Composition of *O*-Alkyl and *O*-Alk-1-enyl Moieties in the Glycerolipids of the Human Adrenal

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ABSTRACT

A comparison of human adult and fetal adrenals with respect to their levels of glyceryl ether lipids and other lipid components is reported. Fetal glands contained significantly lower levels of alk-1-enyl phosphoglycerides and of cholesterol. Neutral glyceryl ether diesters, and ethanolamine and choline phosphoglycerides were isolated from adult adrenal tissue. The composition of the *O*-alkyl glycerol groups in these lipid fractions was obtained by means of gas chromatography of the trimethylsilyl ethers and diacetyl derivatives; *O*-alk-1-enyl glycerols were analyzed as their diacetates. About one-half of the alkyl and alk-1-enyl glycerol moieties present in glyceryl ether diesters contained hydrocarbon side chains with 20, 22, or 24 carbon atoms. Long hydrocarbon chains (C_{19-24}) were also found in the *O*-alkyl glycerol moieties present in the total lipids of fetal adrenals.

INTRODUCTION

Glycerolipids which contain ether bonds are found ubiquitously in mammalian tissues, but little is known about the ether lipids present in the human adrenal (1). The recent finding that alkyl diacylglycerols accumulated in the adrenals of an infant with Wolman's disease drew attention to the fact that neutral glyceryl ether lipids were also present in this gland, besides the plasmalogens (2). This paper concerns six classes of ether lipids found in normal adrenals. Most of the analyses were done on specimens taken from adults. Fetal adrenals (at 28-36 wk of maturity) were also studied because this gland exhibits a specific pattern of development in the prenatal period (3). Neutral lipids and phospholipids containing *O*-alkyl and *O*-alk-1-enyl moieties were found in both fetal and adult tissues. The compositions of the *O*-alkyl and *O*-alk-1-enyl glycerol moieties in the six lipid classes are reported. The scope of the observations on adrenal ether lipids made earlier by Debuch and Winterfeld (4,5) has therefore been extended.

MATERIALS AND METHODS

Chemicals

The following commercial preparations were used: hexadecyl and octadecyl glycerol, glycerol 1,2-diarachidoyl-3-eicosanyl ether (Analabs, Inc., North Haven, CT); tetradecyl glycerol, 1-octadec-9-enyl glycerol, Sil-Prep (Applied Science Laboratories, Inc., State College, PA); phospholipase C from *B. cereus* (Sigma Chemical Co., St. Louis, MO); phosphatidyl ethanolamine plasmalogen (Serdary Research Laboratories, Inc., London, Ontario, Canada); 1,10-decanediol and 1,12-dodecanediol (Koch-Light Laboratories, Colnbrook, Bucks, England).

Adrenal Specimens

Glands were removed at autopsy, usually within 48 hr of death, from a total of 61 Chinese adults and fetuses without adrenal disease. The adrenals were completely freed from the surrounding fat, weighed, and stored in chloroform (-20 C). Lipid extracts were prepared with chloroform-methanol (2:1) and washed with 0.0015 M $CaCl_2$ (6).

Colorimetric Methods

The estimation of *O*-alkyl and *O*-alk-1-enyl glycerol moieties in neutral lipid and phospholipid fractions (separated on short columns of silicic acid) was carried out on the glyceryl ethers liberated by means of saponification and isolated by means of thin layer chromatography (TLC); the phospholipid fractions were subjected to an additional step of phospholipase C digestion prior to saponification (7). The estimations of *O*-alkyl and *O*-alk-1-enyl glycerol moieties in individual lipid classes were carried out by the same procedures on ether-linked diacylglycerols, and ethanolamine and choline phosphoglycerides which were first isolated with the aid of TLC. Cholesterol was determined by the ferric chloride method (8).

