Formation of tritium-labeled polysialylated gangliosides in the cytosol of rat cerebellar granule cells in culture following administration of [^{3}H]GM1 ganglioside*

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GM1 ganglioside tritium-labeled at C-3 of sphingosine has been administered to rat cerebellar granule cells. Tritiated polysialylated gangliosides were observed in the cytosol of the cells, where they resulted in a higher amount after a short period of chase. This, together with the data showing an increase of the tritiated polysialylated gangliosides in the total particulate fraction in parallel to the prolonging of the chase period, suggests that cytosolic gangliosides could be a way of transporting neosynthesized gangliosides from the Golgi apparatus to the plasma membranes.

Ganglioside; Cytosol; Biosynthesis; Transport

1. INTRODUCTION

Gangliosides are normal components of the vertebrate cells and are particularly abundant in the neurons [1]. Although the main site of ganglioside deposition is the plasma membrane, small amounts of gangliosides occur as soluble complexes with proteins in the brain of different animals as well as in other tissues and cells in culture [2-8].

In experiments where radioactive galactose [5] or *N*-acetylmannosamine [6] were used as precursors for ganglioside biosynthesis, the specific radioactivity was 2-3 times higher for cytosolic than for membrane-bound gangliosides particularly at the initial stages of precursor involvement. This suggested the hypothesis that cytosolic gangliosides are involved with the intracellular traffic of gangliosides linked to their metabolic processing.

Rat cerebellar granule cells were shown [9] to take up administered gangliosides. In the present work we fed cerebellar granule cells in culture with GM1 ganglioside tritium-labeled at C-3 of long chain bases and observed the occurrence of tritiated polysialylated gangliosides in the cell cytosol.

2. MATERIALS AND METHODS

2.1. *Reagents*

Commercial chemicals were the purest available; common solvents were distilled and water was doubly distilled in a glass apparatus. En^{3}Hance spray was from New England Nuclear. X-Omat SO-282 films were from Kodak. All the solutions for cell cultures and washing were from Flow Laboratories. GM1 ganglioside was extracted from calf brain [10], purified over 99% and characterized [11]. GM1 ganglioside was tritium-labeled at position 3 of long chain bases according to the dichlorodicyanobenzoquinone/[^{3}H]sodium borohydride procedure [12]. The used molecular species that contained C-18 sphingosine with the *erythro* configuration was prepared by reversed phase HPLC purification [13]. Standards GM3, GM2, GD1a, GD1b, GT1b were prepared from calf brain and *O*-Ac-GT1b from rat brain [11].

2.2. *Cell cultures and cell culture treatments*

Granule cells, obtained from the cerebellum of 8-day-old rats, were prepared and cultured as already described [14]. Treatment with radiolabeled ganglioside was carried out on day 7 in culture. To this purpose, dishes were washed twice with prewarmed supplemented basal modified Eagle's medium without fetal calf serum (FCS) and then incubated in the same medium (2 ml/dish) containing 15×10^6 dpm of tritiated ganglioside. After 15 h, the medium containing the radioactive material was discarded, and the cells were washed for 30 min with supplemented basal modified Eagle's medium containing 10% FCS to eliminate the pericellular, loosely bound micelles of gangliosides. During the 15-h incubation in the absence of FCS no visible alterations in cell morphology were observed. Chase was done with 5 ml of 10% FCS-containing medium for 4 or 24 h. At the end the medium was carefully removed, and the cells were rinsed twice with 0.32 M sucrose containing 0.1 mM bisodium EDTA and 1 mM potassium phosphate buffer, pH 7, scraped off the plates, and centrifuged. Pelleted cells were homogenized at 4°C by gentle 50-fold up-and-down pipetting in 200 μl buffered 0.32 M sucrose solution, and the suspension was centrifuged at 105 000 × *g* for 1 h at 4°C. The upper third of the supernatant (cytosolic fraction) was accurately siphoned, the remaining supernatant being disregarded. This precaution was adopted in order to avoid any inadvertent withdrawal of particulated fines floating above the pellet. The collected supernatant was dialysed, frozen, and lyophilized. The 105 000 × *g* pellet (total particulate fraction) and the lyophilized supernatant were suspended (0.1 mg protein/ml) in 0.01 N potassium phosphate buffer, pH 7, and gangliosides were extracted and purified from the other lipids [10].

