerol PE fraction.

Because of difficulties in the separation of intact glycerol- and diol-derived lipids, assignment of radioactivities to specific lipids or lipid moieties required enzymatic or chemical degradation. However, when 1-O-[1'-^{14}C]hexadecyl ethanediol 2-phosphorylethanolamine was incubated *in vitro* with phospholipase C (EC 3.1.4.3) from *Bacillus cereus* (11,22) at pH 7.2 in the presence of Zn^{++} ions and carrier lecithin (25°, 18 hr), conditions suitable to quantitatively cleave 1-O-acyl ethanediol 2-phosphorylethanolamine (22), the diol ether PE was recovered unchanged; no radioactivity was associated with alkyl ethanediol. Similarly, alkyl ethanediol PE was not cleaved by phospholipase D (EC 3.1.4.4) (23).

Lithium aluminum hydride reduction of the lipids of the 3 and 24 hr experiments followed by TLC (hexane-diethyl ether, 70:30, v/v, triple development) showed that 94.7 and 78.1% of the radioactivities, respectively, were associated with O-alkyl ethanediol (R_f 0.37) derived from unmetabolized precursor (Table I). After the same time periods, 4.4 and 19.4% of the label, respectively, had a mobility in TLC similar to that of long-chain alcohol (R_f 0.46) and/or 1-O-alk-1-enyl ethanediol (R_f 0.49). When the reduced lipids of the 24 hr experiment were acetylated, the major portion of label (78.1%) was found in the O-alkyl ethanediol acetates (R_f 0.16; hexane-diethyl ether, 90:10, v/v). The long-chain alkyl acetates (R_f 0.41), representative of the constituent fatty acids, carried 19.2% of the activity, whereas the alk-1-enyl ethanediol acetate fraction (R_f 0.24) was essentially unlabeled ($< 0.3\%$).

These data suggested that alkyl ethanediol PE was not desat-

urated in myelinating rat brain. This was confirmed by subjecting the alkyl/alk-1-enyl ethanediol acetate fraction (R_f 0.16-0.24) to HCl/diethyl ether hydrolysis (1). Only traces (< 0.7%) of the radioactivity present in the hydrolyzed products showed a mobility in TLC (R_f 0.39; hexane-diethyl ether, 90:10, v/v) equal to that of long-chain aldehyde. The absence of significant amounts of labeled aldehydogenic constituents (< 1.3%) in the total lipids of the 24 hr experiment was similarly demonstrated.

In vivo experiments have previously shown that 1-*O*-alkyl glycerol PE is more rapidly converted to 1-*O*-alk-1'-enyl 2-acyl glycerol PE of brain than is 1-*O*-alkyl 2-acyl glycerol PE (24,25), despite the fact that the latter is the true substrate in the desaturation process (2-6,25). The apparently rapid conversion of alkyl glycerol PE to plasmalogen is readily explained by facilitated transport of the lyso compound through brain cell membranes. In view of the structural and functional similarities between lyso glycerophospholipids and the corresponding diol phospholipids, for example in their lytic activities (26,27) and other properties (*vide infra*), it appears most probable that alkyl ethanediol PE is readily available at the potential site of desaturation. Hence, we conclude that alkyl ethanediol PE does not serve as substrate for the 1-*O*-alkyl 2-acyl *sn*-glycero-3-phosphorylethanolamine desaturase present in rat brain.

An alternate pathway of alkyl ethanediol PE metabolism is indicated by the occurrence of labeled fatty acids (19.2%) in the lipids of brain 24 hr after administering the precursor. The major portion of radioactivity was found in the palmitic acid moieties (64.9%) which were analyzed as hexadecyl acetate. Smaller amounts of label were associated with stearic (17.0%) and oleic (12.3%) acids produced through the elongation-desatura-

tion sequence. As the labeled fatty acids in turn were available to enter the pathways of glycerol ether and plasmalogen biosyntheses (2-6,21,25,28), the formation of traces of labeled aldehydogenic lipids in our experiment can readily be explained without assuming alkyl ethanediol desaturation.

Degradation of O-alkyl ethanediol PE is probably brought about by the alkyl ether cleavage enzyme which was originally found in rat liver microsomes (29) and occurs at much lower levels in brain (30,31). This tetrahydropteridine-requiring enzyme system catalyzes the oxidative cleavage of alkyl ethers to carboxylic acids (29). It most effectively utilizes O-alkyl glycerols (29-32) and O-alkyl ethanediol (33) as substrates, but does not directly cleave glycerol-substituted O-alkyl glycerols with the noteworthy exception of 1-O-alkyl sn-glycero-3-phosphorylethanolamine (32), the lysophospholipid similar in structure to alkyl ethanediol PE. As our in vitro and in vivo experiments did not produce any evidence (Table I) that alkyl ethanediol PE is hydrolyzed by phospholipases of the C- and D-type present in brain (34-36), oxidative ether bond cleavage probably on the intact phospholipid is the major pathway of 1-O-alkyl ethanediol 2-phosphorylethanolamine catabolism.

ACKNOWLEDGMENTS

This work was supported in part by PHS Research Grants Nos. CA 12150, CA 13113, and NS 10013, PHS Research Grant No. HL 08214 from the Program Projects Branch, Extramural Programs, National Heart and Lung Institute, and by The Hormel Foundation. We also wish to thank Dr. J. T. Lin for help and advice in several experiments.

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