O-Alkyl and *O*-Alk-1-enyl Glycerols

Total lipids (27.3 g from 350 g of adult adrenals) were separated into neutral lipids and phospholipids fractions by means of solvent partitioning (9). Alkyl and alk-1-enyl diacyl-

glycerols were partially purified from the neutral lipids fraction by column chromatography. A column (126 g silicic acid) prepared in diethyl ether-petroleum ether (60-80 C) in the proportions 1:99 was loaded with 2 g lipids and washed with a further 600 ml of the same mixture; the glyceryl ether diesters together with some triacylglycerols were eluted with 750 ml of diethyl ether-petroleum ether (1:24). Three such eluates were pooled and evaporated to dryness. The residue was saponified with 50 ml of 0.5 M KOH in methanol (50 C, 70 min). Seven volumes of water, methanol, and chloroform in the proportions 12:9:14 were shaken with the hydrolyzate. The lipids recovered from the chloroform layer were extracted with diethyl ether-petroleum ether (9:91) and the extract was applied to a column (18 g silicic acid) previously equilibrated with the same solvent mixture. The column was washed with 120 ml of the 9:91 mixture, followed by 240 ml diethyl ether-petroleum ether (1:3) and 300 ml diethyl ether. The diethyl ether eluate was evaporated to dryness, and the alkyl and alk-1-enyl glycerols contained therein were separated from each other and from other lipid components by TLC (diethyl ether-acetic acid, 99:1).

Ethanolamine and choline phosphoglycerides were isolated from the phospholipids fraction by means of preparative TLC (chloroform-acetone-acetic acid-water, 75:45:12:3). Chloroform-acetone-methanol-acetic acid-water (8:6:2:2:1) was used to test the purity of ethanolamine phosphoglycerides and chloroform-methanol-ammonia (65:25:5) for that of choline phosphoglycerides. Alkyl and alk-1-enyl glycerols were isolated from these fractions by means of a procedure utilizing phospholipase C, saponification, and TLC (diethyl ether-acetic acid, 99:1), and *O*-alkyl glycerols were isolated from total lipids from fetal adrenals by the same procedure (7).

Commercial sources provided the standard alkyl and alk-1-enyl glycerols for TLC and gas chromatography (GLC). Eicosanyl glycerol was prepared from its diacyl derivative by means of saponification and TLC. Standard *O*-alk-1-enyl glycerols were prepared from phosphatidyl ethanolamine plasmalogen (which was a mixture of *O*-alkyl acyl, *O*-alk-1-enyl acyl, and diacyl glycerophosphatides) by the previously specified phospholipase C-saponification-TLC procedure (7).

O-Alkyl glycerols and alkanediols were converted to their trimethylsilyl (TMS) ethers by reacting the dried lipid samples with Sil-Prep (22 C, 15 min). Diacetates of glyceryl ethers and of alkanediols were prepared by the method of Albro and Dittmer (10).

Chromatographic Methods

TLC was carried out on glass plates precoated with Silica Gel G with fluorescence indicator (Macherey Nagel, Düren, Germany). Plates used for the purification of glyceryl ethers were prewashed with chloroform-methanol (2:1). Silver ion-TLC plates were prepared before use by spraying prewashed plates with 10% silver nitrate in methanol-water (3:1) and drying them at 100 C (45 min). Lipids were located either by their fluorescence quenching property or by visualization with the aid of 2,7-dichlorofluorescein. The mercuric ion-dependent fuchsin reaction given by *O*-alk-1-enyl groups and the ninhydrin reaction were also employed where appropriate. Solvent systems used in TLC for the separation of ether-linked diacylglycerols were benzene-hexane-diethyl ether-acetic acid (45:50:5:1) or pure benzene. *O*-Alkyl glycerols were separated according to their degree of unsaturation by means of silver ion-TLC (11).

A Varian Aerograph model 2800 gas chromatograph with flame ionization detector was employed with 5 ft x 1/8 in. columns of 1.5% OV-101 on Chromosorb G and 10% OV-1 on Chromosorb W (220 C). Nitrogen flow rates (per min) were 30 ml for TMS-alkyl glycerols and diacetyl alk-1-enyl glycerols on OV-101; 12 ml for TMS-alkyl glycerols on OV-1; 45 ml for diacetyl alkyl glycerols on OV-101. The quantitative data presented on the composition of the alkyl and alk-1-enyl moieties were based on chromatograms obtained with OV-101 as the liquid phase. Theoretical plate numbers for the TMS ethers of octadecyl and octadecenyl glycerol were, respectively, 2300 and 2100; for the diacetyl derivatives of the corresponding alk-1-enyl glycerols, 1800 and 1700. Near baseline separations were obtained. The comparisons of the retention times of alkanediol and *O*-alkyl glycerol derivatives were obtained on a column of OV-101 (180 C; nitrogen, 25 ml/min).