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*Ganglioside and glycosphingolipids nomenclature is according to Svennerholm (1980) [17]

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Table I

Distribution of cell radioactive gangliosides, after 15 h pulse-administration of tritiated long chain bases containing GM1 to rat cerebellar granule cells in culture

Cell fraction	Chase (hr)	GT1b	O-Ac-GT1b	GD1b	GD1a	GM1	GM2	GM3
		Percent						
cytosol	4	tr[a]	0.65	0.76	0.90	90.49	5.60	1.60
cytosol	24	-	0.33	0.51	0.75	91.25	5.55	1.61
particulate	4	0.45	0.40	0.35	0.77	83.45	10.33	4.25
particulate	24	1.11	1.02	0.80	1.43	81.10	10.31	3.75
		dpm × 10^{-3}/mg protein						
cytosol	4	tr[a]	1.80	2.11	2.50	277.80	15.56	4.45
cytosol	24	-	0.67	1.03	1.51	201.70	11.19	3.25
particulate	4	3.56	3.17	2.27	6.10	792.20	81.81	33.66
particulate	24	8.00	7.35	6.35	10.31	721.10	74.33	27.04

Data are means of 4 experiments; SD for GM1 value <3%, for the other gangliosides ±10%. [a]tr = traces

2.3. *Analytical procedures*

Total ganglioside radioactivity was determined by liquid scintillation counting.

Gangliosides were separated by TLC using 10 cm long HPTLC plates developed at room temperature, the solvent system chloroform/methanol/0.2% aqueous $CaCl_2$ (50:42:11 v/v), reaching the top edge of the plate. Gangliosides were identified by chromatographic comparison with standard gangliosides. Radioactive spots were quantified by radiochromatographic scanning of the plate using a Berthold TLC linear analyzer LB282 equipped with an Apple II data system. Protein content was determined in solubilized pellet and cytosolic fraction [15,16], bovine serum albumin being used as the reference standard.

3. RESULTS AND DISCUSSION

After 15 h pulse and 4 h chase, 95–96% of the total ganglioside radioactivity was in the total particulate fraction and 4–5% in the cytosol. Prolonging the chase up to 24 h, the soluble radioactivity remained constant, this reflecting the long period of pulse. Six gangliosides different from GM1 were found in radioactive form and identified, in both total particulate fraction and cytosol: GM3, GM2, GD1a, GD1b, GT1b and *O*-Ac-GT1b. The ganglioside distribution, in the particulate and cytosol fractions at two used periods of chase, is reported in Table I.

GM3 and GM2 are present at trace level in the granule cells [9] and their high percent value is mainly representative of the GM1 catabolic processing, and their presence in the cytosol is probably due to a partial lysis of the lysosomes wih release of its components.

GD1a, GD1b, GT1b and *O*-Ac-GT1b derive by biosynthetic reactions. The amount of gangliosides GD1a, GD1b, GT1b and *O*-Ac-GT1b prolonging the chase, increased in the total particulate fraction, this in agreement with previous data [9], and decreased in the cytosol. These data would indicate a flow of soluble gangliosides, that according to previous results [3,8] should be a flow of ganglioside/protein complexes, from the biosynthetic compartment to the plasma membrane through the cell cytosol. This flow is high when the availability of radioactive substrates for ganglioside biosynthesis is high, that is when the amount of exogenously administered ganglioside is high such as at a short time of chase after a long pulse. Far from the pulse, the administered ganglioside is to a large extent catabolized, the amount of intermediate for ganglioside biosynthesis reduced and in parallel the amount of soluble polysialylated gangliosides is decreased. As a consequence of these events prolonging the chase time the amount of neosynthesized gangliosides increases at the membrane level. According to the above interpretation, cytosolic gangliosides would be representative, at least in part, of a way to transport neosynthesized gangliosides from the Golgi apparatus to the plasma membranes.

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