The fatty acid composition of brain sphingolipids: sphingomyelin, ceramide, cerebroside, and cerebroside sulfate

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SUMMARY Sphingomyelin, ceramide, cerebroside, and cerebroside sulfate from beef brain were isolated by column chromatography in quantitative yield and in pure form. The fatty acid composition of each sphingolipid was determined by gas–liquid chromatography (GLC). Sphingomyelin contained only nonhydroxy fatty acids, while cerebroside, cerebroside sulfate, and ceramide contained both nonhydroxy and hydroxy fatty acids. The nonhydroxy fatty acid composition of each sphingolipid was similar, except that more stearic acid was present in sphingomyelin and ceramide than in cerebroside and cerebroside sulfate. The hydroxy fatty acid compositions of cerebroside and cerebroside sulfate were, with minor exceptions, similar to each other and to the corresponding nonhydroxy fatty acid compositions. In both the nonhydroxy and hydroxy fatty acid series, both odd and even chain saturated and monounsaturated fatty acids containing 14 to 26 carbons were present.

SPHINGOLIPID FATTY acids are chiefly saturated and monounsaturated long-chain acids. Early work on sphingomyelin indicated that it contained primarily lignoceric acid, but nervonic, palmitic, and stearic acids were present as well (1, 2). Blix found the predominant fatty acid in cerebroside sulfate to be cerebronic acid (3), while Jatzkewitz (4) noted, in addition, the presence of stearic, lignoceric, and nervonic acids. Early work on cerebroside indicated the predominant fatty acids to be lignoceric, cerebronic, and nervonic acids, with smaller amounts of palmitic and stearic acids (5, 6).

More recently Radin and co-workers (7, 8) have determined the quantitative fatty acid composition of brain cerebroside using quantitative column chromatographic techniques combined with gas–liquid chromatography (GLC). Their work established that cerebroside contains a series of fatty acids ranging from 14 to 26 carbons in both the nonhydroxy and α-hydroxy series. The quantitative fatty acid compositions of beef brain sphingomyelin, ceramide, cerebroside, and cerebroside sulfate were reported in a preliminary communication (9). This report presents the details of the quantitative fatty acid analysis of these brain lipids.

MATERIALS AND METHODS

All solvents were reagent grade and the nitrogen used contained less than 5 parts per million of oxygen. All solvent proportions are given as volume ratios.

Column Chromatographic Isolation

Beef brains from mature animals were extracted with chloroform–methanol 2:1 under nitrogen as described previously (10). The total lipid extract was fractionated into its components by column chromatography as described previously (10). One scheme for the isolation of ceramide, cerebroside, and sphingomyelin was as follows. The total lipid was applied to a diethylaminoethyl (DEAE) cellulose column and cholesterol, ceramide, cerebroside, lecithin, and sphingomyelin were eluted together with chloroform–methanol 8:1. This fraction was then applied to a silicic acid–ammonium silicate column and the individual lipids were eluted as follows: cholesterol with chloroform, ceramide with chloroform–methanol 19:1, cerebroside with chloroform–methanol 4:1 plus 0.5% water, lecithin with chloroform–methanol 4:1 plus 1.5% water, sphingomyelin with chloroform–methanol 4:1 plus 2% water.

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The column was cleared of any residual lipid with methanol plus 2% water.

Cerebroside sulfate was isolated by another procedure. Total brain lipid was applied to a magnesium silicate column and cholesterol was eluted with chloroform, ceramide with chloroform–methanol 19:1, and cerebroside plus cerebroside sulfate with chloroform–methanol 2:1. These solvents contained 5% 2,2-dimethoxypropane (10). The fraction containing cerebroside plus cerebroside sulfate was applied to a DEAE column; cerebroside was eluted with chloroform–methanol 2:1 and cerebroside sulfate with chloroform–methanol 4:1 plus 2% ammonium hydroxide. Prior to fatty acid analysis each lipid was examined by silicic acid paper chromatography (11) to ensure that no contamination was present. The quantities of each of these lipids present in beef brain have been presented previously (10).

