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Sphingolipid Uptake by Cultured Cells

COMPLEX AGGREGATES OF CELL SPHINGOLIPIDS WITH SERUM PROTEINS AND LIPOPROTEINS ARE RAPIDLY CATABOLIZED*

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Human fibroblasts, rat neurons, and murine neuroblastoma cells, cultured in the presence of fetal calf serum, were fed with [1-^{3}H]sphingosine to radiolabel sphingolipids. The fate of cell sphingolipids, the release of sphingolipids in the culture medium, the interaction of sphingolipids with the proteins and lipoproteins of fetal calf serum, and the fate of sphingolipids taken up by the cells were investigated. For this latter purpose, the culture medium containing radioactive sphingolipids was delivered to nonlabeled cells. The presence of tritium at position 1 of sphingosine allowed us to follow the extent of sphingolipid catabolism by measuring the production of radioactive phosphatidylethanolamine and proteins by recycling the radioactive ethanolamine formed during sphingosine catabolism and the production of tritiated water. We confirmed that in cells the recycling of sphingosine occurred to a high extent and that only a minor portion of cell sphingolipids was catabolized to the small fragments of ethanolamine and water. Cell sphingolipids were released in the culture medium, where they formed large lipoproteic aggregates at a rate of about 12% per day. Released sphingolipids were taken up by the cells and catabolized to the sphingosine and then to ethanolamine, and recycling of sphingosine was not observed. This suggests that in the presence of fetal calf serum in the culture medium, exogenous sphingolipids directly reach the lysosomes, were they are entirely catabolized. Thus, the trafficking of sphingolipids from cells to the extracellular environment and from this to other cells does not allow the modification of the plasma membrane composition.

Membrane sphingolipids are complex lipids displaying a strong amphiphilic character (1). This implies an equilibrium between plasma membrane molecules and free monomers present in the extracellular environment. The critical aggregative concentration of sphingolipids is in the nano- or picomolar range (1, 2), thus the amount of free monomers in solution is low. Nevertheless, the quantity of free monomers should increase when a favorable equilibrium leads to the formation of stable lipoprotein complexes (3–5). This process should also occur in living organisms, where significant amounts of sphingolipids are detectable in serum and cerebrospinal fluid from normal and pathological subjects (6–8), as components of serum lipoproteins or associated with albumin (4).

The presence of sphingolipids in biological fluids leads to the following considerations. Exogenous sphingolipids administered to cells are taken up by the cells, thus entering the sphingolipid metabolic pathway. Several studies have been carried out by administering sphingolipids to cells (9). Gangliosides administered to cells that are cultured in the absence of fetal calf serum (FCS)[1] or proteins have been shown to become components of the cell membranes, modifying in some cases the quantity and the quality of plasma membrane gangliosides, and to enter into the endogenous sphingolipid metabolic pathways (9–11). The information on the administration of sphingolipids to cells in the presence of FCS is very scant. Some data suggest that under this condition the amount of gangliosides taken up by the cells is much lower, but the rate of the catabolic process higher (11). This is probably because of the formation of stable complexes between gangliosides and the proteins and lipoproteins of FCS (3–5) that reduce the number of free monomers available for a direct interaction with the cell membranes but allows endocytosis of sphingolipid aggregates (12). Sphingomyelin is also taken up by neuronal cells and largely catabolized (13).

In this paper we performed the following: 1) the release of all

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[1] The abbreviations used are: FCS, fetal calf serum; Neu5Ac, *N*-acetylneuraminic acid; Cer, ceramide; *N*-acylsphingosine, (2*S*,3*R*,4*E*)-2-amino-1,3-dihydroxy-octadecene; [1-^{3}H]sphingosine, (2*S*,3*R*,4*E*)-2-amino-1,3-dihydroxy-[1-^{3}H]octadecene; [1-^{3}H]sphinganine, (2*S*,3*R*)-2-amino-1,3-dihydroxy-[1-^{3}H]octadecane; PE, phosphatidylethanolamine; SM, sphingomyelin; BME, basal modified Eagle's medium; DMEM, Dulbecco's modified Eagle's medium; EMEM, Eagle's minimum essential medium; HPTLC, high performance thin layer chromatography; GlcCer, β-Glc-(1–1)-Cer; LacCer, β-Gal-(1–4)-β-Glc-(1–1)-Cer; GM3, II^{3}Neu5AcLacCer, α-Neu5Ac-(2–3)-β-Gal-(1–4)-β-Glc-(1–1)-Cer; GM1, II^{3}Neu5AcGgOse$_4$Cer, β-Gal-(1–3)-β-GalNAc-(1–4)-[α-Neu5Ac-(2–3)]-β-Gal-(1–4)-β-Glc-(1–1)-Cer; GD3, II^3(Neu5Ac)$_2$LacCer, α-Neu5Ac-(2–8)-α-Neu5Ac-(2–3)-β-Gal-(1–4)-β-Glc-(1–1)-Cer; GD1a, IV^{3}Neu5AcII^{3}Neu5AcGgOse$_4$Cer, α-Neu5Ac-(2–3)-β-Gal-(1–3)-β-GalNAc-(1–4)-[α-Neu5Ac-(2–3)]-β-Gal-(1–4)-β-Glc-(1–1)-Cer; GD1b, II^3(Neu5Ac)$_2$GgOse$_4$Cer, β-Gal-(1–3)-β-GalNAc-(1–4)-[α-Neu5Ac-(2–8)-α-Neu5Ac-(2–3)]-β-Gal-(1–4)-β-Glc-(1–1)-Cer; *O*-Ac-GT1b, IV^{3}Neu5AcII^3[α-Neu5,9Ac$_2$-(2–8)-α-Neu5Ac-(2–3)]GgOse$_4$Cer; GT1b, IV^{3}Neu5AcII^3(Neu5Ac)$_2$GgOse$_4$Cer, α-Neu5Ac-(2–3)-β-Gal-(1–3)-β-GalNAc-(1–4)-[α-Neu5Ac-(2–8)-α-Neu5Ac-(2–3)]-β-Gal-(1–4)-β-Glc-(1–1)-Cer; GQ1b, IV^3(Neu5Ac)$_2$ II^3(Neu5Ac)$_2$GgOse$_4$Cer, α-Neu5Ac-(2–8)-α-Neu5Ac-(2–3)-β-Gal-(1–3)-β-GalNAc-(1–4)-[α-Neu5Ac-(2–8)-α-Neu5Ac-(2–3)]-β-Gal-(1–4)-β-Glc-(1–1)-Cer; the ganglioside and glycosphingolipid nomenclature is in accordance with Svennerholm (53) and the IUPAC-IUBMB recommendations (54, 55).

classes of sphingolipids from both normal and tumor cells in culture, 2) the association of sphingolipids released by cells with proteins and lipoproteins of FCS present in the culture medium, and 3) the fate of sphingolipids released by the cells and taken up by the same or different cells. Our results suggest that the endogenous and specific sphingolipid content of plasma membrane cannot be changed *in vivo* by the incorporation into cells of circulating sphingolipids, and suggest that the sphingolipids that are taken up by the cells are largely and rapidly catabolized.

EXPERIMENTAL PROCEDURES

Materials—The commercial chemicals were the purest available, common solvents were distilled before use, and deionized water, obtained by a MilliQ system (Millipore), was distilled in a glass apparatus. High performance silica gel precoated thin layer plates (TLC Kieselgel 60) were purchased from Merck. Basal modified Eagle's medium (BME), Eagle's minimum essential medium (EMEM), Dulbecco's modified Eagle's medium (DMEM), and fetal calf serum (FCS) were from Hyclone. Other reagents for cell cultures, *Vibrio cholerae* sialidase, *Staphylococcus aureus* sphingomyelinase, and bovine serum albumin were from Sigma.

