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Endogenous Glycosphingolipid Acceptor Specificity of Sialosyltransferase Systems in Intact Golgi Membranes, Synaptosomes, and Synaptic Plasma Membranes from Rat Brain†

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ABSTRACT: Preparations highly enriched in Golgi complex membranes, synaptosomes, and synaptic plasma membranes (SPM) by marker enzyme analysis and electron microscopic morphology were made from the brains of 28-day-old rats. These were incubated with cytidine 5′-monophosphate–*N*-acetyl[^{14}C]neuraminic acid (CMP–NeuAc) in a physiologic buffer, without detergents. Glycolipid sialosyltransferase activities (SATs) were measured by analyzing incorporation of radiolabeled NeuAc into endogenous membrane gangliosides. Golgi SAT was diversified in producing all the various molecular species of labeled gangliosides [2.64 pmol of NeuAc transferred (mg of protein)$^{-1}$ h^{-1}]. Synaptosomal SAT exhibited a lower activity [0.66 pmol (mg of protein)$^{-1}$ h^{-1}], but it was highly specific in its labeling pattern, with a marked preference for labeling NeuAcα2→8NeuAcα2→3Galβ1→4Glcβ1→1Cer (GD3 ganglioside). SPM prepared from the synaptosomes retained the GD3-related SAT (or SAT-2), and the total specific activity increased [1.41 pmol (mg of protein)$^{-1}$ h^{-1}], which suggests that the location of the synaptosomal activity is in the SPM. These results indicate that SAT activity in Golgi membranes differs from that in synaptosomes with regard to endogenous acceptor substrate specificity and SAT activity of synaptosomes should be located in the synaptosomal plasma membrane. This SAT could function as an ectoenzyme in concert with ecto-sialidase to modulate the GD3 and other ganglioside population in situ at the SPM of the central nervous system.

Gangliosides are sialic acid containing glycosphingolipid membrane components that exist in high amounts in brain (Brunngraber, 1979; Leskawa & Rosenberg, 1981). With their strategic location in synaptic plasma membrane (SPM), it has been popular to envision ganglioside involvement in synaptic events such as neurotransmitter release and dispersal (Rahman, 1976; Svennerholm, 1980), receptor function (Tamir et al., 1980; Fishman, 1982), Ca^{2+} flux control (Veh & Sander, 1981), and involvement in neurotrophic–neuritogenic events in neuronal development [reviewed by Ledeen (1984)]. However, it is not yet possible to clearly identify even one event in which gangliosides function unequivocally (Wiegandt, 1985).

An SPM ecto-sialidase activity has been described which splits sialic acid residues from gangliosides (Schengrund & Rosenberg, 1970; Tettamanti et al., 1972) and, more recently, an SPM ecto-sialosyltransferase (ecto-SAT) that transfers sialic acid residues to endogenous membrane gangliosides (Den et al., 1975). Through the concerted action of these two enzymes, the ganglioside characteristics of the SPM could be varied or controlled (Schengrund & Nelson, 1975).

Sialosyltransferases, along with other glycosyltransferases, traditionally have been reported as being Golgi apparatus enzymes involved in the de novo biosynthesis of gangliosides and glycoproteins. Some researchers have attributed the measured SAT activity in synaptosomes to artifact due to Golgi contamination (Ng & Dain, 1977). This is difficult to resolve because differentially subfractionated membrane preparations of brain homogenates may never be acceptably homogeneous.

In the present study, we have examined differences between the behavior of Golgi SAT and putative SPM ecto-SAT. Such differences would lend support to the existence of a separate SPM ecto-SAT activity and would lend credibility to a theoretical ganglioside desialosylation–resialosylation system in situ at the synapse.

Materials and Methods

Reagents were of the highest purity available from biochemical suppliers, and solutions were prepared with distilled–deionized water redistilled in a glass apparatus. All homogenization and centrifugation steps in the preparation of the brain subfractions were carried out at 0–4 °C.

