EVIDENCE FOR THE INCORPORATION OF A LONG-CHAIN 1,2-ALKANEDIOL INTO DIOL PHOSPHOLIPIDS BY MAMMALIAN BRAIN

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SUMMARY

[2-^{14}C,2-^{3}H]1,2-Heptadecanediol administered intracerebrally to 18-day-old rats was found to be incorporated as such into phospholipic Chemical and enzymic degradation of the choline phosphatide fraction showed that an appreciable amount of radioactivity was associated with 2-O-acyl heptadecanediol phosphoryl choline.

Long-chain 1,2-alkanediols are constituents of mammalian neutral lipids (1), yet little is known about their biosynthesis and metabolis Previous work from this laboratory has provided evidence for the formation of 1-O-2'-hydroxyalkyl and 1-O-2'-ketoalkyl-2-acyl glycero-phosphatides in myelinating rat brain from intracerebrally injected long-chain 1,2-alkanediols or 1,2-alkaneketols (2-4). When [2-^{14}C]1,2 heptadecanediol was administered (2), hydrogenolysis of the ethanolam phosphatide fraction with lithium aluminum hydride yielded labeled 1,2-heptadecanediol in addition to 1-O-2'-hydroxyheptadecyl glycerol. 1,2-Heptadecanediol as a reduction product of purified ethanolamine phosphatides could not be explained satisfactorily at that time. Recent studies with doubly labeled substrates[1] showed that the 1,2-heptadecanediol derived from both ethanolamine and choline phosphatides by $LiAlH_4$ reduction retained the tritium label at carbon. In this communication, we present evidence for the formation of a new class of choline phospholipid having a long-chain alkanediol backbone

[1] N. Chang and H. H. O. Schmid, manuscript in preparation.

MATERIALS AND METHODS

Fatty acids were obtained from The Hormel Institute Lipids Preparation Laboratory, Austin, Minnesota. [1-^{14}C]Palmitic acid (58 mCi/mmole) and lithium aluminum [^{3}H]hydride (157 mCi/mmole) were purchased from New England Nuclear, Boston, Massachusetts. Male albino rats of the Sprague-Dawley strain were purchased from the Dan Rolfsmeyer Company, Madison, Wisconsin. They were 18 days old at the time of the experiment.

Emulsification and intracerebral injection of precursor and chemical reactions for the preparation of derivatives, such as methanolysis, acetylation, ketalation and hydrogenolysis, were as described earlier (2-5). Adsorption chromatography was carried out on layers of Silica Gel H, 0.5 mm thick, in tanks lined with filter paper. Proportions of developing solvents are given by volume throughout the text. Gas-liquid chromatography was performed with a Victoreen 4000 instrument using an aluminum column, 180 cm x 0.4 cm I.D., filled with ethylene glycol succinate (10% EGSS-X) on Gas Chrom P, 100-120 mesh (Applied Science Laboratories, State College, Penn.)

Radioactivities were determined in a Packard Tri-Carb liquid scintillation spectrometer and were corrected by the use of standards. Counting efficiency for ^{14}C (channel 1) was 62.5% and for simultaneous ^{14}C-^{3}H determination (channel 2) 14.4% and 36.7%, respectively. In addition, ^{14}C and ^{3}H were measured individually after combustion in a Packard Tri-Carb Sample Oxidizer.

[2-^{14}C]1,2-Heptadecanediol (58 mCi/mmole) was prepared as previously described (2). [2-^{3}H]1,2-Heptadecanediol (39.3 mCi/mmole) was prepared by reduction of 1-hydroxy-2-ketoheptadecane (4) with $LiAl^{3}H_4$ (157 mCi/mmole). The product was purified to a radiopurity of >98% by TLC using hexane-diethyl ether, 20:80. A mixture of the labeled diols was emulsified in aqueous sodium choleate (10 mg/ml) and the

radioactivity determined for a 10 µl aliquot: $^{14}C = 1.63 \times 10^6$ dpm, $^{3}H = 7.63 \times 10^6$ dpm; $^{3}H/^{14}C = 4.68$.