Sphingosine was prepared from cerebroside (14). [1-^{3}H]Sphingosine (radiochemical purity over 98%; specific radioactivity, 2.08 Ci/mmol) was prepared by specific chemical oxidation of the primary hydroxyl group of sphingosine followed by reduction with sodium boro[^{3}H]hydride (15). [1-^{3}H]Sphinganine was prepared by quantitative hydrogenation in the presence of 10% activated palladium-charcoal, of [1-^{3}H]sphingosine (10, 16). Ceramide species having fatty acids of different length were prepared by acetylation of sphingosine or [1-^{3}H]sphingosine using the corresponding anhydrides or activated fatty acids (16). Radioactive sphingolipids were extracted from cells fed with [1-^{3}H]sphingosine, purified, characterized as described previously (17, 18), and used as chromatographic standards.

Normal human skin fibroblasts were obtained by the punch technique, cultured, and propagated as described (19) in 100-mm dishes (0.42 ± 0.10 mg of protein/dish), using EMEM supplemented with 10% FCS, and used for the experiments when confluent. Granule cells were obtained from cerebellum of 8-day-old Harlan Sprague-Dawley rats and cultured as described (20). Cells were plated at a density of 9×10^6 cells/dish in 100-mm dishes (0.45 ± 0.05 mg of protein/dish), cultured with BME supplemented with 10% FCS, and used for the experiments between the 6th and 8th day of culture. The murine neuroblastoma cell line clone NB2a (Neuro2a, CCL-131, American Cell Type Culture Collection, Manassas,VA) was cultured in DMEM supplemented with 10% FCS in 100-mm dishes (4.52 ± 0.51 mg of protein/dish).

Cell Feeding with [1-^{3}H]Sphingosine—[1-^{3}H]Sphingosine dissolved in methanol was transferred into a sterile glass tube and dried under a nitrogen stream; the residue was then dissolved in an appropriate volume of pre-warmed (37 °C) 2% FCS-EMEM (fibroblasts) or cell-conditioned medium (granule cells) or 10% FCS-DMEM (neuroblastoma cells) to obtain a final 3×10^{-8} M concentration. After a 2-h incubation (pulse), the medium was removed, and cells were washed with culture medium and incubated for 48 h with culture medium not containing radioactive precursors in the presence of 10% FCS. In some experiments, the chase was prolonged up to 5 days with EMEM or BME. In some others experiments FCS was omitted or substituted with a 0.4% albumin. A few control experiments were carried out feeding [1-^{3}H]sphinganine to human fibroblasts.

Fractionation of Cell Medium—The cell medium from cells pulsed with 3×10^{-8} M [1-^{3}H]sphingosine and subjected to a 48-h chase were centrifuged at 1000 × *g* for 15 min to remove cellular debris. The supernatant was then centrifuged at 150,000 × *g* for 1 h at 4 °C, and carefully collected in 1-ml fractions starting from the top of the tube (21). The pellet and each fraction were analyzed for radioactivity content. Lipoproteins were isolated from the cell-conditioned medium from cells pulsed with 3×10^{-8} M [1-^{3}H]sphingosine and subjected to a 48-h chase, by sequential preparative ultracentrifugation (22, 23).

Laser Light-scattering Measurements—Static and dynamic laser light-scattering measurements were performed on the cell medium collected from cells pulsed with [1-^{3}H]sphingosine and chased up to 48 in the absence of proteins. Details on the apparatus and on the procedures are available from several references reported previously (2).

Determination of Catabolic Water—Tritiated water (10) formed during the metabolic processing of the tritiated sphingolipids and diffused into the cell culture medium was determined as follows. One ml of the cell culture medium was dialyzed overnight against 2 ml of water. The dialysate was distilled, and the radioactivity associated with the 100 °C fraction was determined by liquid scintillation counting.

Cell Cultures in Radioactive Conditioned Medium—The medium from cultured cells pulsed with 3×10^{-8} M [1-^{3}H]sphingosine was collected after a 48-h chase. Cells that were never treated before with radioactive precursors were incubated in the presence of these medium for different times (2–24 h). At the end of chase, the cell medium was carefully collected, and cells were repeatedly washed with EMEM, BME, or DMEM, and then with 10% FCS-containing medium (30 min) to remove ganglioside aggregates loosely bound to the cell surface. In some experiments a final 5-min treatment with 0.1% trypsin was performed to remove sphingolipids bound to surface proteins (11, 24).

Extraction and Analysis of Radioactive Lipids—At the end of all experiments, the cells were carefully separated from the medium and then were harvested in ice-cold water (2 ml) by scraping with a rubber policeman. Lipids present in both cells and medium were extracted, characterized, and analyzed for radioactivity content. Delipidized pellets were analyzed for radioactivity content. The cell suspensions were frozen and lyophilized, and the residues were subjected to lipid extraction. Cell lipids were extracted according to the chloroform/methanol/water procedure as described previously (25). The cell medium from cells fed with [1-^{3}H]sphingosine were dialyzed for 2 days, frozen, and lyophilized, and the residues were subjected to lipid extraction with chloroform/methanol/water, 20:10:1 by volume. The resulting total lipid extracts were dried under nitrogen stream and dissolved in chloroform/methanol, 2:1 by volume.

The main radioactive sphingolipids extracted from cells and cell medium were purified by the HPTLC-blotting technique (26) and characterized as described previously (17, 18). Lipids were analyzed by HPTLC using the solvent systems chloroform, methanol, 0.2% aqueous $CaCl_2$, 55:45:10 by volume, to separate fibroblast lipids, chloroform, methanol, and 0.2% aqueous $CaCl_2$, 50:42:11 by volume, to analyze separate granule and neuroblastoma cell lipids, and ethyl acetate, chloroform, methanol, 50 mM KCl, 50:40:10:1.4 by volume, to analyze ceramide and radiolytic byproducts. The absence of [^{3}H]sphingosine in the total lipid extracts from cells fed 3×10^{-8} M [1-^{3}H]sphingosine was determined after analysis of the total radioactive lipid extract by two-dimensional TLC performed with chloroform, methanol, 0.2% aqueous $CaCl_2$, 55:45:10 by volume, and chloroform, methanol, 32% NH_4OH, 40:10:1 by volume, respectively for the 1st and 2nd run. The radioactive sphingolipid pattern in neuroblastoma cells fed 3×10^{-8} M [1-^{3}H]sphingosine and that in the corresponding cell medium were confirmed by two-dimensional TLC using chloroform/methanol/water, 110:40:6 by volume, and chloroform, methanol, 0.2% aqueous $CaCl_2$, 50:42:11 by volume, respectively, for the 1st and 2nd run.

After separation, radioactive lipids were visualized with a Beta-Imager 2000 (Biospace). The radioactivity associated with individual lipids was determined with the specific β-Vision software (Biospace).

Other Analytical Methods—Radioactivity associated with cells, with medium, with medium fractions, and with lipid extracts was determined by liquid scintillation counting. The protein content was determined according to Lowry *et al.* (27) with bovine serum albumin as reference standard.

