THE OCCURRENCE OF III^3-α-FUCOSYLLACTONEOTETRAOSYLCERAMIDE IN HUMAN BRAIN

M. T. VANIER+, J. E. MÅNSSON and L. SVENNERHOLM

+*Department de Biochimie, INSERM U189, Faculté de Médecine Lyon-Sud, BP 12, F-69600 Oullins, France* and *Department of Neurochemistry, Psychiatric Research Centre, St. Jörgen Hospital, S-42203 Hisings Backa, Sweden*

Received 10 January 1980

1. Introduction

In our study of the minor neutral glycolipids of human infant brain [1], the higher oligoglycosylceramides could not be isolated in pure form and no detailed chemical characterization was possible. By improvement of the isolation methods we have now been able to isolate and characterize all the neutral glycolipids occurring in at >1 nmol/g tissue [2]. This unexpectedly led to identification of III^3-α-fucosyllactoneotetraosylceramide as the most common neutral glycolipid among those with more than two sugar residues. Fucose-containing neutral glycolipids have been isolated from various human organs particularly of entodermal origin, such as intestine and pancreas (reviewed [3]), but not from brain tissue.

2. Experimental

2.1. *Material*

The material consisted of human brains from subjects who had died from disorders not affecting the central nervous system and who had been autopsied at the Departments of Forensic Medicine in Göteborg and Lyon.

Silicic acid, Biosil A 200–400 mesh was obtained from Bio Rad Labs, Sephadex G-25 and DEAE–Sephadex A-25 from Pharmacia Fine Chem. Florisil 200–300 mesh from Floridin, and thin-layer chromatography (TLC) and high-pressure liquid chromatography (HPTLC) plates precoated with Silica gel 60, 0.25 mm thick, from Merck AG. α-L-Fucoside fucohydrolase from bovine kidney (EC 3.2.1.51) was from Boehringer, Mannheim. α-Galactosidase isolated from fig ficin, and β-galactosidase and *N*-acetyl-β-hexosaminidase from jack beans were gifts from Drs S. C. and Y. T. Li, Tulane University, LA.

2.2. *Isolation of the fucose-containing glycolipid*

Total brain lipids were isolated from 15–200 g brain tissue extracted twice with 20 vol. chloroform/methanol/water (4:8:3, v/v/v). After evaporation, dissolution in chloroform/methanol/water (60:30:4.5, v/v/v) and removal of an insoluble residue by centrifugation, the major gangliosides were removed by partition in a final solvent ratio chloroform/methanol/0.1 mol/l aqueous KCl (4:2:1, v/v/v). The lower phase was evaporated to dryness and redissolved in chloroform/methanol/water (60:30:4.5, v/v/v) and low molecular contaminants were removed on Sephadex G-25 [4]. Sulfatides, acidic phospholipids and remaining gangliosides were then retained on a DEAE–Sephadex A-25 column [5]. The neutral glycosphingolipids were separated batchwise by column chromatography on silicic acid with the following elution scheme: chloroform, 10 vol.; chloroform/methanol (9:1, v/v) 10 vol.; chloroform/methanol (4:1, v/v) 10 vol.; chloroform/methanol (1:1, v/v). The glycolipids of the chloroform/methanol (1:1, v/v) eluate were freed from phospholipids by peracetylation and column chromatographic separation of the acetylated glycolipids on Florisil as in [6]. After saponification the fucose-containing glycolipid was isolated by preparative TLC with chloroform/methanol/water (65:25:4, v/v/v) as developing solvent.

2.3. *Analytical methods*

The component analysis and the partial acid and enzymic hydrolyses were done as in [2]. The fuco-

Elsevier/North-Holland Biomedical Press

lipid was permethylated as in [7], hydrolysed, reduced, acetylated and analysed by gas chromatography–mass spectrometry (GC–MS) as in [2]. The brain concentration of the fucolipid was determined by TLC on ordinary TLC or HPTLC plates with standards of authentic III^3-α-fucosyllactoneotetraosylceramide. The plates were sprayed with the cupric acetate reagent [8], heated for 25 min at 140°C and recorded with a Zeiss KM3 chromatogram spectrophotometer at 450 nm.

3. Results

3.1. *Chemical composition*

Component analysis of the new fucolipid, isolated from cerebral white matter, showed sphingosine, glucose, galactose, *N*-acetylglucosamine and fucose in the molar ratio 1.0:1.0:1.8:0.9:1.0. The fucolipid showed only one band on TLC in chloroform/methanol/2.5 mol/l ammonia (40:80:25, v/v/v). Partial acid hydrolysis gave a mixture of glycolipids with the same TLC mobilities as gal–glcNAc–gal–glc–cer, glcNAc–gal–glc–cer, gal–glc–cer and glc–cer. Degradation of the fucolipid with α-fucosidase converted it into tetraglycosylceramide with the same mobility as neolactotetraosylceramide. Further degradation of the tetraglycosylceramide with β-galactosidase, β-galactosidase followed by β-*N*-acetyl-hexosaminidase, and the combined action of β-galactosidase and β-*N*-acetyl-hexosaminidase yielded glycolipids with the same mobility as glcNAc–gal–glc–cer, gal–glc–cer, and glc–cer. The GC–MS analysis of the permethylated fucose-containing glycolipid showed it to contain 2,3,4-tri-*O*-methylfucitol, 2,3,4,6-tetra-*O*-methylgalactitol, 2,4,6-tri-*O*-methylgalactitol and 6-*O*-methyl-2-*N*-methylacetamidoglucitol. Analysis of the permethylated sugars from the defucosylated substance showed no change in the substitution pattern of the galactose and glucose molecules, but the permethylated amino sugar was now 3,6-di-*O*-methyl-2-*N*-methylacetamidoglucosaminitol. No 2,3,4-tri-*O*-methylfucitol was detected. The results of the structural determinations suggest the following structure of the fucolipid of human normal brain:

