NEUTRAL GLYCEROGLUCOLIPIDS OF THE HUMAN GASTRIC CONTENT

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SUMMARY - Two major neutral glycolipids of the human gastric content have been isolated and partially characterized. Both glycolipids were found to contain glucose, glyceryl ethers and fatty acids. Based on the data of chemical analyses we suggest that these glycolipids are monoalkyl-monoacyl-glyceryl hexaglucoside and monoalkyl-monoacyl-glyceryl octaglucoside.

INTRODUCTION - Our recent studies on glycolipids of human gastric content (1,2) indicated that these compounds differ from the glycolipids of gastric mucosa (3,4) with respect to their sugar composition and the nature of the lipid core. Whereas majority of glycolipids found in the gastric mucosa contain sphingosine, and thus belong to the glycosphingolipids, the lipid core of glycolipids from gastric content consist of diglyceride (1,2). The glycoglycerolipids, in the mammalian species, so far are represented only by neutral and sulfated monogalactosyl diglycerides (5-8). In this paper we describe the isolation and partial characterization of two major neutral glycolipids present in human gastric secretion.

MATERIALS AND METHODS - A 100 ml of pentagastrin-stimulated human gastric secretion,obtained from the individuals with the normal gastric pathology, was dialyzed against distilled water and lyophilized. The powder was extracted twice, each time for 24h with 500 ml of chloroform-methanol (2:1 v/v), and filtered through a sintered glass funnel. The lipids in the filtrates were concentrated, dissolved in a small volume of methanol-chloroform water (60:30:8 v/v) and fractionated on DEAE-Sephadex column. The neutral glycolipids were eluted from the column with the above solvent mixture, and the acidic glycolipids with 0.4 M sodium acetate in methanol-chloroform-water (60:30:8 v/v). Further fractionation of the lipids, present in the neutral eluate from DEAE-Sephadex, was accomplished on Silicic acid. The column was developed first with chloroform, followed by acetone and acetone-methanol (9:1 v/v). The neutral glycolipids, eluted with the last solvent system, were further separated into individual components by thin-layer chromatography in chloroform-methanol-water (65:30:8 v/v) and chloroform-acetone-methanol-water (50:40:20:5 v/v).

Mild alkaline methanolysis of the studied glycolipids was performed with 0.3 M NaOH in chloroform-methanol (9). After neutralization and removal of fatty acid methyl esters with hexane, the glycolipids present in methanolic phase were chromatographed on thin-layer plates developed in chloro-

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form-methanol-water (65:35:8 v/v). Methyl esters of fatty acids, glyceryl ethers and methyl glycosides were obtained by methanolysis of the glycolipids in 1.2 M methanolic HCl at 80°C for 24 hours. Following extraction of glyceryl ethers and fatty acid methyl esters with hexane, the methanolic phases were neutralized with silver carbonate, dried and analyzed for methyl glycosides (10). The glyceryl ethers present in the hexane extracts were separated from the methyl esters of fatty acids by thin-layer chromatography in hexane-diethyl ether-acetic acid (70:30:1 v/v). An aliquots of acid methanolysates prior to hexane extraction were dried and treated with BCl_3 (11). Following extraction of fatty acid methyl esters and alkyl chlorides with hexane, the methanolic phase was analyzed for glycerol (10).

Glyceryl ethers derived from the studied glycolipids and the glyceryl-monoalkyl standards (Supelco) were oxidized with 0.2 M sodium metaperiodate in aqueous chloroform-methanol (12), at room temperature for 24 hours. The products of oxidation were recovered from the lower phase of the chloroform-methanol-water partition system.

Gas-liquid chromatography were performed with a Beckman GC-65 instrument equipped with glass columns (6 ft. x 1/8 inch) packed with 3% SE-30 on chromosorb, W, AW, DMCS (80-100 mesh). For analysis of trimethylsilyl derivatives of glycerol and methyl glycosides, the temperature was programmed at 2°C/min. from 100-210°C; program from 190-270°C at 2°C/min. was used for trimethylsilyl derivatives of glyceryl ethers. For analysis of fatty acid methyl esters, alkyl chlorides and alkoxyacetaldehydes, temperature programmings were 170-270°C at 2°C/min., 130-240°C at 3°C/min. and 150-260°C at 2°C/min., respectively.