RESULTS

Metabolic Labeling of Cell Sphingolipids—[1-^{3}H]sphingosine fed to cells in culture at a final concentration of 3×10^{-8} M was taken up by cells and entered into the complex sphingolipid biosynthetic pathway, in agreement with previous data (17, 18). After a 2-h pulse, 45 ± 5% of the tracer was incorporated into fibroblasts, 55 ± 5% into neurons, and 52 ± 5% into neuroblastoma cells and used for the synthesis of cell sphingolipids. Fig. 1, *panel A*, shows the radioactive sphingolipid patterns obtained from human fibroblasts, differentiated rat cerebellar granule cells, and murine Neuro2a neuroblastoma cells. Some radioactivity was associated with phosphatidylethanolamine (PE), due to recycling of radioactive ethanolamine produced in the catabolism of [1-^{3}H]sphingosine. The solvent systems based on a mixture of chloroform, methanol, and water are very powerful for the separation and chromatographic identification of complex mixtures of radioactive sphingolipids, both acidic and neutral sphingolipids, with the exemption of ceramide. In fact, in the presence of chloroform, some radical reactions on radioactive lipids occur, yielding a minor quantity of

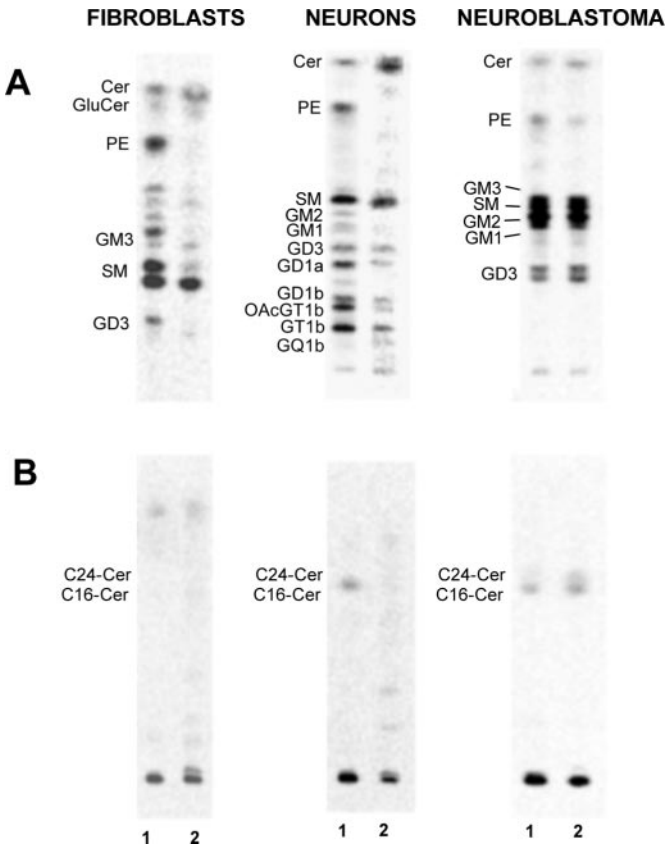

FIG. 1. **TLC patterns of radioactive lipids present in human fibroblasts, rat cerebellar granule cells, murine Neuro2a neuroblastoma cells, and their cell-conditioned medium after feeding 3×10^{-8} M [1-^{3}H]sphingosine, (2-h pulse followed by 48 h-chase).** Lipids from cells (*lanes 1*) and from cell-conditioned media (*lanes 2*) were extracted and separated by HPTLC by using the solvent systems chloroform, methanol, 0.2% aqueous $CaCl_2$, 55:45:10 by volume, to separate fibroblast lipids, chloroform, methanol, 0.2% aqueous $CaCl_2$, 50:42:11 by volume, to separate granule and neuroblastoma cell lipids (*panel A*), and ethyl acetate, chloroform, methanol, 50 mM KCl, 50:40:10:1.4 by volume (*panel B*). The chase was performed in the presence of 10% FCS in the medium. Radioactive lipids were detected by digital autoradiography; 100–200 dpm/lane; time of acquisition, 48 h. Patterns are representative of those obtained in five different experiments.

radiolytic apolar compounds of unknown structure that chromatographically overlap with ceramide. Thus, to specifically recognize and quantify ceramide, TLC were performed with a solvent system containing ethyl acetate (Fig. 1, *panel B*). Fig. 1, *panel B,* shows that in fibroblasts ceramide is under the detectable limit. Instead ceramide was recognized and quantified in neurons and neuroblastoma cells.

A precise qualitative and quantitative evaluation of the sphingolipid pattern of neuroblastoma cells was carried out after two-dimensional TLC separations (Fig. 2, *panel A*). This was necessary to better separate GM2 from SM. The quantitative radioactive lipid composition of human fibroblasts, differentiated rat cerebellar granule cells and murine Neuro2a neuroblastoma cells, as derived by five experiments, is reported in Tables I, II and III, respectively.

Release of Sphingolipids from Cultured Cells—As known, some sphingolipids are released from cells (28–31). The metabolic labeling of cell sphingolipids allowed us the comparative qualitative and quantitative analysis of this event in different cell types. The radioactive sphingolipids obtained from the cell culture media after [1-^{3}H]sphingosine 2/48-h pulse/chase experiments were analyzed by TLC, as shown in Figs. 1 and 2. The radioactive sphingolipid patterns were in some way different

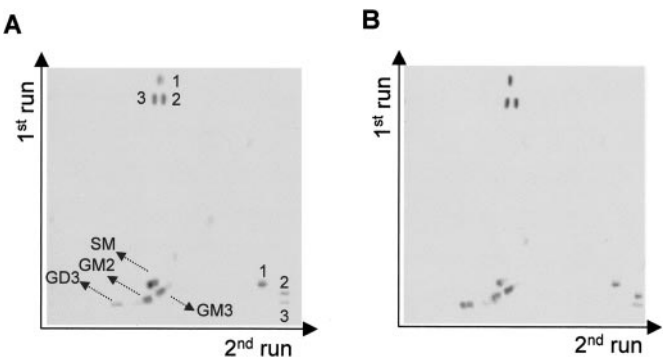

FIG. 2. **Two-dimensional TLC patterns of radioactive lipids present in murine Neuro2a neuroblastoma cells and their cell-conditioned medium after feeding 3×10^{-8} M [1-^{3}H]sphingosine, (2-h pulse followed by 48 h-chase).** Lipids from cells (*panel A*) and from cell-conditioned medium (*panel B*) were extracted and separated by two-dimensional TLC by using the following solvent systems: 1st run, chloroform/methanol/water, 110:40:6 by volume; 2nd run, chloroform, methanol, 0.2% aqueous $CaCl_2$, 50:42:11 by volume. The chase was performed in the presence of 10% FCS in the medium. Radioactive lipids were detected by digital autoradiography; 100–200 dpm/lane; time of acquisition, 48 h. The positions of standard lipids are indicated by the following numbers: *1*, SM; *2*, GM3; and *3*, GM2. Patterns are representative of those obtained in five different experiments.

from those of the corresponding cells. The quantitative distribution patterns of radioactive sphingolipids found in the cell medium, as derived by five experiments, are reported in Tables I–III, compared with those of the corresponding cell samples.

The release of sphingolipids from cells into the medium was studied in detail for up to 4 days of culture. Fig. 3 shows that the release process was very similar within the three cell lines, cultured in the presence of 10% FCS. Substitution of 10% FCS with 0.4% albumin did not change the extent of the process. In the absence of proteins, the quantity of sphingolipids in the cell medium was quite low, even if not negligible.

When incubated in protein-free medium, cells released small amounts of radioactive sphingolipids, about 2% of starting cell radioactivity per day. Laser light-scattering measurements revealed that lipids were present in these samples as a mixture of different aggregates characterized by high polydispersity, with hydrodynamic radius ranging from 6 to 90 nm. This is not surprising, due to the large content of sphingomyelin in the sphingolipid mixture. In the case of protein-containing media, laser light-scattering experiments were not reliable due to the extreme heterogeneity in the aggregate size. In this case, media were investigated by sedimentation experiments.

Ultracentrifugation of the culture medium from neuroblastoma cells fed with radioactive sphingosine pelleted about 50% of the total radioactivity, the remaining being homogeneously distributed in the solution. When the neuroblastoma cell-conditioned medium was kept at room temperature for 48 h before centrifugation, the percent of sedimentable radioactivity decreased to 22%. Fibroblasts and granule cell-conditioned medium did not sediment radioactive particles by ultracentrifugation, and all the radioactivity remained in the solution. The supernatants from cell-conditioned medium were subjected to sequential preparative ultracentrifugation to separate serum lipoproteins. Four fractions at increasing density corresponding to very low density lipoprotein, low density lipoprotein, high density lipoprotein, and to serum essentially depleted of lipoproteins were obtained. Radioactivity was associated with all fractions as follows: 5–7% with very low density lipoprotein; 20–27% with low density lipoprotein; 12–17% with high density lipoprotein; and 45–58% with the lipoprotein-depleted fraction. According to previous results, this latter part should correspond to complexes between sphingolipids and albumin (2–4).

