Subcellular biosynthesis and transport of gangliosides formed from exogenous lactosylceramide in rat liver

Marco TRINCHERA and Riccardo GHIDONI*

Dipartimento di Chimica e Biochimica Medica, Facoltà di Medicina, Università di Milano, Via Saldini 50, 20133 Milano, Italy

In order to clarify the mechanisms of ganglioside biosynthesis and transport we intravenously administered a liposomal dispersion of radiolabelled lactosylceramide (LacCer) to rats and then followed the time course of the individual gangliosides which became radioactive in the Golgi-apparatus and plasma-membrane fractions prepared from the liver. After administration of radiolabelled LacCer the liver retained a substantial amount of radioactivity, which was distributed among an organic phase (mainly residual LacCer), a fraction containing low- M_r substances (mainly $^3\text{H}_2\text{O}$) and a ganglioside fraction. The hepatocytes were found to provide the bulk of gangliosides biosynthesized from exogenous LacCer. After subcellular fractionation, the total radioactive gangliosides increased in the Golgi apparatus up to 8 h, to then decrease and practically disappear at 24 h; in the plasma membranes they were progressively concentrated, accounting for high absolute values. Ganglioside patterns were greatly modified with time in both the Golgi apparatus and plasma membrane, but without significant differences between them. Biosynthesis in the Golgi apparatus and accumulation in the plasma membrane of each individual ganglioside followed a precursor-product relationship. The obtained results indicated that once a ganglioside is biosynthesized in the Golgi apparatus, it is in part made available for translocation to the plasma membrane, which rapidly occurs, and is in part retained in the Golgi apparatus, where it acts as a precursor for the biosynthesis of more glycosylated gangliosides.

INTRODUCTION

It has been postulated that gangliosides are biosynthesized in the Golgi apparatus, where specific glycosyltransferases are localized (Keenan *et al.*, 1974), and successively transported to the plasma membrane, which is the site of their elective deposition (for a review, see Tettamanti, 1984). How such transport occurs is still unknown. In accordance with the membrane-flow model (Morre *et al.*, 1979), budding and fusion of vesicles has been proposed as a reliable mechanism (Miller-Podraza & Fishman, 1984; Landa *et al.*, 1981), but a system involving proteic transporters cannot be excluded (Gammone *et al.*, 1987). Moreover, it is unclear whether the different gangliosides leave the Golgi apparatus prevailing at one sorting face, as has been proposed for glycoproteins (Pfeffer & Rothman, 1987).

The use of exogenous radiolabelled gangliosides has been introduced as a suitable approach for studying glycosylation reactions *in vitro* (Sonderfeld *et al.*, 1985) and *in vivo* (Ghidoni *et al.*, 1983). Thus it has been demonstrated that exogenous GM1 is sialylated to GD1a in the rat liver Golgi apparatus (Ghidoni *et al.*, 1986). Furthermore, exogenously administered neutral glycosphingolipids are also taken up by cells and tissues and metabolized (Pentchev *et al.*, 1981; Saito & Rosenberg, 1985; Chatterjee *et al.*, 1986; Datta *et al.*, 1986); in particular, liposomes containing lactosyl-

ceramide (LacCer) have been reported to be actively incorporated by hepatocytes *in vivo* (Soriano *et al.*, 1983; Grosse *et al.*, 1984). On this basis, to study the mechanisms of ganglioside biosynthesis and transport, we intravenously administered a liposomal dispersion of radiolabelled LacCer to rats and then monitored the time course of the individual gangliosides which became radioactive in the Golgi-apparatus and plasma-membrane fractions prepared from the liver.

EXPERIMENTAL

Materials

Asialofetuin, ovalbumin, nucleotide sugars and bovine testis β -galactosidase (EC 3.2.1.23) were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). High-performance t.l.c. plates, silica-gel 100 and chemicals were from Merck G.m.b.H. (Darmstadt, Germany). *Vibrio cholerae* sialidase (EC 3.2.1.18) was from Behringwerke (Marburg, Germany). NaB^3H_4 (6.5 Ci/mmol), UDP-[U- ^{14}C]galactose and CMP-*N*-acetyl[4,5,6,7,8,9- ^{14}C]-neuraminic acid were from Amersham International (Amersham, Bucks., U.K.). DEAE-Sepharose CL-6B was from Pharmacia (Uppsala, Sweden).