Hydrolysis and Isolation of Nonhydroxy and Hydroxy Acid

Each sphingolipid was hydrolyzed in 3 N HCl for 2 hr at 110° in a sealed tube at a concentration of 2 mg of lipid per milliliter of aqueous acid. These conditions gave virtually complete release of fatty acids (95% of theory). After hydrolysis, the fatty acids with some accompanying sphingosine were extracted into petroleum ether (bp 30–60°), evaporated to dryness, and applied to a silicic acid column. Nonhydroxy fatty acids were eluted with chloroform–hexane 1:1 and hydroxy acids plus sphingosine with methanol. Hydroxy acids were then methylated and reapplied to a second silicic acid column, eluting hydroxy fatty acid esters with chloroform–hexane 1:1 and sphingosine with methanol. The details of this method are given in a separate report (12).

TABLE 1 NONHYDROXY FATTY ACID COMPOSITION OF SPHINGOLIPIDS*

Fatty Acid	Ceramide	Sphingomyelin	Cerebroside	Cerebroside Sulfate
14:0	0.6	0.3	0.2	0.8
15:0	0.2	0.1	tr.	0.3
16:0	4.5	5.9	3.5	4.1
16:1	0.4	tr.	0.1	0.7
17:0	tr.	0.3	tr.	tr.
18:0	35.2	40.1	5.8	3.3
18:1	2.1	0.7	2.7	5.2
19:0	0.1	0.1	0.2	0.3
20:0	1.0	0.4	0.6	0.3
20:1	0.5	tr.	0.3	0.4
21:0	tr.	tr.	—	—
22:0	3.3	3.5	3.6	3.5
22:1	0.5	0.6	0.6	0.6
23:0	3.7	2.8	5.2	3.1
23:1	1.0	1.0	1.2	0.8
24:0	9.8	10.7	17.4	17.8
24:1	18.4	24.6	39.3	39.5
25:0	1.4	1.5	3.6	2.2
25:1	3.0	3.3	6.2	5.2
26:0	2.3	0.6	1.7	2.3
26:1	3.6	2.6	7.6	8.5
27:0	2.9	—	—	—
27:1	1.8	—	tr.	—

* Each fatty acid is expressed as per cent of the total nonhydroxy fatty acids of each lipid.

Gas–Liquid Chromatography (GLC) of Fatty Acids

Nonhydroxy fatty acids were converted to methyl esters prior to GLC. Hydroxy fatty acid methyl esters were acetylated and chromatographed as acetoxy methyl esters. Fatty esters were chromatographed at two column temperatures, one for analyzing C_{12}–C_{20}, the other for C_{18}–C_{27}. The details of these procedures and the GLC method are described elsewhere (12). Nonhydroxy fatty acid methyl esters and acetoxy fatty acid methyl esters were chromatographed on both polar (10% diethylene glycol succinate polyester) and nonpolar (3% Apiezon L) columns. Peaks were identified by comparing their retention times with those of standard fatty acids. Where standards were unavailable, peaks were tentatively identified by carbon numbers determined from plots of chain length versus logarithm of retention time. Samples were chromatographed before and after hydrogenation over a palladium catalyst (12) as an aid in the identification of unsaturated fatty acids.

Quantification was achieved by triangulating each peak, cutting it out, and weighing it on an analytical balance. Since the detector response was not identical for each fatty acid, correction factors were applied using pure standards[1] of 14:0, 16:0, 16:1, 18:0, 18:1, 20:0, 21:0, 23:0, and 24:0.[2] In those instances where fatty acid standards were unavailable, the closest corresponding correction factor was used.

RESULTS

The nonhydroxy fatty acid composition of each sphingolipid included a wide range of long-chain acids from 14 to 26 or 27 carbons. Even numbered saturated acids were present as well as the corresponding monounsaturated homologues. No polyunsaturated fatty acids were detected, although polyenoic long-chain acids were recently reported to be present in small amounts in cerebrosides (13). These acids must have been present in proportions below the limit of detection of the methods used herein. The nonhydroxy fatty acid compositions of cerebroside, cerebroside sulfate, ceramide, and sphingomyelin were similar. In each, 24:0 and 24:1 were quantitatively important (Table 1). Ceramide and

[1] Obtained from Applied Science Laboratories, State College, Pa.

[2] Chain lengths and degree of unsaturation are indicated by numerals, i.e. 18:1 is an 18-carbon monounsaturated acid; h indicates a hydroxy acid.

sphingomyelin contained more stearic acid and less of the longer chain acids than cerebroside and cerebroside sulfate. Except for this major difference in 18:0, the quantitative pattern was very similar for all four sphingolipids. Also of interest was the finding that ceramide (and perhaps cerebroside) contained small proportions of C_{27} acids. These fatty acids have not previously been reported to occur in the brain.