TABLE I

Radioactive sphingolipid patterns in normal human fibroblasts and their cell culture medium

Cells were incubated for 2 h in the presence of 3×10^{-8} M [1-^{3}H]sphingosine, followed by a 48-h chase in the presence of 10% FCS. The radioactivity associated with each lipid was determined after extraction from a pool of 10 dishes (0.42 ± 0.10 mg of protein/dish), HPTLC separation, and digital autoradiography as described under "Experimental Procedures." GM3, SM, and GD3 were resolved in two bands by HPTLC ("upper" and "lower"), containing longer and shorter acyl chains, respectively. Total radioactivity was determined by liquid scintillation counting. Data are the means ± S.D. of five different experiments.

	Cell homogenate (539,824 ± 27,182 dpm/mg cell protein)	Cell-conditioned medium (208,656 ± 12,668 dpm/mg cell protein)	Radioactivity in the medium on total radioactivity (homogenate + medium), %
	%, on total	%, on total	
Cer	Traces		
GlcCer	3.6 ± 0.3	8.4 ± 0.9	47.0 ± 0.6
PE	16.4 ± 0.9	1.5 ± 0.2	3.5 ± 0.3
GM3 upper	8.7 ± 0.6	5.2 ± 0.6	18.7 ± 0.8
GM3 lower	2.4 ± 0.3	10.8 ± 0.7	36.2 ± 1.2
SM upper	20.5 ± 0.9	9.7 ± 0.9	15.4 ± 0.7
SM lower	31.4 ± 1.4	47.7 ± 2.2	37.0 ± 0.1
GD3 upper	4.9 ± 0.6	2.3 ± 0.4	15.6 ± 0.8
GD3 lower	1.2 ± 0.2	2.7 ± 0.3	46.8 ± 1.9
Others[a]	10.9 ± 0.7	11.7 ± 0.8	29.3 ± 0.2
Total	100 ± 5.0	100 ± 6.1	27.9 ± 0.2

[a] Others indicates three not characterized compounds chromatographically migrating between PE and GM3.

TABLE II

Radioactive sphingolipid patterns in differentiated rat cerebellar granule cells and their cell culture medium

Cells were incubated for 2 h in the presence of 3×10^{-8} M [1-^{3}H]sphingosine, followed by 48-h chase in the presence of 10% FCS. The radioactivity associated with each lipid was determined after extraction from a pool of 10 dishes (0.45 ± 0.05 mg protein/dish), HPTLC separation, and digital autoradiography as described under "Experimental Procedures." Total radioactivity was determined by liquid scintillation counting. Data are the means ± S.D. of five different experiments.

	Cell homogenate (545,640 ± 38,666 dpm/mg cell protein)	Cell-conditioned medium (209,136 ± 10,437 dpm/mg cell protein)	Radioactivity in the medium on total radioactivity (homogenate + medium), %
	%, on total	%, on total	
Cer	5.6 ± 0.4	Traces	Traces
GlcCer	Traces	10.9 ± 0.7	>99
PE	11.2 ± 0.8	Traces	Traces
SM	20.8 ± 1.3	33.7 ± 2.7	38.3 ± 0.5
GM2	3.6 ± 0.3	Traces	Traces
GM1	5.2 ± 0.4	1.6 ± 0.5	10.8 ± 2.5
GD3	5.5 ± 0.4	16.8 ± 0.8	53.6 ± 0.6
GD1a	9.8 ± 0.7	8.7 ± 0.8	25.3 ± 0.4
GD1b	7.8 ± 0.5	6.4 ± 0.3	23.9 ± 0.3
O-Ac-GT1b	8.7 ± 0.7	5.3 ± 0.3	18.9 ± 0.2
GT1b	17.5 ± 1.4	10.6 ± 1.1	18,9 ± 0.3
GQ1b	1.6 ± 0.1	5.9 ± 0.3	58.1 ± 0.9
Others[a]	1.8 ± 0.2	Traces	Traces
Total	100 ± 7.1	100 ± 5.0	27.7 ± 0.4

[a] Others indicates a not characterized compound chromatographically migrating between GD1a and GD1b.

TABLE III

Radioactive sphingolipid patterns in murine neuroblastoma (Neuro2a and CCL-131) cells and their cell culture medium

Cells were incubated for 2 h in the presence of 3×10^{-8} M [1-^{3}H]sphingosine, followed by a 48-h chase in the presence of 10% FCS. The radioactivity associated with each lipid was determined after extraction from a pool of 10 dishes (4.52 ± 0.51 mg protein/dish), HPTLC separation, and digital autoradiography as described under "Experimental Procedures." GM3, SM, GM2, and GD3 were resolved in two bands by HPTLC ("upper" and "lower"), containing longer and shorter acyl chains, respectively. Total radioactivity was determined by liquid scintillation counting. Data are the means ± S.D. of five different experiments.

	Cell homogenate (64,671 ± 4,278 dpm/mg cell protein)	Cell-conditioned medium (12,665 ± 1,017 dpm/mg cell protein)	Radioactivity in the medium on total radioactivity (homogenate + medium), %
	%, on total	%, on total	
Cer	4.8 ± 0.9	6.4 ± 1.2	20.8 ± 0.2
PE	5.3 ± 0.6	6.1 ± 0.9	18.4 ± 0.5
GM3 upper	23.0 ± 1.7	11.8 ± 2.0	9.1 ± 0.8
GM3 lower	Traces	14.3 ± 1.3	>99
SM upper	13.9 ± 1.2	Traces	Traces
SM lower	21.5 ± 1.8	16.5 ± 2.2	13.0 ± 0.6
GM2 upper	10.0 ± 0.6	10.0 ± 2.2	16.2 ± 2.1
GM2 lower	9.5 ± 0.9	10.0 ± 1.9	17.0 ± 1.3
GM1	4.1 ± 1.2	Traces	Traces
GD3 upper	2.5 ± 0.6	14.1 ± 0.9	52.7 ± 4.8
GD3 lower	5.5 ± 0.9	10.0 ± 2.1	26.4 ± 0.7
Total	100 ± 6.6	100 ± 8.0	16.4 ± 0.2

Fate of Cell Sphingolipids—The loss of cell sphingolipids during time was quantitatively studied in detail in fibroblasts fed with [1-^{3}H]sphingosine and cultured for up to 36 days in the presence of 10% FCS. During this time of culture, we determined the radioactivity associated with cell sphingolipids, PE, and the delipidized pellet and the radioactivity associated with sphingolipids and water in the cell medium. Fig. 4, *panel A1*, shows the continuous depletion of the radiolabeled sphingolip-

