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A novel inositol-containing glycosphingolipid isolated from human peripheral nerve

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An inositol-phosphate-containing glycosphingolipid, not reported earlier, was isolated from human cauda equina. Structural characterization showed the glycosphingolipid to be inositol-phosphoryl-2(3) galactosylceramide. The concentration varied between 25 and 30 nmol/g fresh tissue.

Inositol-phosphate; Galactosylceramide; Glycosphingolipid; Human peripheral nerve

1. INTRODUCTION

Acidic glycosphingolipids in mammals can be divided into 3 groups with regard to their acidic components. These groups are sialic acid-containing (gangliosides), sulfate-containing (sulfatides), and sulfate-glucuronic acid-containing glycosphingolipids [1]. A fourth group of glycosphingolipids has been found in various species of plants, yeasts, bacteria and fungi which contain an inositol-phosphate moiety with the basic core ceramide-phosphate-inositol [1,2]. The present paper describes the isolation and characterization of an inositol-phosphate-containing glycosphingolipid from human cauda equina with the phosphate group bound to galactosylceramide instead of ceramide. This type of acidic glycosphingolipid has neither been reported in mammals nor in any other species.

2. MATERIALS AND METHODS

2.1. *Materials*

Samples of cauda equina were obtained for examination from the Department of Forensic Medicine, from subjects aged 25–90 years, who had died in accidents. Silica gel 60, 230–400 mesh, and glass backed HPTLC plates of silica gel 60 were from Merck, Darmstadt (Germany). Sephadex G-25 and DEAE-Sepharose fast flow were from Pharmacia (Uppsala Sweden). Spherosil-DEAE-Dextran was a gift from Institute Merieux (Lyon, France). All organic solvents used were of HPLC quality. All other chemicals used were of analytical quality and used without further purification. Gangliosides and neutral glycolipids used as references were all isolated from human sources and characterized by FAB-MS in our laboratory.

2.2. *Isolation of the inositol-containing compound*

Samples of cauda equina were extracted as described by Svennerholm et al. (1991). Biochim. Biophys. Acta, manuscript in preparation. A monosialoganglioside fraction was obtained from Spherosil-DEAE-dextran ion-exchange chromatography by our routine method [3]. The monosialoganglioside fraction was desalted by dialysis and after evaporating dissolved in chloroform/methanol/water 65:25:4 (v/v). The extract was applied to a silica gel column (600 × 10 mm) and eluted with the same solvent. Fractions containing orcinol positive material with HPTLC migration slightly faster than ganglioside GM2 ($R_{GM2} \approx 1.05$) in the solvent system chloroform/methanol/0.25% aqueous KCl 50:40:10 (v/v) were collected. Contaminating gangliosides were removed from the combined fractions by treatment with 1 M formic acid for 30 min at 100°C [4]. After neutralization and dialysis, asialoglycolipids formed were removed by ion-exchange chromatography on Spherosil-DEAE-dextran. The acidic orcinol-positive compound was eluted as before with 0.02 M potassium acetate in methanol and desalted by dialysis.

2.3. *Analytical methods*

The quantitative composition of the carbohydrate moiety was determined as alditol acetates by GLC with mannose as internal standard [5]. Sphingosine was assayed by a modification of the methyl orange method of Lauter and Trams [6]. Lipid phosphorous was determined as described by Svennerholm and Vanier [7]. Mild acid hydrolysis was performed with 0.05 M HCl in methanol for 16 h at 20°C, 0.3 M HCl in chloroform/methanol 2:1 (v/v) for 25 min at 60°C, or 0.05 M aqueous H_2SO_4 for 60 min at 80°C. After hydrolysis the samples were neutralized by a small amount of Ag_2CO_3 and purified on 0.3 g of Sephadex G-25 [8]. The glycosphingolipid products were analyzed by HPTLC with chloroform/methanol/0.25% aqueous KCl 50:40:10 (v/v) as developing solvent and visualized with the orcinol reagent [9]. Alkaline treatment was performed with 0.5 M KOH in 50% aqueous methanol for 20 h. After neutralization with acetic acid the samples were desalted and analyzed as above. Permethylation and analysis of partially methylated alditol acetates were carried out as described previously [10]. Fast atom bombardment-mass spectrometry (FAB-MS) of the permethylated intact substance was performed on a VG 7070E mass spectrometer [11]. Analysis of the ceramide composition was carried out as described by Månsson et al. [12].