The hydroxy acid compositions of cerebroside and cerebroside sulfate are given in Table 2. As in the nonhydroxy series, a wide range of acids from 14 to 26 carbons was present, including both odd and even numbered acids and the corresponding monounsaturated homologues. However, the proportion of 24h:1 was smaller than 24:1, and 22h:1 and 23h:1 were not detected. Small quantities of hydroxy acids were found in ceramide; the pattern observed was similar both qualitatively and quantitatively to that of cerebroside and cerebroside sulfate, but sufficient quantities were not isolated for reliable quantification. No hydroxy acids were detected in sphingomyelin after paper, column, or gas–liquid chromatography. The hydroxy fatty acid compositions of cerebroside and cerebroside sulfate were similar. However, cerebroside contained a higher content of hydroxy acids than cerebroside sulfate, also noted in studies of these lipids in human brains (14). The ratio of total nonhydroxy acids to hydroxy acids in cerebroside was 42:58, while for cerebroside sulfate this ratio was 77:13. These values were obtained by weighing the nonhydroxy and hydroxy acid fractions after column chromatographic separation.

TABLE 2 HYDROXY FATTY ACID COMPOSITION OF SPHINGOLIPIDS*

Fatty Acid	Cerebroside	Cerebroside Sulfate
12h:0	0.4	0.3
13h:0	0.2	0.2
14h:0	0.8	3.9
15h:0	tr.	0.3
16h:0	0.1	0.7
16h:1	tr.	1.7
17h:0	0.2	0.9
18h:0	16.2	12.3
19h:0	0.4	0.3
20h:0	0.5	0.7
22h:0	5.8	7.7
23h:0	8.9	6.5
24h:0	30.1	26.0
24h:1	14.6	16.7
25h:0	7.0	4.5
25h:1	3.0	2.8
26h:0	3.9	3.8
26h:1	6.3	6.5

* Each fatty acid is expressed as per cent of the total hydroxy fatty acids in each lipid.

DISCUSSION

This study establishes for the first time the quantitative fatty acid compositions of beef brain sphingomyelin, ceramide, and cerebroside sulfate. In agreement with the reports of earlier investigators (1–4), sphingomyelin and cerebroside sulfate were found to contain long-chain saturated fatty acids. The pattern was one of odd and even numbered acids, both saturated and monounsaturated, ranging from 14 to 26 carbon atoms in the nonhydroxy series. α-Hydroxy acids found in ceramide, cerebroside, and cerebroside sulfate, but not in sphingomyelin, showed a similar pattern except for reduced amounts of 24h:1 and undetectable 22h:1 and 23h:1. This general type of fatty acid composition is similar to that reported earlier by Radin and co-workers (7, 8) for rat and human brain cerebroside. Recently Gatt and Berman (15) have reported that human brain ceramide also possesses a fatty acid composition of similar type. These authors point out that ceramide and sphingomyelin contain higher proportions of 18:0 than cerebroside and cerebroside sulfate, in agreement with the results reported here. Svennerholm (16) has also reported a similar nonhydroxy fatty acid composition for human brain sphingomyelin.

Long-chain saturated odd and even numbered acids are present in sphingolipids from tissues other than the brain, e.g. aortic sphingomyelin (17), serum sphingomyelin (18, 19), ceramide, cerebroside, and ceramide dihexoside from spleen (20, 21), equine erythrocyte gangliosides (22), and Gaucher's cerebroside from spleen (23).[3] The notable exception to this rule is brain gangliosides, which contain primarily stearic acid (24).[3] It is probable that the long-chain saturated character of the fatty acids of these sphingolipids is important in their biologic function, perhaps in their role as membrane components.

The qualitative similarities of fatty acid composition of sphingomyelin, cerebroside, and cerebroside sulfate prompted us to suggest (9) that these lipids arise from a common acylated precursor, ceramide. Enzymic studies indicate that ceramide is the precursor of sphingomyelin (25); however, cerebrosides are formed from psychosine (galactosyl sphingosine) in vitro (26). If sphingomyelin and cerebrosides are synthesized by two different metabolic pathways, the systems for their fatty acid chain elongation must either be the same or act similarly to account for the similarities in their fatty acid compositions.

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[3] J. S. O'Brien and G. Rouser. Unpublished data.

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