Radiolabelling of LacCer and gangliosides

LacCer was prepared by controlled acid hydrolysis

Abbreviations used: this paper follows the ganglioside nomenclature of Svennerholm (1964) and the IUPAC-IUB Recommendations (1977); GM3, NeuAca2 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4Glc β 1 $\rightarrow$ 1'Cer; GM2, GalNAc β 1 $\rightarrow$ (NeuAca2 $\rightarrow$ 3)4Gal β 1 $\rightarrow$ 4Glc β 1 $\rightarrow$ 1'Cer; GM1, Gal β 1 $\rightarrow$ 3GalNAc β 1 $\rightarrow$ (NeuAca2 $\rightarrow$ 3)4Gal β 1 $\rightarrow$ 4Glc β 1 $\rightarrow$ 1'Cer; GD1a, NeuAca2 $\rightarrow$ 3Gal β 1 $\rightarrow$ 3GalNAc β 1 $\rightarrow$ (NeuAca2 $\rightarrow$ 3)4Gal β 1 $\rightarrow$ 4Glc β 1 $\rightarrow$ 1'Cer; GD1b, Gal β 1 $\rightarrow$ 3GalNAc β 1 $\rightarrow$ (NeuAca2 $\rightarrow$ 8NeuAca2 $\rightarrow$ 3)4Gal β 1 $\rightarrow$ 4Glc β 1 $\rightarrow$ 1'Cer; lactosylceramide (LacCer), Gal β 1 $\rightarrow$ 4Glc β 1 $\rightarrow$ 1'Cer; designation of glycosphingolipid glycosyltransferases (SAT-1, SAT-4, GalT-3) follows the indications of Basu *et al.* (1987).

* To whom correspondence and reprint requests should be sent.

(0.5 M-HCl, 4 h, 80 °C) of a bovine brain ganglioside mixture. At the end of the reaction, after neutralization with NaOH, the solution was dialysed overnight against distilled water and finally freeze-dried. LacCer was purified by silica-gel column chromatography using chloroform/methanol/water 125:30:2, by vol, as eluting solvent. The molecular species containing C₁₈ sphingosine and stearic acid was obtained by reversed-phase h.p.l.c. as described by Hirabayashi *et al.*, 1986. It was successively ³H-labelled on the C-6 position of the galactose residue by the galactose oxidase/NaB³H₄ method of Leskawa *et al.* (1984). The specific radioactivity was 1.11 Ci/mmol and the radiochemical purity, assessed immediately before use, was better than 99 %. Standard radiolabelled GM3, GM2, GM1, GD1a and GD1b were prepared as reported by Gazzotti *et al.* (1984).

Animals and animal treatment

Adult male Wistar rats (Charles River, Milan, Italy), 60 days old and 100 g average body weight, were employed. The animals were kept until the day before injection and during treatment under the following conditions: light, from 06:00 to 18:00 h; temperature, 20 °C; food and water *ad libitum*. During the 24 h before injection no food was given. Each animal was intravenously injected in the tail, between 8:00 and 10:00 h, without anaesthesia, with 0.1 ml of a liposomal solution freshly prepared as described by Soriano *et al.* (1983), containing 50 µCi of [Gal-³H]LacCer/0.1 ml. At different times after injection, the animals were killed by decapitation and their livers were removed, weighed and immediately processed. The parenchymal-cell fraction was prepared from the liver of treated animals by the modified (Seglen, 1973) collagenase-perfusion technique of Berry & Friend (1969). Homogeneity, purity and viability of the hepatocytes were assessed as previously described (Ghidoni *et al.*, 1986).