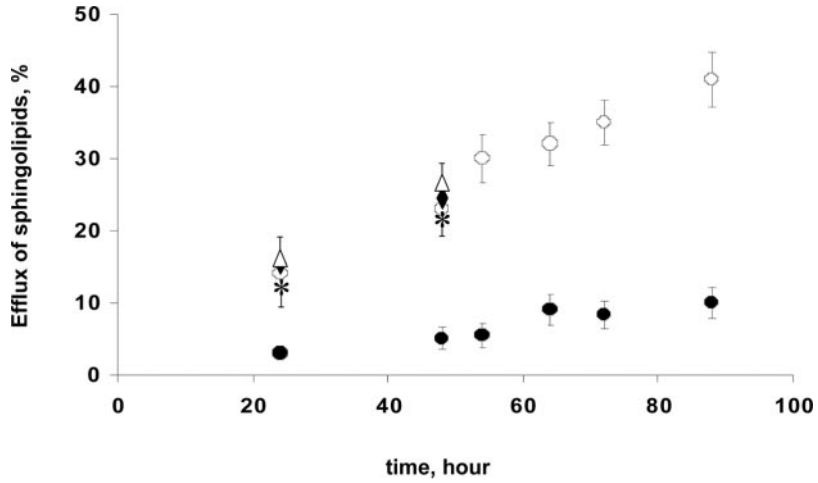

FIG. 3. **Effect of proteins on the efflux of metabolically radiolabeled sphingolipids, from human fibroblasts in culture.** Cells cultured in the presence (*open circles*) or in the absence (*closed circles*) of 10% FCS-containing medium were fed 3×10^{-8} M [1-^{3}H]sphingosine for 2 h (pulse) and then incubated for up to 48 h (chase). At different times, cells and cell-conditioned medium were collected and analyzed for their radioactivity content as described under "Experimental Procedures." In two experiments (*asterisk*), after pulse, cells were then incubated for up to 48 h (chase) in albumin-containing EMEM (4 mg/ml). Two data are related to rat cerebellar granule cells (*triangle*) and neuroblastoma cells (*diamond*) cultured in the presence of 10% FCS. Data are the means of three different experiments ± S.D.

ids from the cells by a daily removal of the radioactive medium and addition of unlabeled medium. Four components contributed to the progressive reduction of radioactivity associated with cell sphingolipids as follows: 1) about 12% of the radioactivity associated with cell sphingolipids was released daily in the culture medium (Fig. 4, *panel B1*); 2) about 12% of the cell radioactivity was associated with PE (Fig. 4, *panel C1*), indicating that a certain amount of catabolic sphingosine is cleaved to ethanolamine that is recycled for the synthesis of PE; 3) a tiny amount of radioactivity was associated with the delipidized pellets, possibly due to recycling of ethanolamine for the synthesis of glycine and serine, *i.e.* of proteins (Fig. 4, *panel D1*); 4) a tiny amount of radioactivity was present in the medium as tritiated water (Fig. 4, *panel D1*). Control experiments showed that radioactive sphingolipids added to the culture medium or cell-conditioned medium were stable and did not lead to the formation of tritiated water. Therefore, tritiated water is produced from the cells and represents the final tritiated cell catabolite. Similar behavior was observed in one experiment performed by feeding cells with [1-^{3}H]sphinganine instead of [1-^{3}H]sphingosine (Fig. 4, *panel 2*).

Re-uptake and Metabolic Fate of Released Lipids—The cell-conditioned chase medium obtained after labeling of cell lipids was used as a source of radioactive sphingolipids to be administered to cells not subjected previously to lipid metabolic radiolabeling. Cells were maintained in the radioactive sphingolipid-containing medium for up to 24 h. After 24 h of incubation and cell washing with culture medium, the radioactivity associated with fibroblasts, neurons, neuroblastoma cells, and fibroblasts cultured in the conditioned medium from neuroblastoma cells was 12, 11, 14, and 7%, respectively, with respect to total radioactivity present in the cell-conditioned medium at the beginning of the incubation. When neuroblastoma cells were also washed with FCS-containing medium and trypsin, the radioactivity associated with cells decreased from 14 to 12 and 9%, respectively, suggesting that a part of the cell radioactivity was loosely associated and was not inside the cells. Fig. 5 shows that 30 min of washing with FCS-containing medium and trypsin treatment progressively removed a part of the cell-associated sphingolipids with a parallel increase of the percent of radioactivity associated to PE. PE can be produced only inside the cells and cannot be removed by the washing process. This confirms that a part of sphingolipids associated with cells is bound to the cell surface but does not belong to the cells.

Fig. 6 shows the HPTLC radioactive patterns from cells cultured in the cell-conditioned medium containing radioactive sphingolipids and washed with culture medium before analysis. The sphingolipids taken up by the cells were progressively catabolized with the formation of radioactive PE. This occurred in all the three cell lines, but the catabolic process was very rapid in neurons, and after 24 h PE was the only detectable radioactive compound. Fibroblasts were also incubated with the radioactive medium from neuroblastoma cells that contained radioactive gangliosides in quantity much higher than the other media. Also in this case, the sphingolipids taken up by the cells, both gangliosides and sphingomyelin, entered the catabolic pathway yielding progressively higher quantities of radioactive PE.

DISCUSSION

Minor quantities of sphingolipids are circulating in the blood system as components of serum lipoproteins (22–23); we determined the content of 62.8 nmol of SM/ml of FCS, and the serum gangliosides are 2 nmol of Neu5Ac/ml of FCS (32). To study the fate of serum sphingolipids taken up by the cells, we used the following experimental model: 1) cells were fed with [1-^{3}H]sphingosine to metabolically label all sphingolipids; 2) feeding was followed by a chase time, allowing sphingolipids to reach the extracellular environment and to largely associate with the serum proteins and lipoproteins present in the medium; 3) the radioactive medium was collected and incubated with new cell populations; and 4) the fate of radioactivity taken up by the cells was determined.

Cell sphingolipids were found into the FCS containing cell medium in different quantities. Thus, the radioactive sphingolipid pattern present in the cell-conditioned medium was significantly different from that of the cell homogenate (Figs. 1 and 2). These sphingolipids are expected to be taken up by the cells (9–11, 13, 25, 33–37).

Sphingolipids administered to cells in the absence of proteins in the medium of culture are taken up by the cells, thus entering into the general sphingolipid metabolic pathway (9–11, 13). A common feature of the cellular metabolism of gangliosides is represented by the extensive re-use of catabolic fragments (9–11, 34, 38–42). In fibroblasts, the extent of the recycling proc-