RESULTS

Table I shows the lipid composition of representative adult and fetal adrenal specimens used in this study. The levels of cholesterol and other neutral lipid components varied markedly among the adult specimens, which were all taken from men (age 40-55 yr). This might have been expected since normal adrenals are rarely found post-mortem, and specimens taken routinely usually exhibit one of several types of lipid depletion (12). The lipid composition of fetal adrenals (28-36 wk) was less variable. On the average, fetal adrenals had significantly

TABLE I

Lipid Composition of Adult and Fetal Adrenal Specimens[a]

	Adult	Fetal	Difference[b]
Total lipids	101 ± 40 (27-142)	38 ± 15 (20-59)	P<0.05
Neutral lipids	87 ± 42 (6-130)	24 ± 13 (11-44)	P<0.05
Cholesterol	15 ± 21 (1-66)	7 ± 2 (4-9)	P<0.01
Phospholipids	14 ± 4 (9-21)	14 ± 4 (9-18)	NS
Neutral alkyl glycerols	58 ± 32 (10-102)	71 ± 61 (17-175)	NS
Neutral alk-1-enyl glycerols	42 ± 31 (4-93)	68 ± 53 (13-154)	NS
Alkyl phosphoglycerides	60 ± 48 (11-156)	61 ± 26 (39-107)	NS
Alk-1-enyl phosphoglycerides	205 ± 108 (89-380)	98 ± 46 (42-156)	P<0.05

[a]The values, expressed as mean ± SD (range), were based on analyses of 8 specimens from adults and 5 fetal specimens. The levels of total lipids, neutral lipids, cholesterol, and phospholipids are given in mg/g wet weight tissue; those of ether-linked glycerolipids in μg/g, computed as octadecyl or octadecenyl glycerol.

[b]Statistical significance of difference between the means obtained by means of the variance ratio test or Student's *t* test. P, probability level; NS, not statistically significant.

TABLE II

Composition of *O*-Alkyl and *O*-Alk-1-enyl Moieties in Ether-linked Glycerolipids of the Human Adrenal[a]

Side chain	Adult adrenals						Fetal adrenals
	Diacylglycerols		Ethanolamine phosphoglycerides		Choline phosphoglycerides		Total lipids
	Alkyl	Alk-1-enyl	Alkyl	Alk-1-enyl	Alkyl	Alk-1-enyl	Alkyl
16:1[b]	--	--	t[c]	t	t	t	t
16:0	6	9	52	52	42	61	16
17:1	t	t	t	t	t	t	--
17:0	t	t	t	t	t	t	1
18:1	21	17	15	20	47	16	50
18:0	17	26	33	28	11	15	15
19:1	4	--	--	t	t	8	--
19:0	t	--	t	--	t	--	3
20:1	4	5	--	--	t	t	2
20:0	4	7	t	--	t	--	2
21:1	t	--	--	--	--	t	--
21:0	t	--	--	--	--	--	t
22:1	16	14	--	--	t	--	4
22:0	7	7	t	t	t	--	4
24:1	21	15	--	--	t	--	3
24:0	t	--	--	--	--	--	--

[a]The 6 lipid fractions from adult adrenals were prepared from a single pool of tissues from 48 individuals. The total lipids of 7 fetal adrenal specimens were pooled in order to obtain the preparation of *O*-alkyl glycerols.

[b]The numbers before and after the colon refer to the number of carbon atoms in the side chain and the number of double bonds present in the chain at positions other than carbon 1, respectively.

[c]Trace component (t), amounting to less than 0.01 of the sample; identified solely on the basis of retention time. The figures denote area percentage, computed from gas chromatograms of the TMS ethers of *O*-alkyl glycerols and of diacetyl *O*-alk-1-enyl glycerols.

lower concentrations of alk-1-enyl phosphoglycerides, neutral lipids, and cholesterol. About half of the neutral alkyl glycerols in adult glands could be accounted for as alkyl diacylglycerols. Alk-1-enyl diacylglycerols were not detected in some specimens; in others, they amounted to almost half of the neutral alk-1-enyl glycerols present. The molar proportions of alkyl and alk-1-enyl glycerophosphatides in ethanolamine phospholipids were, respectively, 1.2 and 5.6%. Alkyl and alk-1-enyl phosphoglycerides comprised 0.9 and 1.8% of choline phospholipids.

