1-Docosanol and Other Long Chain Primary Alcohols in Developing Rat Brain

ABSTRACT

Long chain alcohols were detected in developing rat brain at ages ranging from 5 to 40 days. They were at their highest level of 0.0109% of the total lipids at the age of 10 days and decreased to 0.0036% at the age of 40 days. They consisted mainly of hexadecanol, octadecanol, octadecenol, eicosanol, docosanol, and tetracosanol. The fact that substantial amounts of fatty alcohols having more than 20 carbon atoms were present in myelinating rat brain indicated a chain length specificity in their utilization for *O*-alkyl and *O*-alk-1-enyl glycerolipid biosynthesis.

INTRODUCTION

Fatty alcohols are important intermediates in the biosynthesis of *O*-alkyl and *O*-alk-1-enyl glycerolipids (1). Small amounts of alcohols varying in chain length from C_{16} to C_{22} were detected in adult mammalian brain and heart (2,3), and somewhat larger amounts in transplantable neoplasms (3). Their biosynthesis from the corresponding fatty acids was demonstrated in vitro in a variety of mammalian (4-6) and nonmammalian (7-9) systems.

Developing rat brain was shown to produce hexadecanol from palmitic acid, both in vivo (10,11) and in vitro (6), and the rate of this reduction was highest at the age of 15 days (6). In rat brain, phospholipid biosynthesis reaches highest levels during myelination between the ages of 10 and 20 days. Thus, it was of interest to determine the levels of free alcohols present in the brain during this period and to compare their composition with that of the *O*-alkyl and *O*-alk-1-enyl glycerols.

In the present communication, we report the amounts and composition of fatty alcohols in developing rat brain between the ages of 5 and 40 days. Their composition did not correspond to that of the *O*-alkyl and *O*-alk-1-enyl glycerols. The fact that relatively large amounts of alcohols having more than 20 carbon atoms were present during myelination indicates a distinct chain length specificity in their utilization for *O*-alkyl and *O*-alk-1-enyl glycerolipid biosynthesis.

EXPERIMENTAL PROCEDURES

Male albino rats (ARS Sprague Dawley, Madison, WI), 5 to 40 days old, were killed by decapitation in groups of 7 to 20, brains from each group were pooled and the lipids were extracted (12). To each of the lipid extracts, 50 μg of 1-heptadecanol was added as internal standard. Lipids from an additional group of 20-day-old rats were analyzed without adding the internal standard in order to establish the level of heptadecanol among the brain alcohols.

In each case, the lipids were fractionated on layers of Silica Gel H (Merck), 3 mm thick (13), essentially as described previously (2), but using hexane:diethyl ether:acetic acid 40:60:1 v/v/v as developing solvent. The leading edge of the cholesterol fraction, including the fatty alcohols, was scraped off and eluted with chloroform:methanol 4:1 v/v. This crude alcohol fraction was refractionated by thin layer chromatography (TLC) on layers, 0.5 mm thick, and the fatty alcohols were eluted as described above.

Preparation of the alkyl acetates and analysis by gas liquid chromatography (GLC) were as described previously (2) using ethylene glycol succinate (10% EGSS-X on Gas Chrom P, 100-120 mesh) at 190 C and silicone gum rubber (SE-30 on Anachrom ABS, 90-100 mesh) at 220 C. Weight percentages of alcohols were calculated by triangulation of peak areas;

TABLE I

Fatty Alcohol Concentration in Developing Rat Brain

Age (days)	Number of rats	Total brain weight (g)	Total lipids (mg/g wet tissue)	Fatty alcohols (% of total lipids)	Fatty alcohols (nmol/g wet tissue)
5	20	9.61	24.5	0.0071	6.52
10	10	9.30	35.2	0.0109	14.03
15	10	11.72	45.6	0.0071	12.34
20	10	11.48	50.9	0.0065	12.26
40	7	8.47	68.8	0.0036	9.42

TABLE II

Fatty Alcohol Composition in Developing Rat Brain

Age (days)	Composition (wt %) of alcohols[a]					
	16:0	18:0	18:1	20:0	22:0	24:0
5	28.8	46.9	8.9	5.0	10.4	trace
10	24.6	35.3	13.7	3.5	22.9	trace
15	34.7	45.7	10.6	3.1	5.9	trace
20	54.9	25.9	6.4	1.5	6.1	5.2
40	42.0	33.5	11.1	2.7	9.3	1.4

[a]Traces (<0.5%) of 15:0, 17:0, 19:0 and 21:0 were also detected.

absolute amounts were determined by comparison of peak areas with that of the internal standard.

Trimethylsilyl (TMS) ethers of the brain alcohol fraction as well as of synthetic standards were prepared by reaction with hexamethyl disilazane and trimethylchlorosilane in dry pyridine (14). The TMS derivatives were analyzed on an LKB gas chromatograph-mass spectrometer, Type 9000. The GLC column (3% OV-1) was operated from 150 C to 300 C at a temperature program of 8 C/min. Mass spectra were taken at 20 eV and 70 eV.

RESULTS

Long chain alcohols were found in developing rat brain in the amounts listed in Table I. They were at their highest level of 0.0109% w/w of the total lipids at the age of 10 days, the beginning of myelination.

With increasing age the total amount of free alcohols present in rat brain decreased to 0.0036% w/w of the total lipids, which is comparable to the amount of 0.002% found previously for adult bovine and porcine brain (2). On the basis of total brain tissue, this decrease was less pronounced due to the concomitant increase in total brain lipids.

The relative amounts of the individual fatty alcohols of rat brain are listed in Table II. As is evident from Table II, substantial amounts of 1-docosanol were present among the fatty alcohols at all ages examined, reaching a maximum at the age of 10 days.

Preparation of the TMS ethers of the alcohol fraction from the 20-day-old rat brain and analysis by GLC-mass spectrometry confirmed their structures. Comparison of the spectra obtained from TMS ethers of synthetic 1-hexadecanol and 1-docosanol with those obtained from the appropriate GLC fractions of the natural mixture showed identity of the diagnostic peaks. All long chain alcohols showed molecular ions of low intensity but very intense M-15 ions which represented the major peaks in the high mass unit region of each spectrum.

DISCUSSION

The presence of significant amounts of long chain alcohols of chain lengths C_{20} to C_{24} in developing rat brain is of interest because the corresponding *O*-alkyl or *O*-alk-1-enyl moieties are not found among the glycerophosphatides of mammalian brain (15). Analysis of the *O*-alk-1-enyl glycerols derived from the total lipids of each group of animals used in this study confirmed the fact that mainly $C_{16:0}$, $C_{18:0}$, and $C_{18:1}$ structures were present with only traces of $C_{20:0}$ homologues. Since ether lipid biosynthesis is proceeding at a high rate during the period of myelination, we postulate a distinct chain length specificity for the biosynthesis of *O*-alkyl glycerolipids. In contrast, previous findings demonstrated a lack of specificity with regard to certain structural features of the long chain alcohols. The presence of additional double bonds (16) or certain functional groups (17,18) in primary alcohols ranging in chain length from C_{16} to C_{18} did not impede their incorporation into *O*-alkyl glycerolipids.

Although some fatty alcohols could be formed in rat brain by degradation of sphinganine (19) or *O*-alkyl glyerol (20), those actually used for ether lipid biosynthesis during myelination are apparently produced by reduction of fatty acids (21). Our data imply that this reduction involves mainly saturated and certain monounsaturated fatty acids ranging from C_{16} to C_{24}.

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