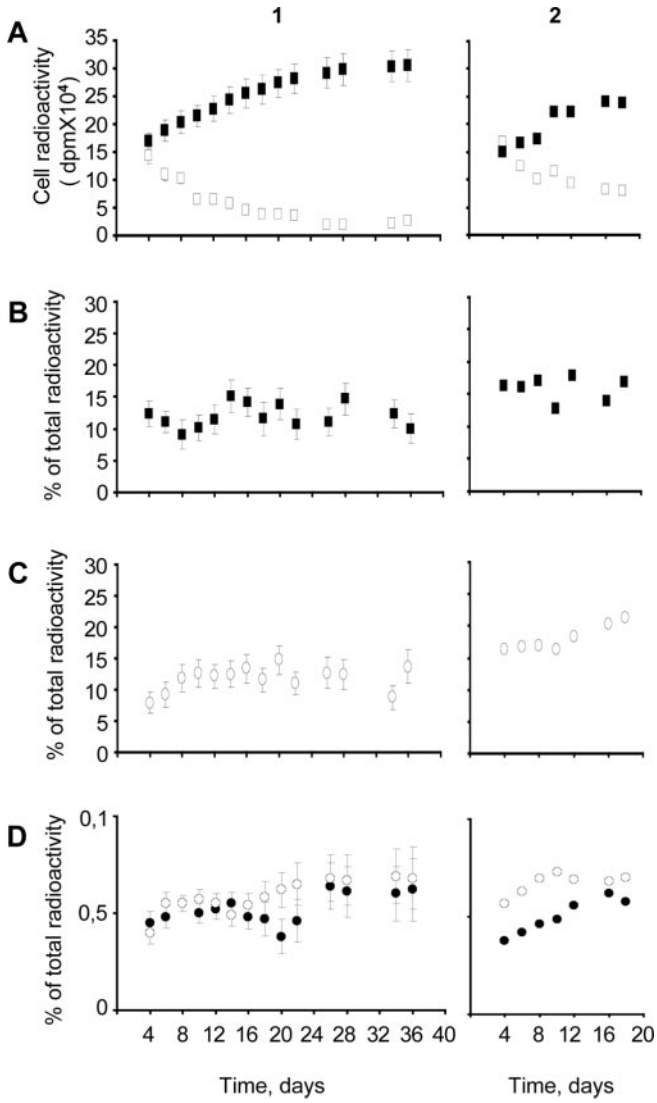

FIG. 4. **Fate of metabolically radiolabeled sphingolipids of human fibroblasts in culture: release into the cell medium, recycling of the catabolic fragment ethanolamine, and production of tritiated water during time.** Fibroblasts cultured in 10% FCS-containing medium were fed 3×10^{-8} M [1-^{3}H]sphingosine (*panels 1*) for 2 h (pulse), and then incubated for up to 36 days (chase). Every day, the chase medium was collected and analyzed. Cells were further incubated with fresh culture medium not containing radioactive lipids or were collected and analyzed for their radioactivity content as described under "Experimental Procedures." Data are the means of three different experiments ± S.D. A further experiment, performed by administering [1-^{3}H]sphinganine instead of [1-^{3}H]sphingosine and with a chase up to 20 days is shown in *panels 2*. *Panel A,* continuous depletion of radiolabeled sphingolipids from the cells by a daily removal of the radioactive medium and addition of unlabeled medium. *Open squares*, radiolabeled sphingolipids associated with cells; *closed squares*, sphingolipids released into the cell medium (each point of the curve is the addition of all the ahead points). *Panel B,* daily efflux of radiolabeled sphingolipids from cells. The amount of radioactivity released each day from cultured fibroblasts in the medium is expressed as percent of total radioactivity (radioactivity associated with cells and medium). *Panel C,* radiolabeled cellular PE, by recycling of catabolic radioactive ethanolamine, is expressed as percent of total radioactivity. *Panel D, closed circles*, tritiated water formed during metabolic processing of sphingolipids, expressed as percentage of total radioactivity; *open circles*, radioactivity associated with the delipidized pellets, expressed as percent of total radioactivity.

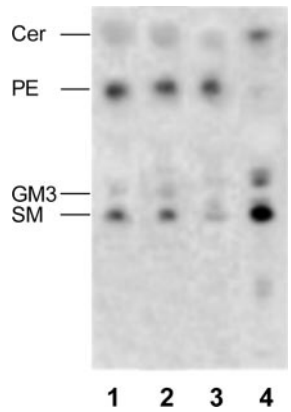

FIG. 5. **TLC patterns of radioactive lipids present in murine Neuro2a neuroblastoma cells (*lanes 1–3*) and their cell-conditioned medium (*lane 4*) after cell feeding with 3×10^{-8} M [1-^{3}H]sphingosine followed by a 48-h chase.** Before lipid extraction, cells were washed with EMEM (*lane 1*), 10% FCS containing EMEM (*lane 2*), and 10% FCS containing EMEM followed by 0.1% trypsin treatment (*lane 3*). Radioactive lipids were detected by digital autoradiography; 100–200 dpm/lane; time of acquisition, 48 h. Patterns are representative of those obtained in three different experiments.

ess of sphingosine has been shown to be relatively high, comprising about 80% of the total catabolic sphingosine (10). In slowly dividing cells (including human foreskin fibroblasts and NB41A3 neuroblastoma cells), the *de novo* pathway contribution to the synthesis of glycosphingolipids could be irrelevant (38). This information suggests that cellular sphingolipids could be predominantly synthesized by using sphingoid bases recycled from the degradation pathway. Also in the case of SM, it has been shown in Sertoli cells that SM is largely synthesized by recycling of sphingosine and ceramide (42). Instead, in the presence of FCS in the cell culture medium, the sphingolipid containing lipoproteins are taken up by the cells in low amounts and probably reach the lysosomes via an endocytotic process (12).

Human fibroblasts, neurons and neuroblastoma cells were cultured in the presence of chase medium that was collected from cell cultures in which sphingolipids were metabolically radiolabeled. Some radioactive sphingolipids present in these cell-conditioned medium were taken up by the cells and catabolized following a time-dependent process. We did not observe the recycling of sphingosine, which was largely catabolized to ethanolamine and recycled for the biosynthesis of PE. In some cases, the production of ceramide preceded the production of PE. The catabolic process also occurred to the sphingolipids taken up by fibroblasts when they were cultured in the medium from neuroblastoma cells. Most interestingly fibroblasts were able to catabolize radioactive GM2, GM3, and SM with a very similar rate. This is not so obvious, because the three compounds display similar radioactivity, but their mass amount is dramatically different. This is particularly true for GM2, which is a very minor component of both fibroblasts and FCS. This led us to hypothesize that sphingolipids taken up by the cells enter cells without becoming components of the plasma membranes. Thus, the rate of catabolism is only determined by the time necessary for the aggregates internalized by endocytosis to reach the lysosomes and by the lysosomal catabolic capacity. Of course, the production of radioactive PE is expected in the case of a catabolic process and can be considered the mirror of the total catabolism of complex sphingolipids and sphingosine, when tritium is in position 1. Other groups studied the metabolic use of exogenous sphingosine, labeled in position 3, with results similar to ours (43) but with no possibility to follow the formation of PE.

The total amount of radioactivity released from the cells, with respect to the cell radioactivity, was constant at about 12% (Fig. 4). Thus the cell radioactivity content decreased following a hyperbolic trend, whereas the total radioactivity

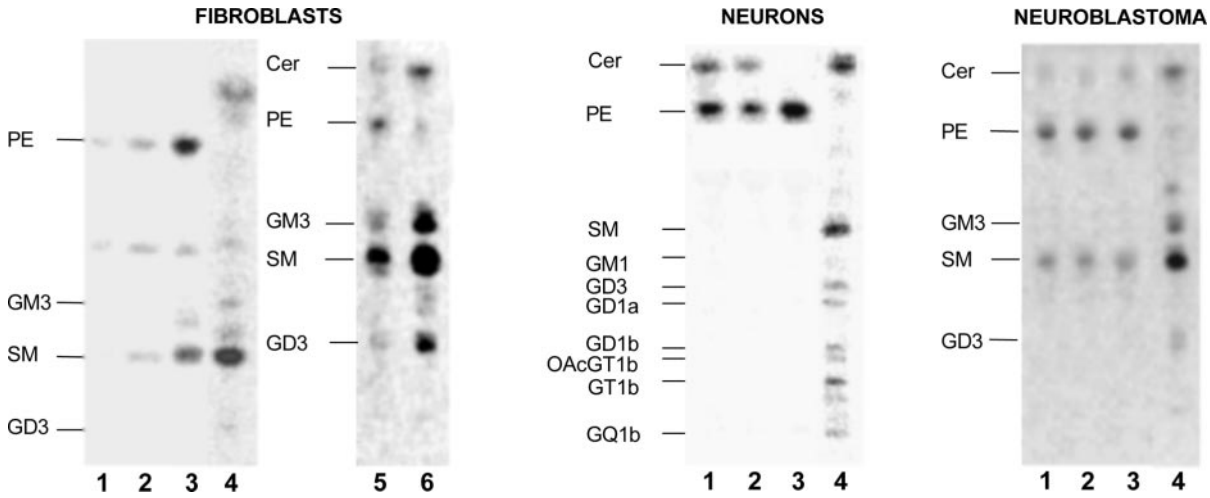

FIG. 6. **Pattern of radioactive lipids from human fibroblasts, rat cerebellar neurons, and neuroblastoma cells that were incubated for 2 (*lane 1*), 4 (*lane 2*), and 24 h (*lane 3*) with the cell-conditioned medium (*lane 4*) from [1-^{3}H]sphingosine-labeled cells (2 h for 3 × 10^{-8} M [1-^{3}H]sphingosine followed by a 48-h chase).** Shown is the pattern of radioactive lipids from human fibroblasts (*lane 5*) that were incubated for 24 h with the cell-conditioned medium from murine Neuro2a neuroblastoma cells fed with 3 × 10^{-8} M [1-^{3}H]sphingosine (*lane 6*). Solvent systems chloroform, methanol, 0.2% aqueous $CaCl_2$, 55:45:10 by volume, to separate fibroblast lipids, chloroform, methanol, 0.2% aqueous $CaCl_2$, 50:42:11 by volume, to separate granule and neuroblastoma cell lipids. Patterns are representative of those obtained in three different experiments.