The compositions of *O*-alkyl and *O*-alk-1-enyl moieties in several types of glycerolipids found in the adult adrenal are shown in Table II. These analyses were carried out on pooled specimens of adrenals taken from 48 individuals (age 59 ± 16 yr), the majority of whom were male. The *O*-alkyl and *O*-alk-1-enyl glycerols prepared from ether-linked diacylglycerols both contained significant proportions of compo-

TABLE III

Characterization of Glyceryl Ether Moieties in Ether-linked Diacylglycerols[a]

Assigned side chain composition	*O*-Alkyl glycerols (Trimethylsilyl ethers)	*O*-Alk-1-enyl glycerols (diacetates)
	Equivalent chain length (retention time, min)[b]	
16:0	16.00 (15.0)[c]	16.02 (16.5)
18:1	17.69 (25.4)[d]	17.73 (28.3)
18:0	18.02 (27.7)[c]	18.00 (30.8)
19:1	18.67 (34.2)[d]	18.74 (38.7)
20:1	19.68 (46.5)[d]	19.72 (52.3)
20:0	20.00 (51.0)[c]	20.00 (57.2)
22:1	21.71 (86.0)[d]	21.73 (96.4)
22:0	22.04 (94.5)[c]	22.04 (106)
24:1	23.72 (157.5)[d]	23.74 (178)

[a]Prepared from pooled adrenal tissues from 48 adults.

[b]Gas liquid chromatography on OV-101 as described under Methods.

[c,d]Found in the saturates (c) or the monoenes (d) fraction obtained by silver ion-thin layer chromatography.

nents with side chains having 20, 22, or 24 carbon atoms. These compounds included glyceryl ethers with a double bond at positions other than carbon 1, and they are represented in the table as 20:1, 22:1, and 24:1. The 22:1 and 24:1 groups, found in the *O*-alkyl glycerols fractions in the proportions of 16% and 21%, respectively, were also present in comparable amounts in the *O*-alk-1-enyl fraction. Glyceryl ethers with C_{20}, C_{22}, and C_{24} groups accounted for 52% of the total complement of alkyl glycerols and for 48% of the alk-1-enyl glycerols in ether-linked diacylglycerols. The commonly occurring 16:0, 18:1, and 18:0 groups were also found in these neutral glyceryl ether diesters. The ether-linked side chains in the phospholipid fractions consisted mainly of 16:0, 18:1, and 18:0 groups. Both the *O*-alkyl and *O*-alk-1-enyl fractions derived from ethanolamine phospholipids contained 52% of components with 16:0 groups and about 30% with 18:0 groups. The alkyl and alk-1-enyl glycerols derived from choline phospholipids contained different proportions of 16:0 and 18:1 groups, and the alk-1-enyl fraction also included a 19:1 component found only in trace quantities in the corresponding alkyl fraction.

O-Alkyl glycerols with long hydrocarbon side chains were also found in the total lipids of the fetal adrenals. Compounds with C_{19}, C_{20}, C_{22}, and C_{24} groups comprised 18% of the total complement of alkyl glycerols in the fetal gland (Table II).

The characterization of the individual glyceryl ether components in the adrenal lipids was obtained by a combination of GLC and TLC. The key observations are summarized in Table III. The equivalent chain length (ECL) of the individual alkyl glycerols was obtained from a plot of the log of the retention times of standard alkyl glycerols (14:0, 16:0, 18:0, and 20:0) versus the length of the side chains. The ECL of standard octadec-9-enyl glycerol (TMS ether) chromatographed on OV-101 was 17.67, indicating a fractional chain length (13) of about -0.3 for the monoene. Alkyl glycerols prepared from adrenal alkyl diacylglycerols and chromatographed in the same manner exhibited several components which had ECL corresponding to either saturates or monoenes. The assigned side chain compositions were confirmed by GLC of the saturates and the monoenes separated from a sample of the unknown alkyl glycerols by means of silver ion-TLC. The same complement of alkyl glycerols was also found by GLC on OV-1 of the TMS ethers and by analysis of the diacetyl derivatives on OV-101. The possibility that long-chain alkanediols might have been mistaken for some of the higher homologs of alkyl glycerols was ruled out by determinations of the ECL values of alkanediol derivatives, using the same procedure as that applied to the unknowns. The values, 8.16 (TMS ether) and 8.26 (diacetate) for 1,10-decanediol, 10.12 (TMS ether) and 10.17 (diacetate) for 1,12-dodecanediol, showed that the slopes of the log retention time-carbon number curves were different from those of the corresponding derivatives of *O*-alkyl glycerols; secondly, the apparent fractional chain length values for alkanediols were substantially different from those found for the various adrenal lipid components (Table III).