Unlabeled 2-O-hexadecanoyl 1,2-heptadecanediol was prepared according to the method of Baumann and Madson[2] by reacting 1,2-heptadecanediol with hexadecanal to form the cyclic acetal, followed by ring cleavage through ozonolysis. 2-O-Hexadecanoyl 1,2-heptadecanediol (Rf = 0.54) was separated from the small amount of 1-O-hexadecanoyl 1,2-heptadecanediol (Rf = 0.65) by TLC using hexane-diethyl ether, 50:50, and was characterized by its IR spectrum and the mass spectrum of its TMS derivative[2]. The 2-acyl alkanediol was acetylated with acetic anhydride in pyridine at 80°C for 2 hours.

The choline phosphatide fraction was isolated from the total brain lipids by preparative TLC using chloroform-methanol-water, 65:25:4, and repurified with chloroform-methanol-acetic acid-water, 50:25:4:2.

Hydrolysis of 5 mg of the choline phosphatide fraction with 5 mg of phospholipase C (Cl. welchii)[3] was carried out at room temperature in a mixture of 50 ml of Tris buffer, pH 7.6, containing 44.4 mg of $CaCl_2$ and 25 ml of diethyl ether. After 20 hours, the ether phase was removed and the aqueous phase extracted with diethyl ether. The combined ether phases were washed with water and dried over Na_2SO_4.

RESULTS AND DISCUSSION

[2-^{14}C,2-^{3}H]1,2-Heptadecanediol ($^{3}H/^{14}C = 4.68$) was administered intracerebrally to six 18-day-old rats and its incorporation into the brain choline phosphatides was determined after 24 hours. Detailed results of the metabolic degradation and incorporation of the doubly labeled substrate into other complex lipids will be published else-

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[2] W. J. Baumann and T. H. Madson, manuscript in preparation.

[3] Nutritional Biochemicals Co., Cleveland, Ohio.

where[1]. Approximately 30% (4.87×10^5 dpm) of the ^{14}C radioactivity injected per rat (1.63×10^6 dpm) was recovered in total lipids. About 50% of this radioactivity was in the nonmetabolized substrate, 21% in a crude ethanolamine phosphatide fraction and 24% in a crude choline phosphatide fraction. The choline phosphatides were isolated and purified to a radiopurity of better than 95% (total ^{14}C radioactivity 6.96×10^5 dpm).

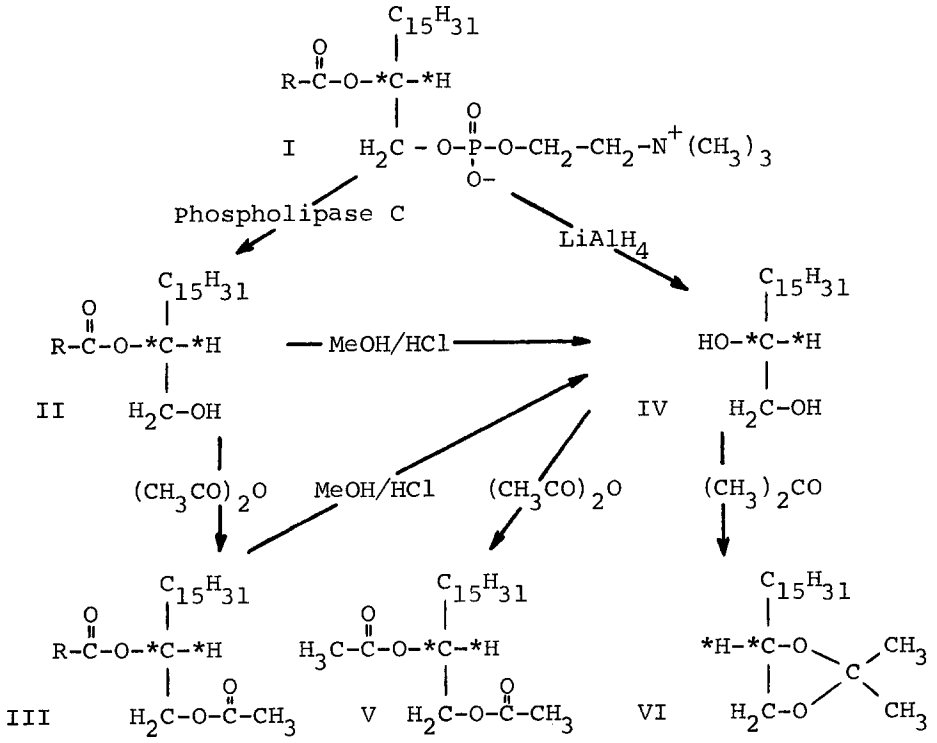
A portion of the choline phosphatides was hydrolyzed with phospholipase C and the distribution of radioactivity in the reaction products was checked by TLC (hexane-diethyl ether, 15:85). Major amounts of radioactivity were in fractions corresponding to diglycerides (43%), 1-O-2'-ketoheptadecyl-2-acyl glycerols (31%), and 1-O-2'-hydroxyheptadecyl-2-acyl glycerols (24%); 2% of the ^{14}C radioactivity was present in unhydrolyzed material.