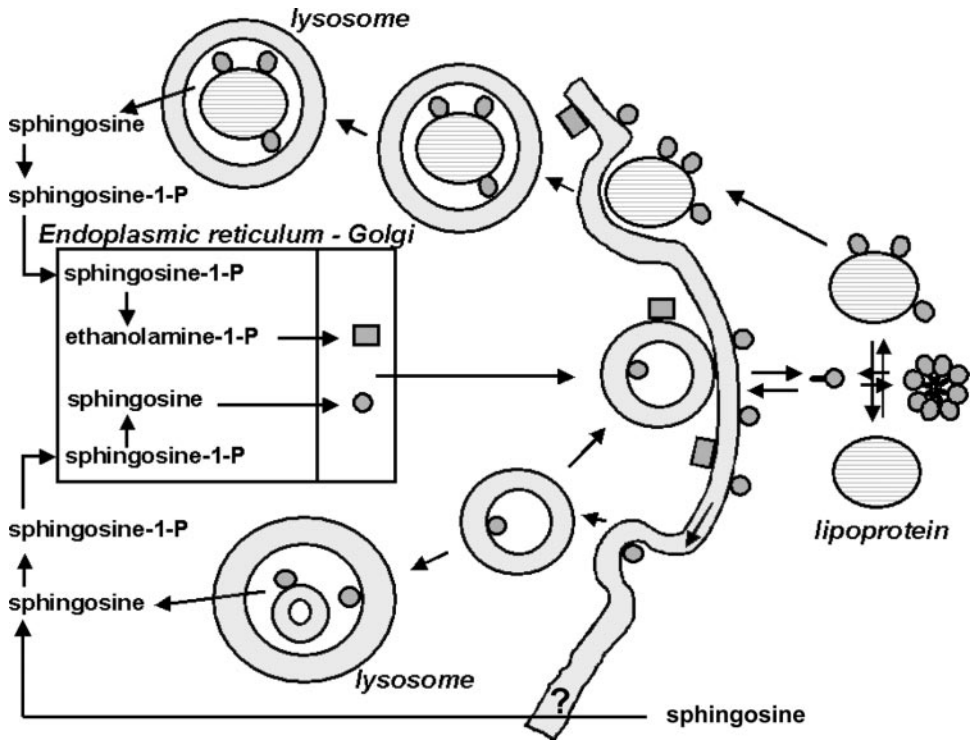

FIG. 7. **Scheme of the metabolic processing of sphingosine exogenously administered to cells in culture.** *Square*, phosphatidylethanolamine; *circle*, sphingolipids. The model shows the hypothetic trafficking comprising the early lysosomes. In the conventional model, the sphingolipids of the outer layer of plasma membranes become components of the endosomal concave inner membrane layer. The membrane in part forms intralysosomal small vesicles with the membrane enriched of bis(monoacylglycero)phosphoric acid. In the second model, during endocytosis large aggregates containing sphingolipids are trapped into the early endosomes so that sphingolipids face the lysosol, remaining inserted into a convex membrane layer. In both cases, sphingosine is formed. Exogenous sphingosine or cell sphingolipid catabolic sphingosine reaches the endoplasmic reticulum where they are phosphorylated and dephosphorylated to enter in the sphingolipid biosynthetic pathway. Sphingosine deriving from the catabolism of the extracellular complex of sphingolipid with lipoproteins/proteins is phosphorylated in the cytosol but is not dephosphorylated, thus being substrate for the lyase. In this case ethanolamine 1-phosphate is formed and recycled for the biosynthesis of phosphatidylethanolamine.

released by the cells followed a parallel opposite trend. At every checked day, a minor part of cell radioactivity comprised, for radioactive PE, about 12% of cell radioactivity and a very minor quantity for tritiated proteins. Finally, in the medium we found a very minor quantity of tritiated water. All this suggests that the total catabolism of sphingolipids is a minor process and that the majority of catabolic sphingosine is recycled; this is in agreement with previous data (38), suggesting that the sphingoid base salvage could represent the predominant pathway of complex sphingolipid biosynthesis. In addition to this, the radioactivity associated with PE, in cells metabolically labeled with [1-^{3}H]sphingosine, was quantitatively very similar, in

percentage, to that associated with PE derived from catabolism of exogenous sphingolipids taken up by the cells incubated with conditioned medium. This suggests that the larger part of PE is derived by the catabolism of radioactive sphingolipids released and then taken up by the cells and not by catabolism of endogenous cell sphingolipids.

Our observations on the efflux of sphingolipids from cells in culture and on the cell catabolism of these sphingolipids when reentering into the cells present a question. Exogenous sphingosine is taken up by the cells and is largely utilized for the biosynthesis of complex sphingolipids (10, 13, 17, 43). Sphingolipids have a turnover of hours in neuronal cells (34) and a few days in human fibroblasts (10, 44). Thus, after administration of sphingosine to the cells and metabolic labeling of all sphingolipids, in a long chase experiment, sphingosine is produced in the sphingolipid lysosomal catabolism, released from lysosomes, recycled, and only in minor part catabolized to small fragments and water. Nevertheless, sphingolipids that enter the cells as lipoproteic complexes are also catabolized to sphingosine in the lysosomes, but the sphingosine derived from this process is largely catabolized, rather than being recycled. To explain this, an hypothesis could be that presented in Fig. 7. The endogenous sphingolipids reach lysosomes as components of the plasma membrane (45), and in lysosomes result in part as the component of the inner layer of lysosomal membrane and in part as component of the external layer of intralysosomal vesicles. Exogenous sphingolipids that enter the plasma membrane as monomers, becoming indistinguishable from the membrane endogenous components, follow a similar fate. But large lipoproteic aggregates containing sphingolipids are trapped by the plasma membrane by endocytosis, thus being present in lysosomes only as big intralysosomal structures, resulting as differently oriented. The existence of intralysosomal structures (46) containing the extracellularly derived biotinylated GM1 ganglioside has been described by electron microscopy immunocytochemistry. Thus, the fate of the catabolic sphingosine could be dependent on a different organization of sphingolipids or on specific interactions occurring between sphingolipids and proteins, inserted into a negative membrane, the inner layer of the lysosomal membrane, or into a positive membrane, the surface of the vesicles in the lysosol. Sphingosine is formed from endogenous sphingolipids as well as from exogenous aggregates containing sphingolipids in the lysosomes, and then it is released into the cytosol. There is the notion that, when sphingosine is phosphorylated in the cytosol (47–49), it subsequently reaches the endoplasmic reticulum, and at the cytosolic side it is transformed into ethanolamine phosphate by a lyase (50) or it is dephosphorylated by a specific phosphatase (23, 51, 52), and then enters into the biosynthetic pathway of complex sphingolipids. Thus, the same molecule, sphingosine 1-phosphate anchored to the cytoplasmic face of the endoplasmic reticulum, is a potential substrate of both the phosphatase and the lyase. From this, it follows that the topology of the two enzymes should be different. An hypothesis could be that a specific organization of sphingolipids in the lysosomes can determine the formation of the specific sphingosine-protein complex that allows us to send sphingosine 1-phosphate to different areas of the endoplasmic membrane, and then being a substrate of the lyase or the phosphatase.