Preparation of Golgi-apparatus and plasma-membrane fractions from rat liver

Golgi-apparatus and plasma-membrane fractions were prepared from a single homogenate by the method of Croze & Morré (1984); briefly, minced livers were homogenized in 2 vol. of 37.5 mM-Tris/maleate buffer, pH 7.0, containing 1 % dextran, 0.5 M-sucrose, 5 mM-MgCl₂ and 0.1 % 2-mercaptoethanol and spun at 6000 g for 15 min. The upper yellow-brown portion of the pellet obtained was resuspended and layered on 1.2 M-sucrose and spun at 100000 g for 30 min. Material from the 1.2 M-sucrose/homogenate interface was collected and pelleted to obtain the Golgi-apparatus fraction. The lower half of the same first differential pellet was resuspended in 1 mM-bicarbonate and spun at 4500 g for 10 min. The top half of this pellet was resuspended and mixed with 5 vol. of 81 % (w/v) sucrose and overlaid with 3.5 ml each of 53.4, 48, 46, 44, 42.6 and 40 % (w/v) sucrose. This gradient was centrifuged for 90 min at 90000 g and the material from the 40/42.6 %-sucrose interface collected and finally pelleted to obtain the plasma-membrane fraction.

Marker-enzyme assays

Glucose-6-phosphatase (EC 3.1.3.9) (Nordlie & Arion, 1966), acid phosphatase (EC 3.1.3.2) (Morré *et al.*, 1983),

5'-nucleotidase (EC 3.1.3.5) and (Na⁺ + K⁺)-dependent ATPase (EC 3.6.1.3) (Emmelot *et al.*, 1964) were assayed as described in the cited references. Galactosyltransferase using ovalbumin as acceptor (EC 2.4.1.38) was assayed as described by Brew *et al.* (1975); sialyltransferase using asialofetuin as acceptor (EC 2.4.99.1) was assayed by the method of Briles *et al.* (1977). Glycosyltransferases using glycosphingolipid acceptors [CMP-NeuAc:LacCer sialyltransferase (or SAT-1), UDP-Gal:GM2 galactosyltransferase (or GalT-3) and CMP-NeuAc:GM1 sialyltransferase (or SAT-4)] were assayed as described by Basu *et al.* (1987). In each case, the glycosyltransferase activity towards endogenous acceptors was measured and subtracted by using a reference incubation mixture that lacked the exogenous acceptor.

Protein was determined by the method of Lowry *et al.* (1951), bovine serum albumin being used as standard.

Fractionation of rat liver

Total lipids were extracted and partitioned by the phosphate buffer/tetrahydrofuran procedure of Tettamanti *et al.* (1973). After partitioning, the aqueous phase containing gangliosides was evaporated to dryness, resuspended in methanol and loaded on to a DEAE-Sepharose CL-6B column (Ghidoni *et al.*, 1986), 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 dialysed against distilled water and finally freeze-dried; this was referred to as the 'ganglioside fraction'. The organic phase, containing lipids of a non-ganglioside nature, was evaporated to dryness before processing; the lost radioactivity after evaporation and dialysis was accounted for as the 'low-M_r-substance fraction'.

Analysis and identification of radiolabelled lipids of rat liver

Separation of the individual compounds contained in the ganglioside fraction was attained by high-performance t.l.c. using the solvent system chloroform/methanol/0.2 % CaCl₂ (60:40:9, by vol.). The purification of the radiolabelled gangliosides was performed by column chromatography on silica-gel 100 (solvent system: chloroform/methanol/water, 12:7:1, by vol.). Identification of radiolabelled gangliosides was accomplished by submitting the isolated compounds to enzyme action. In particular, *Vibrio cholerae* sialidase was used for the hydrolysis of GM3, GD1a and GD1b (Ghidoni *et al.*, 1987) and β-galactosidase for the hydrolysis of GM1 (Cahan *et al.*, 1982). The analysis of the lipids contained in the organic phase was performed as previously reported (Ghidoni *et al.*, 1986).

Intramolecular radioactivity distribution

The intramolecular distribution of radioactivity of each isolated ganglioside was done by radio-g.l.c. analysis of the trifluoroacetyl O-methylglycosides released (Zanetta *et al.*, 1972) from known amounts of radioactivity (about 50000 d.p.m.). G.l.c. conditions were those reported by Zanetta *et al.* (1972). The temperature program was 2 °C/min from 120 to 210 °C. Radioactivity detection was done with a radio-gas-chromatography analyser, model RAGA, from Raytest (Essen, Germany) equipped with a reduction reactor using hydrogen as the auxiliary gas.

