Extensive precursor-product relationship between gangliosides formed from exogenous glucosylceramide in rat liver

Marco Trinchera, Carlo Vallenzasca, Riccardo Ghidoni and Guido Tettamanti

Study Center for the Functional Biochemistry of Brain Lipids, Department of Medical Chemistry and Biochemistry, University of Milan, Italy

Received 5 November 1989

Glucosylceramide, radiolabelled on the glucose residue, was administered to rats and the radioactive gangliosides formed at different time points were chemically characterized. They were identified as GM3, GM1, GD1a and GD1b, each one carrying only radioactive glucose. The time course of each individual ganglioside showed that the simpler gangliosides were formed earlier but were consumed earlier than the more complex ones, resulting in radioactivity patterns that were different at each time point. Only 30 h after injection did it resemble that of endogenous rat liver gangliosides. These results indicate that an extensive precursor-product relationship actually exists in the course of ganglioside biosynthesis.

Glucosylceramide; Ganglioside; Golgi apparatus; Glycosylation

1. INTRODUCTION

The pathway followed in the biosynthesis of individual gangliosides has long been established in the sense that both the acceptor substrate and the product of each glycosyltransferase activity are well known [1]. We recently demonstrated in rat liver that individual $\alpha(2\rightarrow3)$-sialyltransferases are specifically distributed in different compartments of the Golgi apparatus [2], a finding that opens questions about the in situ mechanisms of ganglioside biosynthesis and sorting. First of all, does a ganglioside, once biosynthesized, act as a precursor in the proper pathway for the more glycosylated ones? The establishment of a definite precursor-product relationship during biosynthesis may provide an answer to this question. The metabolism of exogenously administered glucosylceramide has been reported in vitro and in vivo [3,4] and on this basis, we injected rats with glucosylceramide radiolabelled on the glucose residue ([Glc-^{3}H]GlcCer) and followed the time course of the individual ganglioside formation in the liver. Each radioactive compound was also identified.

2. EXPERIMENTAL

2.1. *Radiolabelling of glucosylceramide and gangliosides*

Glucosylceramide, prepared from bovine brain ganglioside [5] was ^{3}H-labelled on the C-6 position of the glucose residue [6]. The specific radioactivity was 0.97 Ci/mmol and the radiochemical purity, assessed immediately before treatment, was better than 99%.

Correspondence address: R. Ghidoni, Dipartimento di Chimica e Biochimica Medica, Via Saldini 50, 20133 Milano, Italy

The ganglioside nomenclature of Svennerholm [(1964) J. Lipid Res. 5, 145-155] was followed.

2.2. *Animal treatment and liver fractionation*

Between 08.00 and 10.00 h, adult male Wistar rats (Charles River, Milan, Italy), 100 g average body weight, were intravenously injected in the tail without anesthesia with 0.1 ml of a freshly prepared solution [4] containing 50 μCi of [Glc-^{3}H]GlcCer. In some cases a parenchymal cell fraction was prepared by the collagenase perfusion method [7] as previously reported [5]. At different times after injection, the animals were sacrificed by decapitation and their livers removed, weighed and immediately processed as follows. Total lipids were extracted and partitioned by the phosphate buffer/tetrahydrofuran procedure [5]. After partitioning, the aqueous phase containing gangliosides was evaporated to dryness, resuspended in methanol and loaded onto a DEAE-Sepharose CL-6B column [5], which was washed in methanol and eluted (in batch) with 0.1 M ammonium acetate. The eluate was then evaporated to dryness, resuspended in distilled water, exhaustively dialyzed against distilled water and finally freeze-dried. This is referred to as the ganglioside fraction.

2.3. *Radiochemical characterization of individual gangliosides*

Analytical separation and purification of the individual compounds contained in the ganglioside fraction were performed as reported [5]. Identification of radiolabelled gangliosides was accomplished by submitting the isolated compounds to enzyme action. In particular *Vibrio Cholerae* sialidase (Behringwerke, Marburg, FRG) was used for the hydrolysis of GM3, GD1a and GD1b [8] and bovine testes β-galactosidase (Sigma, St. Louis, USA) for the hydrolysis of GM1 [9]. The analysis of the endogenous total liver and parenchymal cell ganglioside pattern was performed as previously reported [5].