REFERENCES

1. Formisano, S., Johnson, M. L., Salvatore, G. L., Aloj, M., and Edelhoch, H. (1979) *Biochemistry* **18,** 1119–1124
2. Sonnino, S., Cantù, L., Corti, M., Acquotti, D., and Venerando, B. (1994) *Chem. Phys. Lipids* **71,** 21–45
3. Tomasi, M., Roda, L. G., Ausiello, C., D'Agnolo, G., Venerando, B., Ghidoni, R., Sonnino, S., and Tettamanti, G. (1980) *Eur. J. Biochem.* **111,** 315–324
4. Venerando, B., Roberti, S., Sonnino, S., Fiorilli, A., and Tettamanti, G. (1982) *Biochim. Biophys. Acta* **692,** 18–26
5. Valentino, L. A., and Ladisch, S. (1992) *Cancer Res.* **52,** 810–814
6. Rueda, R., Tabsh, K., and Ladisch, S. (1993) *FEBS Lett.* **328,** 13–16
7. Cotterchio, M., and Seyfried, T. N. (1994) *J. Lipid Res.* **35,** 10–14
8. Valentino, L. A., and Ladish, S. (1994) *Blood* **83,** 2872–2877
9. Saqr, H. E., Pearl, D. K., and Yates, A. J. (1993) *J. Neurochem.* **61,** 395–411
10. Chigorno, V., Riva, C., Valsecchi, M., Nicolini, M., Brocca, P., and Sonnino, S. (1997) *Eur. J. Biochem.* **250,** 661–669
11. Chigorno, V., Pitto, M., Cardace, G., Acquotti, D., Kirschner, G., Sonnino, S., Ghidoni, R., and Tettamanti, G. (1985) *Glycoconj. J.* **2,** 279–291
12. Giglioni, A., Chigorno, V., Pitto, M., Valsecchi, M., Palestini, P., and Ghidoni, R. (1990) *Chem. Phys. Lipids* **55,** 207–213
13. Riboni, L., Prinetti, A., Bassi, R., and Tettamanti, G. (1994) *FEBS Lett.* **352,** 323–326
14. Carter, H. E., Rothfus, J. A., and Gigg, R. (1961) *J. Lipid Res.* **2,** 228–234
15. Toyokuni, T., Nisar, M., Dean, B., and Hakomori, S. (1991) *J. Label. Compd. Radiopharm.* **29,** 567–574
16. Sonnino, S., Chigorno, V., and Tettamanti, G. (2000) *Method Enzymol.* **311,** 639–656
17. Prinetti, A., Chigorno, V., Tettamanti, G., and Sonnino, S. (2000) *J. Biol. Chem.* **275,** 11658–11665
18. Chigorno, V., Palestini, P., Sciannamblo, M. T., Dolo, V., Pavan, A., Tettamanti, G., and Sonnino, S. (2000) *Eur. J. Biochem.* **267,** 4187–4197
19. Leroy, J. G., Ho, W. M., McBrinn, M. C., Zielke, K., Jacob, J., and O'Brien, J. S. (1972) *Pediatr. Res.* **6,** 752–757
20. Gallo, V., Ciotti, M., Coletti, A., Aloisi, F., and Levi, G. (1982) *Proc. Natl. Acad. Sci. U. S. A.* **79,** 7919–7923
21. Kong, Y., Li, R., and Ladisch, S. (1998) *Biochim. Biophys. Acta* **1394,** 43–56
22. Havel, R., Eder, H., and Bragdon, J. (1955) *J. Clin. Investig.* **34,** 1345–1353
23. Mandala, S. M., Thornton, R., Tu, Z., Kurtz, M. B., Nickels, J., Broach, J., Menzeleev, R., and Spiegel, S. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 150–155
24. Mraz, W., Schwarzmann, G., Sattler, J., Momoi, T., Seemann, B., and Wiegandt, H. (1980) *Hoppe-Seyler's Z. Physiol. Chem.* **361,** 177–185
25. Riboni, L., Bassi, R., Sonnino, S., and Tettamanti, G. (1992) *FEBS Lett.* **300,** 188–192
26. Taki, T., Handa, S., and Ishiwaka, D. (1994) *Anal. Biochem.* **223,** 232–238
27. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275
28. Nakamura, O., Iwamori, M., Matsutani, M., and Takakura, K. (1991) *Acta Neurochir.* **109,** 34–36
29. Li, R., Gage, D., and Ladisch, S. (1993) *Biochim. Biophys. Acta* **1170,** 283–290
30. Olshefski, R., and Ladisch, S. (1996) *FEBS Lett.* **386,** 11–14
31. Chang, F., Li, R., and Ladisch, S. (1997) *Exp. Cell Res.* **234,** 341–346
32. Harpin, M. L., Boutry, J.-M., Hauw, J. J., Baumann, N., Ben Younes-Chennoufi, A., Goujet-Zalc, C., and Yavin, E. (1990) *In Vitro Cell. Dev. Biol.* **26,** 217–219
33. Gillard, B. K., Clement, R., Colucci-Guyon, E., Babinet, C., Schwarzmann, G., Taki, T., Kasama, T., and Marcus, D. M (1998) *Exp. Cell Res.* **242,** 561–572
34. Riboni, L., Bassi, R., Prinetti, A., and Tettamanti, G. (1996) *FEBS Lett.* **391,** 336–340
35. Riboni, L., Bassi, R., Conti, M., and Tettamanti, G. (1993) *FEBS Lett.* **322,** 257–260
36. Schwarzmann, G. (2001) *Cell. Dev. Biol.* **12,** 163–171
37. Riboni, L., Prinetti, A., Pitto, M., and Tettamanti, G. (1990) *Neurochem. Res.* **15,** 1175–1183
38. Gillard, B. K., Clement, R. G., and Marcus, D. M. (1998) *Glycobiology* **8,** 885–890
39. Trinchera, M., Ghidoni, R., Sonnino, S., and Tettamanti, G. (1990) *Biochem. J.* **270,** 815–820
40. Schwarzmann, G., and Sandhoff, K. (1990) *Biochemistry* **29,** 10865–10871
41. Hoekstra, D., and Kok, J. W. (1992) *Biochim. Biophys. Acta* **1113,** 277–294
42. Ziulkoski, A. L., *et al*. (2001) *Biochem. Biophys. Res. Commun.* **281,** 971–975
43. Van Echten, G., Iber, H., Stotz, A., Takatsuki, A., and Sandhoff, K. (1990) *Eur. J. Cell Biol.* **51,** 135–139
44. Riboni, L., Bassi, R., Prinetti, A., Viani, P., and Tettamanti, G. (1999) *Biochem. J.* **338,** 147–151
45. Huwiler, A., Kolter, T., Pfeilschifter, J., and Sandhoff, K. (2000) *Biochim. Biophys. Acta* **1485,** 63–99
46. Mobius, W., Herzog, V., Sandhoff, K., and Schwarzmann, G. (1999) *J. Histochem. Cytochem.* **47,** 1005–1014
47. Stoffel, W., Assman, G., and Birczek, E. (1970) *Hoppe-Seyler's Z. Physiol. Chem.* **351,** 635–642
48. Louie, D. D., Kisic, A., and Schroepfer, G. I. (1976) *J. Biol. Chem.* **251,** 4557–4564
49. Olivera, A., and Spiegel, S. (1993) *Nature* **365,** 557–559
50. Van Veldhoven P. P., and Mannaerts, G. P. (1991) *J. Biol. Chem.* **266,** 12502–12507
51. Funato, K., Lombardi, R., Vallée, B., and Riezman, H. (2003) *J. Biol. Chem.* **278,** 7325–7334
52. Mao, C., Wadleigh, M., Jenkins, G. M., Hannun, Y. A., and Obeid, L. M. (1997) *J. Biol. Chem.* **272,** 28690–28694
53. Svennerholm, L. (1980) *Adv. Exp. Biol. Med.* **125,** 11
54. IUPAC-IUBMB Joint Commission on Biochemical Nomenclature (1997) *Pure Appl. Chem.* **69**: 2475–2487
55. IUPAC-IUBMB Joint Commission on Biochemical Nomenclature (1998) *Carbohydr. Res.* **312,** 167–175

Sphingolipid Uptake by Cultured Cells: COMPLEX AGGREGATES OF CELL SPHINGOLIPIDS WITH SERUM PROTEINS AND LIPOPROTEINS ARE RAPIDLY CATABOLIZED

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