The unknown alk-1-enyl glycerols were identified by reference to the retention times of the diacetyl derivatives of alk-1-enyl glycerols prepared from beef brain ethanolamine plasmalogen, which have been identified and quan-

titated (10,14). The reference preparation was first standardized on OV-101 against the diacetyl derivatives of hexadecyl and octadecyl glycerol. The three major peaks in the chromatogram exhibited ECL values of 15.48 (area percentage, 32), 17.30 (41%) and 17.53 (27%), which were consistent with their identification as hexadec-1-enyl glycerol (represented in Table III as 16:0), octadec-1,9-dienyl glycerol (18:1), and octadec-1-enyl glycerol (18:0), respectively. A semilog plot of the retention times of these alk-1-enyl glycerol reference compounds versus the number of carbon atoms in the side chain was used to compute the ECL values shown in Table III.

DISCUSSION

The adult adrenal is composed of the medulla and the cortex, which differ in their embryological origin, structure, and function. Because of their close intermingling, it would have been difficult to obtain in quantity cleanly dissected cortical or medullary tissue. The higher levels of alk-1-enyl phosphoglycerides in adult glands, as compared to that in fetal adrenals, might be explained by their greater proportion of medullary tissue. The fetal adrenal is largely occupied by the fetal zone, which is probably the source of most of the lipids extracted from the fetal glands.

Alkyl and alk-1-enyl glycerol groups with 20 and 22 carbons have been found in the proportions of about 2% each in the ether-linked diacylglycerols of human perinephric fat (15). This is the first report of the co-occurrence of alkyl glycerol moieties with C_{24} groups in lipids from normal mammalian tissues. Such moieties were found in the alkyl diacylglycerols present in harderian gland tumors of mice (16). Saturated and unsaturated aldehydes with C_{24} groups have been isolated from human placental plasmalogens (17). Because transfer of certain lipids (such as dehydroepiandrosterone) between the placenta and the fetal adrenal is known to occur (18,19), there is the possibility that some of the ether lipids found in these two organs may not have been synthesized in situ. The presence of diacylglycerols with C_{22} and C_{24} alkyl and alk-1-enyl groups in the adult adrenal would indicate that the fetal adrenal at least had the latent ability to synthesize these unusual lipid components.

A pathway in mammalian cells for the synthesis of glycerolipids which contain ether bonds has been described (20). The ether bond is formed between a fatty alcohol and monoacyl dihydroxyacetone phosphate. The product, alkyl dihydroxyacetone phosphate, subsequently undergoes reduction, esterification at position 2, and dephosphorylation to form 1-alkyl-2-acyl-*sn*-glycerol. This compound can react with cytidine diphosphate- (CDP-) ethanolamine, CDP-choline, or acyl CoA. It is known that the resulting ethanolamine phosphoglyceride can undergo desaturation to yield the corresponding alk-1-enyl derivative (21). According to this scheme, the composition of the *O*-alkyl group in ether-linked diacylglycerols or glycerophosphatides would be determined at the step of synthesis of alkyl dihydroxyacetone phosphate. The observed differences in the distribution of the aliphatic moieties at position 1 in alkyl diacylglycerols, alkyl acyl glycerophosphoryl ethanolamines and alkyl acyl glycerophosphoryl cholines would suggest that they originated from different metabolic pools of 1-alkyl-2-acyl-*sn*-glycerol. Considering the complex structure of the gland, this result is not surprising. It is, of course, possible that the enzymes which add the radical at position 3 of 1-alkyl-2-acyl-*sn*-glycerol show preferences for certain *O*-alkyl groups on the substrate; so far this aspect of lipid synthesis has not received attention. The idea that glyceryl ether diesters and ether-linked phosphoglycerides do not share the same metabolic fate gains support from the fact that tissue levels of the former may vary independently in certain pathological conditions, namely, in malignancy (22) and in Wolman's disease (2).

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