The diglyceride fraction was isolated by preparative TLC (hexane-diethyl ether, 15:85). Part of it was subjected to acidic methanolysis and the reaction products were fractionated by TLC (hexane-diethyl ether, 80:20); ^{14}C radioactivity was found in methyl esters of fatty acids (80%) and in 1,2-heptadecanediol (20%). The identity of the diol was established by TLC and GLC of its diacetate and isopropylidene derivative using unlabeled 1,2-heptadecanediol as carrier. The isopropylidene derivative recovered after preparative GLC exhibited a $^{3}H/^{14}C$ ratio of 5.26.

Refractionation of a small aliquot of the diglyceride fraction with hexane-diethyl ether, 50:50, showed essentially all ^{3}H radioactivity at Rf = 0.54 ($^{3}H/^{14}C = 5.20$), corresponding to 2-O-hexadecanoyl 1,2-heptadecanediol, and the major ^{14}C radioactivity at Rf = 0.43, corresponding to a 1,2-diglyceride.

It therefore appeared that some of the [2-^{14}C,2-^{3}H]heptadecanediol

Scheme 1



administered was esterified with a fatty acid at carbon-2 and with phosphoryl choline at carbon-1 (Scheme 1, structure I).

Phospholipid I can be expected to be inseparable from choline glycerophosphatides by adsorption chromatography. Phospholipase C hydrolysis should produce II, a compound somewhat less polar than a 1,2-diglyceride, but not separable from 1,2-diglycerides by TLC using hexane-diethyl ether, 15:85. Acetylation of the "diglycerides" and refractionation of the acetates by TLC (hexane-diethyl ether, 80:20) produced two radioactive fractions, one (III) corresponding to 1-O-acetyl-2-O-hexadecanoyl heptadecanediol (Rf = 0.54) and another corresponding to 1,2-dipalmitoyl-3-acetyl glycerol (Rf = 0.36); their $^{3}H/^{14}C$ ratios were 5.27 and 0.23, respectively. Methanolysis of the less polar fraction, isolated by TLC, yielded doubly labeled

1,2-heptadecanediol; the more polar fraction yielded ^{14}C-labeled methyl esters of fatty acids.

When a portion of the choline phosphatide fraction was reacted with $LiAlH_4$, about 8% of the ^{14}C in the reaction products was found in the 1,2-heptadecanediol fraction. The isopropylidene derivative was prepared and isolated: $^{3}H/^{14}C = 5.30$.

As in previous experiments (2-4), only traces of radioactivity were associated with glycerol ethers. Both diacetates (2) and isopropylidene derivatives of long-chain alkyl glycerols can easily be separated by TLC and GLC from the corresponding derivatives (V, VI) of long-chain 1,2-alkanediols. The enrichment of ^{3}H in the 1,2-heptadecanediol recovered from the choline phospholipids ($^{3}H/^{14}C = 5.20$-5.30) compared to the precursor ($^{3}H/^{14}C = 4.68$) remains unexplained.

In proposing structure I, one has to assume that phospholipase C is capable of catalyzing the hydrolysis of the phosphoester bond of a long-chain alkanediol. Such a reaction has not been described; however, phospholipase C is known to be relatively nonspecific (6) and, especially in the presence of choline glycerophosphatides, can be expected to catalyze the hydrolysis of a compound of structure I, as it catalyzes the hydrolysis of sphingomyelin (7).

Phospholipids containing a long-chain alkanediol backbone have not been described previously. One can assume that they are produced in a mammalian system in analogy to established pathways leading to glycerophosphatides (8) or sphingomyelin (7). They may be natural constituents of tissues in which long-chain alkanediols are available as precursors.

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