galβ1→4(fucαl→3)glcNAcβ1→3galβ1→4glc–cer

3.2. *Quantitative distribution*

The concentration of the fucolipid was studied in the cerebral cortex from 2 subjects, aged 4 and 12 months, and in the white matter from 7 subjects, aged 3 months to 52 years. The results are shown in table 1. The content of the fucolipid was lower in the cerebral cortex than in the cerebral white matter, where it varied from 1.3–6.5 nmol/g wet wt.

Table 1
Concentration of III^3-α-fucosyllactoneotetraosylceramide in human brains at different ages

Age	Cerebral cortex	Cerebral white matter
3 months	–	1.5
4 months	1.0	6.5
12 months	0.6	2.4
13 months	–	2.0
3 years	–	1.5
12 years	–	3.0
52 years	–	1.3

All values are given as nmol/g wet wt

4. Discussion

This study has shown that III^3-α-fucosyllactoneotetraosylceramide is a minor but normal constituent of human brain. Fucolipids had not been reported to occur in normal brain, in line with the concept that fucolipids occur in glandular epithelial tissues, such as stomach, intestine and pancreas, but only in very small quantities in parenchymatous organs [9]. The major groups of fucolipids possess activity as ABH and Lewis antigens and they are characterized by the presence of 1 or 2 fucose residues which are linked to galactose in an α1→2 glycosidic linkage or in an α1→4 glycosidic linkage to *N*-acetylglucosamine. III^3-α-Fucosyllactoneotetraosylceramide was first characterized in material from a case of human adenocarcinoma [10], but it has since been found also in hog gastric mucosa [11] and dog small intestine [3]. It has been synthesized in vitro with the use of human serum as a source of fucosyltransferase and lactoneotetraosylceramide as an acceptor [12]. Its presence in brain might have been anticipated, since in rat brain a glycoprotein has been isolated with the same terminal sequence of neutral sugars as in the present fucolipid, namely:

NeuAcα2→3galβ1→4(fucα1→3)glcNAc– [13]

The occurrence of both a glycolipid and a glycoprotein with an α1→3 glycosidic linkage to *N*-acetylglucosamine in brain tissue suggests that the predominating fucosyltransferase of brain tissue has an α1→3 specificity for the *N*-acetylglucosamine residue.

A ganglioside with the same terminal structure as that in the rat brain fucoglycoprotein has been isolated from human kidney [14]. The sialic acid or the fucose residue could have been added as the last sugar, but since the sialic acid is on the terminal galactose, the fucose residue is assumed to be added first. This implies that minor amounts of the neutral fucolipid occur in all the organs containing the corresponding ganglioside. The presence of this ganglioside can also be anticipated in organs with a high sialyltransferase activity and the simultaneous occurrence of the neutral fucolipid. In fact, the corresponding ganglioside occurs in minor amounts in human brains, but it will migrate in neutral solvents together with the GM1-ganglioside and its detection requires the use of an alkaline chromatographic solvent.

References

[1] Vanier, M.-T., Holm, M., Månsson, J.-E. and Svennerholm, L. (1973) J. Neurochem. 21, 1375–1384.
[2] Svennerholm, L., Vanier, M.-T. and Månsson, J.-E. (1980) J. Lipid Res. 21, 53–64.
[3] McKibbin, J. M. (1978) J. Lipid Res. 19, 131–147.
[4] Wells, M. A. and Dittmer, J. C. (1963) Biochemistry 2, 1259–1263.
[5] Yu, R. K. and Ledeen, R. W. (1972) J. Lipid Res. 13, 680–686.
[6] Saito, T. and Hakomori, S.-I. (1971) J. Lipid Res. 12, 257–259.
[7] Hakomori, S.-I. (1964) J. Biochem. 55, 205–208.
[8] Fewster, M., Burns, B. J. and Mead, J. F. (1969) J. Chromatog. 43, 120–126.
[9] Hakomori, S.-I. and Andrews, H. D. (1970) Biochim. Biophys. Acta 202, 225–228.
[10] Yang, H. J. and Hakomori, S.-I. (1971) J. Biol. Chem. 246, 1192–1200.
[11] Słomiany, B. L., Słomiany, A. and Horowitz, M. I. (1975) Eur. J. Biochem. 56, 353–358.
[12] Pacuszka, T. and Koscielak, J. (1976) Eur. J. Biochem. 64, 499–506.
[13] Krusius, T. and Finne, J. (1978) Eur. J. Biochem. 84, 395–403.
[14] Rauvala, H. (1976) J. Biol. Chem. 251, 7517–7520.