The following analytical methods were also used: sphingosine (13), alkenyl ether group (14), phosphorous (15) and sulfate (16). Alkyl-1-chlorides were obtained from the authentic glyceryl ethers (Supelco) by BCl_3 treatment.

RESULTS AND DISCUSSION - The neutral glycolipids have been isolated from the lipid extract of human gastric content by the procedure involving column fractionations on DEAE-Sephadex, silicic acid and thin-layer chromatography. Two major components (glycolipids A and B) were obtained from 100 ml of gastric content in a yield of 5.2 mg, and 4.7 mg., respectively (Fig. 1). The third glycolipid (spot #1, Fig. 1), isolated in a yield of 1.2 mg., was not studied further.

Analyses of the methanolysis products of glycolipids A and B revealed the presence of glucose, alkyl ethers and fatty acids. Only glucose and glyceryl ethers were found among the methanolysis products of deacylated glycolipids. Sphingosine, sulfate, phosphorus and alkenyl ethers were not detected. Thus, the glycolipids studied here differed from the glycolipids of gastric mucosa (3,4,17) primarily with respect to the lipid core and were similar in composition to the major sulfated glyceroglucolipid isolated by us recently from the same source (1,2,18), but did not contain sulfate.

Figure 1. Thin-layer chromatography of the neutral glycolipids purified from human gastric content.

1, minor neutral glycolipid (not studied); 2, glycolipid A; 3, glycolipid B. Conditions: Silica gel HR 250 nm developed in acetone-methanol-water (50:40:20:5 v/v). Visualization: orcinol reagent.

TABLE 1. Fatty Acids and Glyceryl Ethers of the Isolated Glycolipids

Short hand formula	Fatty acid (%)		Glyceryl ether (%)	
	Glycolipid A	Glycolipid B	Glycolipid A	Glycolipid B
16:0	10.5	16.1	36.8	38.7
18:0	23.3	28.7	21.1	20.2
18:1	7.0	12.9		
20:0	14.5	25.6	22.0	25.6
20:1	1.4	1.7		
22:0	29.1	3.2		
22:1	0.9	4.1		
unidentified	13.3	7.7	20.1	15.5

Gas-liquid chromatography analyses of trimethylsilyl derivatives of glycerol and methyl glucoside showed that these components are present in a molar ratio of 1.0 : 6.3, in glycolipid A and 1.0 : 8.2, in glycolipid B. The major glyceryl ethers (Table I) identified from the purified glyceryl ether fractions of glycolipids A and B were glyceryl-monohexadecyl, glyceryl-monooctadecyl and glyceryl-monoeicosyl. The length of the alkyl chains was also confirmed by the results of analysis of alkyl chlorides. Hexadecyl-1-chloride, octadecyl-1-chloride and eicosanyl-1-chloride were identified among the products of BCl_3 treated glyceryl ether fractions. Oxidation of the glyceryl ether fractions with periodate, in both glycolipids, resulted in conversion of glyceryl-monooctadecyl ether to octadecyoxyacetaldehyde, glyceryl-monoeicosyl ether to eicosyloxyacetaldehyde, whereas glyceryl-monohexadecyl ether was not oxidized. These data indicated that the diglyceride portion of the studied glycolipids consists of a mixture of

glyceryl-1-octadecyl, glyceryl-1-eicosyl and glyceryl-2-hexadecyl ethers, with the former type being predominant.

Fatty acid composition of the isolated glycolipids is given in Table I. The major fatty acids present in glycolipid A, in order of abundance, were docosanoate, octadecanoate and eicosanoate. Octadecanoate, eicosanoate and hexadecanoate were the predominant fatty acids of glycolipid B.

Based on the data presented, we suggest that glycolipid A is a monoalkyl-monoacyl-glyceryl hexaglucoside and glycolipid B is a monoalkyl-monoacyl-glyceryl octaglucoside. Further work is in progress to determine the structure of carbohydrate portions of these new glycolipids.

To our knowledge, with the exception of our preliminary abstracts (1,2), glyceroglucolipids have not been previously described in mammalian secretions. Compounds of similar chemical compositions to these described here are also present in the secretions from the dog Heidenhain fundic pouch and rat stomach (unpublished observations). It is therefore conceiveable that these glycolipids form an essential component of digestive secretions of mammals.

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