3. RESULTS AND DISCUSSION

The isolated glycosphingolipid contained sphingosine, galactose, phosphate and myo-inositol in the ratio

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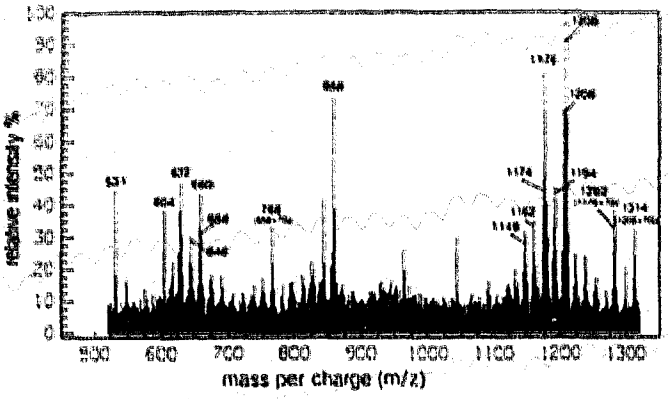

Fig. 1. Positive ion FAB-MS spectrum of permethylated inositol-containing glycosphingolipid from human peripheral nerve.

1.0:0.9:1.0:1.1. Acidic treatment of the substance with 0.05 M HCl in methanol at 20°C yielded 50% of a product with the same HPTLC migration as reference galactosylceramide while the product yields with 0.3 M HCl in chloroform/methanol 2:1 at 60°C or 0.05 M aqueous H_2SO_4 at 80°C were 100%. Alkaline treatment yielded 90% of the same product. FAB-MS of the permethylated product showed molecular ions corresponding to monohexosyl ceramide. The most prominent ones were found at *m/z* 896,894,864 (896-32) and 862 (894-32) representing monohexosyl ceramide with fatty acids C 24:0 and C 24:1 and 4-sphingenine as sphingosine base. Ions at *m/z* 219 and 187 (219-32) showed a terminal hexose. Analysis of the partially methylated alditol acetates gave 2,3,4,6-Me_4-Gal as the only sugar, which shows that the obtained product after acid methanolysis was galactosylceramide.

When the intact permethylated glycosphingolipid was analyzed by FAB-MS (Fig. 1) the most abundant molecular ion (M+H) was found at *m/z* 1208 corresponding to the composition inositol-phosphate-galactose-ceramide with 24:0 fatty acid and 4-sphingenine. Loss of methanol resulted in the ion at *m/z* 1176. Ions at *m/z* 1206 and 1176 represented the same composition with 24:1 fatty acid. Addition of thioglycerol to the molecular species containing 24:1 fatty acid resulted in ions at *m/z* 1314 and 1282. The ion found at *m/z* 858 represented the molecular ion species after loss of the acyl chain (M+2H-acyl) confirming 4-sphingenine as the most abundant long chain base. The ion found at *m/z* 531 is the oxonium ion (inositol-phosphate-galactose) obtained after elimination of the ceramide portion from the molecular ions. The most intense ions in the ceramide region were found at *m/z* 660 and 658 corresponding to the fatty acids 24:0 and 24:1 with 4-sphingenine as the long chain base.

Permethylation analysis of the intact glycospingolipid showed a mixture of 3,4,6-Me_3-Gal and 2,4,6-Me_3-Gal in the ratio 0.7:0.3 showing substitution of the galactose moiety both at C-2 and C-3 position. This mixture may be caused by phosphate ester migration during the permethylation procedure [13]. Analysis of the long chain base composition showed two major components, 4-sphingenine (63%) and sphinganine (20%). The unsubstituted fatty acids 24:0 (33%) and 24:1 (26%) were the dominating ones while h 24:0 constituted 47% of the 2-hydroxy fatty acids. The proportion between unsubstituted and 2-hydroxy fatty acids was 4:1.

The combined structural analyses of the unknown glycosphingolipid isolated from human cauda equina suggests the following structure for the major component, inositol-phosphate-2Gal-Cer and inositol-phosphate-3Gal-Cer for the minor component.

The concentration of these inositol-phosphate-containing glycosphingolipids varied between 25 and 30 nmol/g fresh tissue. A possible function of this new glycosphingolipid, inositol-phosphoryl-galactosyl-ceramide, is to anchor proteins to the plasma membrane in a similar way as described for the glycosyl-phosphatidyl inositol anchors (for review, see [14]). In these structures the C-terminal carboxyl group of a protein is attached to a carbohydrate chain linked to a phosphatidyl inositol molecule located in the lipid layer. The lipid portion of the new lipid contains, except sphingosine, very long-chain unsubstituted- and 2-hydroxy fatty acids. This composition will give a less fluid lipid portion than that of phosphatidyl inositol, which contains predominantly stearic acid and arachidonic acid. With the larger rigidity of the lipid portion of inositol-phosphoryl-galactosylceramide a new glycosphingolipid might be a more stable anchor in the membrane than phosphatidyl inositol.

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