Determination of radioactivity

The determination of radioactivity by fluorography, radiodensitometry or liquid-scintillation counting in solution was performed as previously described (Ghidoni *et al.*, 1986).

RESULTS

Time course of radioactivity incorporation into the different liver fractions

After i.v. injection of 50 μ Ci of [^{3}H]LacCer, the liver retained a substantial amount of radioactivity, maximal incorporation being reached 4 h after injection (Fig. 1a). At each investigated time the radioactivity was mainly (85–95%) recovered in the total lipid extract. After partitioning, the radioactivity of the organic phase (mainly residual LacCer) progressively decreased from 4 to 24 h, whereas that of the low- M_r -substance fraction (mainly $^3\text{H}_2\text{O}$) was almost constant. A low but definitely measurable amount of radioactivity was found in the ganglioside fraction (Fig. 1b).

On the basis of recovery of ganglioside-associated radioactivity and glucose-6-phosphatase activity in the parenchymal-cell fraction with respect to the whole homogenate, it appeared that hepatocytes contributed 80–90% of ganglioside biosynthesis at each investigated time.

Verification of the chemical identity of individual radiolabelled gangliosides and intramolecular radioactivity distribution

In the ganglioside fraction, four major radioactive spots, which co-migrated with reference standards GM3, GM1, GD1a and GD1b (Fig. 2, lanes 1 and 2), were present. After purification, the spots co-migrating with GM3, GD1a and GD1b were treated with sialidase and gave rise to radioactive LacCer (spot corresponding to

Fig. 2. Radioactivity in rat liver gangliosides and verification of the chemical identity, by enzymic treatment, of the individual compounds 24 h after injection of 50 μ Ci of [^{3}H]LacCer

Separation was done by high-performance t.l.c. [solvent system chloroform/methanol/0.2% CaCl_2 (60:40:9, by vol.)] and detection by fluorography. Lane 1, total radioactive liver gangliosides; lanes 2 and 3, reference radiolabelled gangliosides and LacCer; lanes 4 and 5, radioactive GM3 before and after sialidase treatment; lanes 6 and 7, radioactive GM1 before and after β -galactosidase treatment; lanes 8 and 9, radioactive GD1a before and after sialidase treatment; lanes 10 and 11, radioactive GD1b before and after sialidase treatment.

GM3) and GM1 (both spots corresponding to GD1a and GD1b); the spot co-migrating with GM1 was treated with β -galactosidase, giving rise to radioactive GM2 (Fig. 2, lanes 3–11).

No radioactive sialic acid or galactose was detectable on the plate after enzyme action. Moreover, when GD1a and GD1b were treated successively with sialidase and β -galactosidase, giving rise to radioactive GM2, free radioactive galactose was undetectable on the plate (results not shown). Radio-g.l.c. analysis of each ganglioside showed that the radioactivity was located only on the galactose residue (Fig. 3). By the combination of enzymic and radio-g.l.c. data, it was concluded that only the glucose-linked galactose was radioactive in each ganglioside.

Time-course of total ganglioside incorporation in the Golgi-apparatus and plasma-membrane fractions

The purity of the Golgi-apparatus and plasma-membrane fractions prepared from rat liver is reported in Table 1. In the Golgi-apparatus fraction asialofetuin sialyltransferase and ovalbumin galactosyltransferase, as well as glycosphingolipid glycosyltransferases, were up to 60-fold enriched, whereas the marker enzymes of other subcellular fractions were only slightly increased or even diminished. In the plasma-membrane fraction 5'-nucleotidase and $(\text{Na}^+ + \text{K}^+)\text{-dependent ATPase}$ were up to 15-fold concentrated; other marker enzymes and glycosphingolipid glycosyltransferases were not at all enriched.