2.4. *Intramolecular distribution of radioactivity*

The intramolecular distribution of the radioactivity of each isolated ganglioside was determined by radio-GLC analysis of the trifluoroacetyl-*O*-methylglycosides released [10] from known amounts of radioactivity (about 50000 dpm). GLC conditions were as reported [10]. The temperature program was 2°C/min from 120°C to 210°C. Radioactivity detection was done with a radio-gas chromatography analyzer, mod. RAGA, from Raytest (Essen, FRG) equipped with a reduction reactor, using hydrogen as the auxiliary gas.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

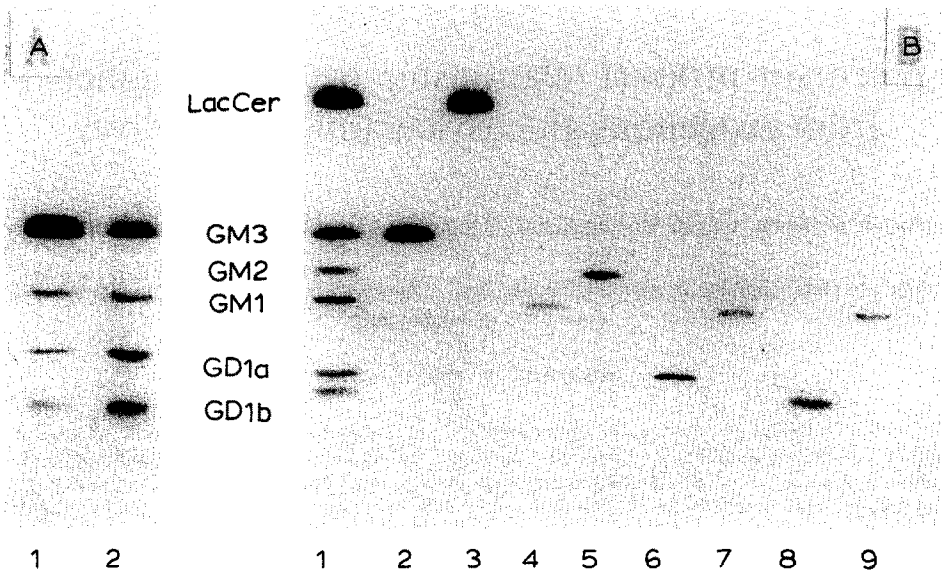

Fig.1. Radioactivity in rat liver gangliosides and verification of the chemical identity, by enzymatic hydrolysis of the individual compounds, 20 h after treatment. Separation was done by HPTLC and detection by fluorography. (A) Solvent system chloroform/methanol/0.2% aq. $CaCl_2$, 50/42/11 by vol.; lane 1: total radioactive liver gangliosides; lane 2: reference gangliosides. (B) Solvent system chloroform/methanol/0.2% aq. $CaCl_2$, 60/35/8, by vol.; lane 1: reference gangliosides and lactosylceramide; lanes 2 and 3: GM3 before and after sialidase treatment; lanes 4 and 5: GM1 before and after β-galactosidase treatment; lanes 6 and 7: GD1a before and after sialidase treatment; lanes 8 and 9: GD1b before and after sialidase treatment.