In the Golgi-apparatus fraction the radioactive gangliosides increased up to 8 h, to then progressively decrease and practically disappear at 24 h. In the plasma-membrane fraction the time course had a quite different shape, characterized by a progressive concentration throughout the time range investigated (Fig. 4a). It appeared useful to calculate the total amount of radio-

Fig. 1. Time course of radioactivity incorporation in the liver homogenate (a) and fractions (b) after injection of 50 μ Ci of [^{3}H]LacCer

Results are the means $\pm$ S.D. for five experiments.

Fig. 3. Intramolecular distribution of radioactivity in the individual gangliosides isolated from rat liver 24 h after injection of 50 μ Ci of [Gal - 3H]LacCer

Analysis was done by radio-g.l.c. The profiles refer to the trifluoroacetyl *O*-methylglycosides obtained after methanolysis of radiolabelled GM3 (a) and GM1 (b). The radioactivity profiles derived from GD1a and GD1b were identical with those presented. The upper profiles of each panel are flame-ionization-detector results; the lower ones are results from the radioactivity detector.

Table 1. Activity of marker enzymes in the Golgi-apparatus and plasma-membrane fractions of rat liver

Values are expressed as nmol of transferred sugar/h per mg of protein for all glycosyltransferases and as μ mol of released phosphate/h per mg of protein for the others. Results are means $\pm$ s.d. for five experiments. RSA, relative specific radioactivity (referred to the whole homogenate). Key to superscripts: ^a Golgi-apparatus marker; ^b plasma-membrane marker; ^c endoplasmic-reticulum marker; ^d lysosome marker.

Enzyme	Fraction ...	Whole homogenate Sp. activity	Golgi apparatus		Plasma membrane	
	Parameter ...		Sp. activity	RSA	Sp. activity	RSA
Asialofetuin sialyltransferase ^a		9.8 $\pm$ 0.8	565.2 $\pm$ 38.8	57.3	9.9 $\pm$ 1.0	1.0
Ovalbumin galactosyltransferase ^a		0.96 $\pm$ 0.11	59.5 $\pm$ 4.1	62.0	0.90 $\pm$ 0.08	0.9
5'-Nucleotidase ^b		2.7 $\pm$ 0.2	4.1 $\pm$ 0.3	1.5	36.1 $\pm$ 3.0	14.4
(Na ⁺ + K ⁺)-dependent ATPase ^b		0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	1.0	5.5 $\pm$ 1.1	13.6
Glucose-6-phosphatase ^c		6.2 $\pm$ 1.1	8.1 $\pm$ 1.2	1.3	3.4 $\pm$ 0.3	0.6
Acid phosphatase ^d		2.4 $\pm$ 0.3	2.1 $\pm$ 0.2	0.9	2.7 $\pm$ 0.3	1.1
SAT-1		0.08 $\pm$ 0.02	5.1 $\pm$ 0.5	63.7	not detectable	
GalT-3		0.49 $\pm$ 0.08	28.5 $\pm$ 3.4	58.5	0.45 $\pm$ 0.09	0.9
SAT-4		0.57 $\pm$ 0.10	35.2 $\pm$ 4.1	61.7	0.58 $\pm$ 0.12	1.0
[Protein] (mg/g of fresh tissue) ...		195.2 $\pm$ 21.4	0.98 $\pm$ 0.21		2.1 $\pm$ 0.5

Fig. 4. Time course of total radioactive gangliosides in the Golgi-apparatus and in the plasma-membrane fraction after injection of 50 µCi of [Gal-³H]LacCer

Values are the means $\pm$ S.D. for three experiments. (a) Ganglioside specific radioactivity; (b) ganglioside total radioactivity (for details on how values were calculated, see the Results section). ●, Plasma membrane; ○, Golgi apparatus.

active gangliosides (TRG) in both subcellular fractions, referring to theoretical recovery of positive markers in the respective fractions. For this purpose ovalbumin galactosyltransferase and 5'-nucleotidase were used for the Golgi-apparatus and plasma-membrane fractions respectively. Calculation was done as follows:

$$\text{TRG} = \frac{\text{Radioactive gangliosides (subcellular fraction)}}{\text{Enzyme marker activity (whole homogenate)}} \times \frac{\text{Enzyme marker activity (sub-cellular fraction)}}{\text{Enzyme marker activity (sub-cellular fraction)}}$$