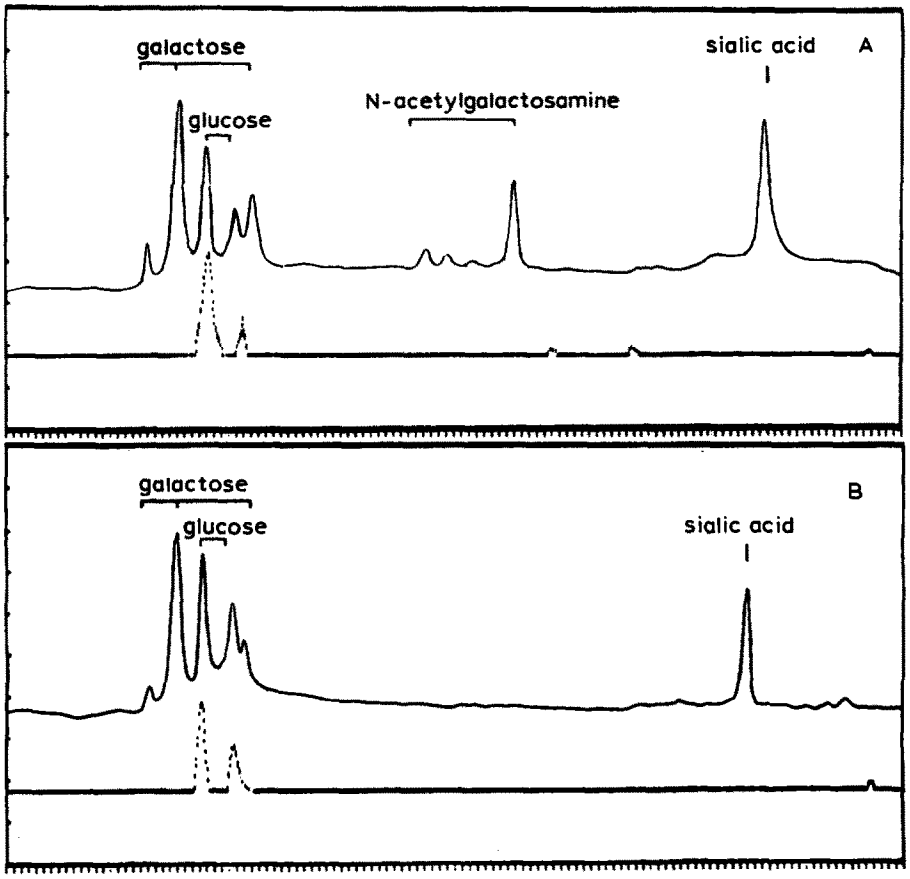

Fig.2. Intramolecular distribution of radioactivity in the individual gangliosides isolated from rat liver 20 h after treatment. Analysis was done by radio-GLC. The profiles refer to GM1 (A) and GM3 (B). The radioactivity profiles derived from GD1a and GD1b were identical to those presented. The upper profile of each panel refers to the flame ionization detector, the lower to the radioactivity detector.

2.5. *Determination of radioactivity*

The determination of radioactivity by fluorography, radiodensitometry or scintillation counting in solution was performed as previously described [5].

3. RESULTS

After i.v. injection of 50 μCi of [Glc-^{3}H]GlcCer, a low but definitely measurable amount of radioactivity was found to be incorporated in the ganglioside fraction at each time point; the largest incorporation was reached 20 h after injection (71 800 dpm/g fresh tissue). Four major radioactive spots which co-migrated with the reference standard, GM3, GM1, GD1a and GD1b (fig.1A), were present. After purification, the spots co-migrating with GM3, GD1a and GD1b were affected by sialidase and gave rise to radioactive LacCer (the spot like GM3) and GM1 (the spots like GD1a and GD1b); the spot co-migrating with GM1 was affected by β-galactosidase and gave rise to radioactive GM2 (fig.1B).

Radio-GLC analysis of each ganglioside showed that the radioactivity was located only on the glucose residue (fig.2). The parenchymal cell fraction accounted for 80% (referred to the total liver) of the endogenous individual ganglioside content and of the ganglioside bound radioactivity.

GM3 increased up to 10 h and then progressively decreased; GM1 increased later, reached a maximum at 20 h and then began to decrease. GD1a increased more slowly than GM1 for up to 20 h and then remained constant. GD1b progressively increased for up to 30 h (fig.3). The above time courses created a ganglioside pattern that was different at each time point. The final one (at 30 h after injection) resembled that of endogenous rat liver gangliosides (table 1).

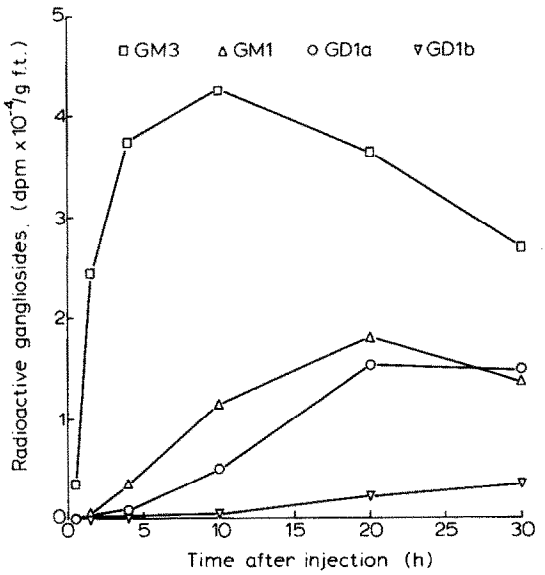

Fig.3. Time course of individual radioactive gangliosides in the rat liver after treatment. Values are the means of three experiments. The SD was less than 15% of the mean value.