'Radioactive gangliosides' were expressed as d.p.m./g of fresh tissue and 'Enzyme marker activity' as nmol of transferred sugar/h per g of fresh tissue for ovalbumin galactosyltransferase and as µmol of released phosphorous/g of fresh tissue for 5'-nucleotidase. The results are shown in Fig. 4b. In the Golgi apparatus, radioactive gangliosides accounted for modest absolute values, whereas in the plasma membrane they were present in considerable amounts, especially at the long time points.

Time course of individual ganglioside incorporation in the Golgi-apparatus and plasma-membrane fractions

Whereas the ganglioside patterns were greatly modified with time, only negligible differences were found between the two subcellular fractions. GM3 largely predominated over all the other gangliosides at 4 h after injection; successively levels of GM1 (earlier) and disialogangliosides (later) became progressively more consistent, together accounting for the same percentage as GM3 24 h after injection (Fig. 5a). In terms of absolute radioactivity (Fig. 5b), calculated as described above for 'Total gangliosides', the patterns for the Golgi-apparatus and plasma-membrane fraction were greatly different. In fact, in the Golgi apparatus, GM3 continuously decreased from 4 to 24 h, GM1 increased up to 12 h then decreased and the disialogangliosides progressively increased for up to 24 h. Conversely, in the plasma-membrane fraction each ganglioside continuously accumulated for up to 24 h.

DISCUSSION

In our experimental model a liposomal dispersion of radiolabelled LacCer was efficiently taken up by the hepatocytes; this is probably due to a galactose-binding receptor which is present on the surface of this cell type (Spanjer & Scherphof, 1983). After internalization, LacCer is metabolically involved in both catabolic and anabolic events. In fact, a considerable amount of radioactivity associated to low- M_r substances, mainly ³H₂O deriving from [³H]galactose degradation (Ghidoni *et al.*, 1986), was found to be formed in the liver, as well as a low, but definitely significant, amount of radioactive gangliosides.

The Golgi-apparatus and the plasma-membrane fractions are expected to be the intracellular sites of ganglioside biosynthesis and deposition respectively. In our Golgi-apparatus preparation a 60-fold enrichment of glycosphingolipid glycosyltransferases was obtained, enrichment that was lacking in the plasma-membrane fraction, as expected (Keenan *et al.*, 1974). The ganglioside-associated radioactivity in these two subfractions clearly provided a direct confirmation that gangliosides are biosynthesized in the Golgi apparatus and then transported to the plasma membrane and that the Golgi-associated gangliosides are only transient. Accordingly, a literature datum on the endogenous ganglioside distribution in rat liver has attributed 1% of the total sialic acid to the Golgi apparatus (Mathyas & Morré, 1987).

The individual ganglioside that became radioactive were GM3, GM1, GD1a and GD1b. It is worth noting that the precursor LacCer was prepared so as to carry a homogeneous ceramide moiety; this ensured that there would be no influence of the heterogeneity of the lipid moiety in the biosynthesis rate of individual gangliosides. The time courses of individual gangliosides in Golgi apparatus indicated that GM3 acted as a precursor for GM1, the same GM1 as precursor for GD1a. A similar precursor-product relationship in cultured hepatocytes fed with radioactive ManNAc has already been reported by Rump *et al.* (1986); moreover, Miller-Podraza & Fishman (1983) and Chatterjee *et al.* (1986) found an analogous relationship among neutral glycosphingolipids in neuroblastoma cells and proximal-tubular cells re-

Fig. 5. Time course of individual radioactive gangliosides in the Golgi-apparatus (left side) and plasma-membrane (right side) fractions after injection of 50 μ Ci of [3 H]LacCer

(a) Separation by high-performance t.l.c. (solvent system as in Fig. 2) of the total ganglioside mixture and detection by radiodensitometry; (b) incorporated radioactivity in individual gangliosides. For details of how values were calculated see the Results section.

spectively. Conversely, in the nervous system a precursor-product relationship in the course of ganglioside biosynthesis is still controversial (Maccioni *et al.*, 1971; Holm *et al.* 1974; Miller-Podraza *et al.* 1982).

The radioactive ganglioside pattern in the plasma membrane resembled that of the Golgi apparatus at the respective time points. This is an interesting finding, as it suggests a sorting and a translocation to the cell membrane as soon as a ganglioside is biosynthesized. A transit time of 20 min, reported by Miller-Podraza & Fishman (1982) in neural cells, is compatible with our case. The time course of individual ganglioside accumulation on the membrane reflected the above model.

In conclusion, our results indicate that once a ganglioside is biosynthesized in the Golgi apparatus, it is in part made available for translocation to the plasma membrane, which rapidly occurs, and in part is retained in the Golgi apparatus, where it acts as a precursor for the biosynthesis of more glycosylated gangliosides. This is in agreement with the concept that each glycosylation step takes place in specific Golgi cisternae, as already established to occur for glycoprotein oligosaccharide processing in some tissues, including rat liver (Roth, 1987). At this regard we recently localized two glycosphingolipid sialyltransferases in different com-

partments of the Golgi stack (Trinchera & Ghidoni, 1989), thus supporting previous suggestions based on metabolic data obtained in the presence of Golgi dissecting drugs (Saito *et al.*, 1984; van Echten & Sandhoff, 1989). Further important questions are unanswered: for example, where is the sub-Golgi sorting site of each ganglioside? and do these ganglioside movements follow a non-selective membrane flow or do they require specific targeting?

We thank Professor Guido Tettamanti for encouragement and helpful advice. The skilful technical assistance of Miss Nadia Toppi was also greatly appreciated.

REFERENCES

- Basu, M., De, T., Das, K. K., Kyle, J. W., Chon, H., Schaeper, R. J. & Basu, S. (1987) *Methods Enzymol.* **138**, 575-607
- Berry, M. N. & Friend, D. S. (1969) *J. Cell Biol.* **43**, 506-520
- Brew, K., Shaper, J. H., Olsen, K. W., Trayer, I. P. & Hill, R. L. (1975) *J. Biol. Chem.* **250**, 1434-1444
- Briles, E. B., Li, E. & Kornfeld, S. (1977) *J. Biol. Chem.* **252**, 1107-1116
- Cahan, L. D., Irie, R. F., Singh, R., Cassidenti, A. & Paulson, S. C. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7629-7633