Table 1

Relative percent of radioactive gangliosides in the rat liver after treatment

Ganglioside	Time after injection		Endogenous pattern
	4 h	30 h	
GM3	85.1	41.3	44.5
GM1	7.0	21.7	18.8
GD1a	1.5	23.7	21.8
GD1b	tr	6.4	5.5
GT1b	nd	tr	3.9
others	6.4	6.9	5.5
	100.0	100.0	100.0

nd, not detectable; tr, traces

4. DISCUSSION

In the present report we demonstrate the occurrence of an extensive precursor-product relationship between gangliosides biosynthesized in the rat liver starting from exogenous glucosylceramide. All the liver gangliosides became radioactive but the relative content of each individual radioactive ganglioside resembled that of the endogenous ones only 30 h after the injection of [Glc-^{3}H]-GlcCer. Since hepatocytes were mostly responsible for glucosylceramide metabolism, as expected [4], the contribution of different liver cell populations should be ruled out.

Analysis of the intramolecular distribution of radioactivity showed that each ganglioside carried only radioactive glucose, thus confirming that gangliosides were formed by successive glycosylations starting from the intact administered glucosylceramide. In the course of such glycosylations, GM3 acted as a precursor for GM1 and the same GM1 as a precursor of GD1a in the 'a' pathway of ganglioside biosynthesis. Moreover, GM3 was a precursor for GD1b in the 'b' pathway. This is the first metabolic evidence of a biosynthetic transport of gangliosides in accordance with the sub-Golgi compartmentation of the related processing enzymes [2]. A precursor-product relationship in the course of neutral glycosphingolipid biosynthesis has already been established [11,12], whereas in the course of ganglioside biosynthesis it is questioned [13]. The precursor-product relationship during ganglioside biosynthesis was verified in rat liver by our in vivo approach, which permits unambiguous radiochemical characterization of the formed compounds.

REFERENCES

[1] Fishman, P.H. and Brady, R.O. (1976) Science 194, 906–914.
[2] Trinchera, M. and Ghidoni, R. (1989) J. Biol. Chem. 264, 15766–15769.
[3] Saito, M. and Rosemberg, A. (1985) J. Biol. Chem. 260, 2295–2300.
[4] Tokoro, T., Gal, A.E., Gallo, L.L. and Brady, R.O. (1987) J. Lipid Res. 28, 968–972.

[5] Ghidoni, R., Trinchera, M., Venerando, B., Fiorilli, A., Sonnino, S. and Tettamanti, G. (1986) Biochem. J. 237, 147–155.
[6] McMaster, M.C. and Radin, N.S. (1976) J. Labelled Comp. Radiopharm. 23, 353–357.
[7] Berry, M.N. and Friend, D.S. (1969) J. Cell Biol. 43, 506–520.
[8] Ghidoni, R., Trinchera, M., Sonnino, S., Chigorno, V. and Tettamanti, G. (1987) Biochem. J. 247, 157–164.
[9] Cahan, L.D., Irie, R.F., Singh, R., Cassidenti, A. and Paulson, S.C. (1982) Proc. Natl. Acad. Sci. USA 79, 7629–7633.
[10] Zanetta, J.P., Breckenridge, W.C. and Vincendon, G. (1972) J. Chromat. 69, 291–304.
[11] Miller-Podraza, H. and Fishman, P.H. (1982) J. Neurochem. 41, 860–867.
[12] Chatterjee, S., Clarke, K.S. and Kwiterovich, P.O. jr. (1986) J. Biol. Chem. 261, 13480–13486.
[13] Miller-Podraza, H., Bradley, R.M. and Fishman, P.H. (1982) Biochemistry 21, 3260–3265.