- Chatterjee, S., Clarke, K. S. & Kwiterovich, P. O., Jr. (1986) *J. Biol. Chem.* **261**, 13480–13486
- Croze, E. M. & Morré, D. J. (1984) *J. Cell. Physiol.* **119**, 46–57
- Datta, S. C., Snider, R. M. & Radin, N. S. (1986) *Biochim. Biophys. Acta* **877**, 387–398
- Emmelot, P., Bos, C. J., Benedetti, E. L. & Rümke, P. (1964) *Biochim. Biophys. Acta* **90**, 126–145
- Gammon, C. M., Vaswani, K. K. & Ledeen, R. W. (1987) *Biochemistry* **26**, 6239–6243
- Gazzotti, G., Sonnino, S., Ghidoni, R. & Tettamanti, G. (1984) *Glycoconjugate J.* **1**, 111–121
- Ghidoni, R., Sonnino, S., Chigorno, V., Venerando, B. & Tettamanti, G. (1983) *Biochem. J.* **213**, 321–329
- Ghidoni, R., Trinchera, M., Venerando, B., Fiorilli, A., Sonnino, S. & Tettamanti, G. (1986) *Biochem. J.* **237**, 147–155
- Ghidoni, R., Trinchera, M., Sonnino, S., Chigorno, V. & Tettamanti, G. (1987) *Biochem. J.* **247**, 157–164
- Grosse, E., Kieda, C. & Nicolau, C. (1984) *Biochim. Biophys. Acta* **805**, 354–361
- Hirabayashi, Y., Hamaoka, A., Matsumoto, M. & Nishimura, K. (1986) *Lipids* **21**, 710–714
- Holm, M., Mannson, J.-E. & Svennerholm, L. (1974) *FEBS Lett.* **38**, 271–273
- IUPAC-IUB Recommendations (1977) *Lipids* **12**, 455–468
- Keenan, T. W., Morré, D. J. & Basu, S. (1974) *J. Biol. Chem.* **249**, 310–315
- Landa, C. A., Defilpo, S. S., Maccioni, H. J. & Caputto, R. (1981) *J. Neurochem.* **37**, 813–823
- Leskawa, K. C., Dasgupta, S., Chien, J.-L. & Hogan, E. L. (1984) *Anal. Biochem.* **140**, 172–177
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Maccioni, H. J., Arce, A. & Caputto, R. (1971) *Biochem. J.* **125**, 1131–1137
- Mathyas, G. R. & Morré, D. J. (1987) *Biochim. Biophys. Acta* **921**, 599–614
- Miller-Podraza, H. & Fishman, P. H. (1982) *Biochemistry* **21**, 3265–3270
- Miller-Podraza, H. & Fishman, P. H. (1983) *J. Neurochem.* **41**, 860–867
- Miller-Podraza, H. & Fishman, P. H. (1984) *Biochim. Biophys. Acta* **804**, 44–51
- Miller-Podraza, H., Bradley, R. M. & Fishman, P. H. (1982) *Biochemistry* **21**, 3260–3265
- Morré, D. J., Kartenbeck, J. & Franke, W. W. (1979) *Biochim. Biophys. Acta* **559**, 71–152
- Morré, D. J., Morré, D. M. & Heidrich, H. G. (1983) *Eur. J. Cell Biol.* **31**, 263–274
- Nordlie, R. C. & Arion, W. J. (1966) *Methods Enzymol.* **9**, 619–625
- Pentchev, P. G., Gal, A. E., Wong, R., Morrone, S., Neumeyer, B., Massey, J., Kanter, R., Sawitsky, A. & Brady, R. O. (1981) *Biochim. Biophys. Acta* **665**, 615–618
- Pfeffer, S. R. & Rothman, J. E. (1987) *Annu. Rev. Biochem.* **56**, 829–852
- Roth, J. (1987) *Biochim. Biophys. Acta* **906**, 405–436
- Rump, A. J., Philipps, S. & Decker, K. (1986) *Biol. Chem. Hoppe-Seyler* **367**, 425–432
- Saito, M., Saito, M. & Rosenberg, A. (1984) *Biochemistry* **23**, 1043–1046
- Saito, M. & Rosenberg, A. (1985) *J. Biol. Chem.* **260**, 2295–2300
- Seglen, P. O. (1973) *Exp. Cell. Res.* **82**, 391–398
- Sonderfeld, S., Conzelmann, E., Schwarzmann, G., Burg, J., Hinrichs, U. & Sandhoff, K. (1985) *Eur. J. Biochem.* **149**, 247–255
- Soriano, P., Dijkstra, J., Legrand, A., Spanjer, H., Londos-Gagliardi, D., Roerdink, F., Scherphof, G. & Nicolau, C. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7128–7131
- Spanjer, H. H. & Scherphof, G. L. (1983) *Biochim. Biophys. Acta* **734**, 40–47
- Svennerholm, L. (1964) *J. Lipid Res.* **5**, 145–155
- Tettamanti, G. (1984) *Adv. Med. Exp. Biol.* **174**, 197–211
- Tettamanti, G., Bonali, F., Marchesini, S. & Zambotti, V. (1973) *Biochim. Biophys. Acta* **269**, 160–170
- Trinchera, M. & Ghidoni, R. (1989) *J. Biol. Chem.* **264**, 15766–15769
- van Echten, G. & Sandhoff, K. (1989) *J. Neurochem.* **52**, 207–214
- Zanetta, J. P., Breckenridge, W. C. & Vincendon, G. (1972) *J. Chromatogr.* **69**, 291–304

Received 22 June 1989/1 September 1989; accepted 18 September 1989