Subfractionation of Rat Brain. After decapitation, the forebrains of 28-day-old Sprague-Dawley rat pups were removed and placed in 10 volumes of ice-cold 0.32 M sucrose [1.0 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl)–0.1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.2] and were then homogenized and fractionated by

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differential centrifugation according to the method of Gray and Whittaker (1962), to prepare P2 (the mitochondrial pellet containing crude synaptosomes) and P3 (the microsomal pellet). These fractions were further subfractionated by discontinuous density gradient centrifugation, as follows. The P3 fraction (microsomes) was subfractionated on a discontinuous sucrose density gradient, a modification of the continuous density gradient of Siegrist et al. (1979). The layers were 1 mM Tris-HCl in 0.1 mM EDTA, pH 7.2, containing 0.6, 0.8, 0.9, 1.0, 1.1, and 1.2 M sucrose from top to bottom. The P3 fraction suspended in 0.32 M sucrose was layered on the top of the gradients and centrifuged in the SW28 rotor in a Beckman L-8 ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA) at 27 000 rpm (10000*g*) for 2 h. The bands were harvested and washed in 4 volumes of phosphate-buffered saline (PBS) and recentrifuged in the same rotor at the same speed for 30 min. Part of these washed subfractions were used immediately in the enzyme assays below; the rest were stored at −70 °C.

The P2 fraction was subfractionated on an expanded discontinuous ficoll density gradient by a modification of the method of Preti et al. (1981). The gradients had six layers, which were 5%, 7%, 9%, 11%, 13%, and 16% ficoll (g/100 mL of 0.32 M sucrose) from top to bottom. The gradients were centrifuged and the bands harvested and washed as described for P3 subfractionation.

Synaptic plasma membranes (SPM) were prepared and subfractionated as described by Cotman and Mathews (1971). The washed synaptosomal preparation (P2b) was suspended in 30 mL/g (of starting brain material) of hypoosmotic (lysing) solution (0.1 mM Tris-HCl, pH 8.0) for 1 h at 4 °C. This suspension was centrifuged at 17000*g* for 15 min. The supernatant containing the lysed synaptosomes was centrifuged at 30000 rpm (100000*g*) in a Beckman SW41 swing-out rotor for 30 min. The pellet was resuspended in lysing solution and was layered on top of the discontinuous sucrose density gradient. The layers, from top to bottom, were 0.4, 0.6, 0.8, 1.0, 1.1, and 1.2 M sucrose and 50% sucrose. The tubes were centrifuged in the Beckman SW41 rotor at 30000 rpm (100000*g*) for 2 h, and the bands were harvested and washed as described before. No bands could be seen at the top two gradient interfaces.

Aliquots from each of the subfractions were removed for electron microscopic examination (Siegrist et al., 1979) and for protein determination (Hess et al., 1978).

Sialosyltransferase Incubation. The washed membrane subfractions were resuspended in ice-cold Krebs–Henseleit buffer (117 mM NaCl, 4.7 mM $MgSO_4$, 1.9 mM $CaCl_2$, 1.17 mM NaH_2PO_4, 25 mM $NaHCO_3$, and 11.1 mM D-glucose, pH 7.4) in a ratio of 1 mL/g of fresh brain. The Krebs buffer had been saturated with 95% O_2–5% CO_2 by bubbling on ice for several hours to oxygenate and establish the bicarbonate buffering system of the solution. These conditions were those used by Pastuszko et al. (1982) for metabolically active synaptosomal incubation, which is above the reported pH optimum for ganglioside SATs (pH 6.5; Basu et al., 1984), but the intent was to keep conditions as physiologic as possible. The suspensions were divided into 2-mL samples, including heat-denatured control samples (100 °C for 10 min). One-tenth microcurie of cytidine 5′-monophosphate–*N*-acetyl-[^{14}C]neuraminic acid (CMP–NeuAc) from Amersham International, Amersham, U.K. (247 mCi/mmol), was added to each sample. Each vial was sealed, and 95% O_2–5% CO_2 was forced into the vials. Samples were incubated at 37 °C. The reactions were stopped by quick-freezing followed by lyophilization. One milliliter of chloroform–methanol solution (C/M 2:1 v/v) was added to the residues, and the samples were kept at −20 °C.

Ganglioside Extraction and Purification. The procedure used was that of Irwin and Irwin (1979) as modified by Saito and Rosenberg (1982). This included chloroform–methanol extraction of total lipids followed by separation of gangliosides from neutral lipids and neutral glycosphingolipids by activated silica column chromatography. After alkaline hydrolysis of phospholipid contaminants and dialysis, the lyophilized gangliosides were separated into individual species by silica gel high-performance thin-layer chromatography (HPTLC) and were quantitated by densitometric analysis (Saito & Rosenberg, 1982). Radioactivity was located on the plates by scraping sequential 1-mm segments of the silica gel from each lane into scintillation vials and counting (Saito & Rosenberg, 1982). Total lipid-bound NeuAc was determined by the method of Svennerholm (1957).

Marker Enzyme Analysis. Aliquots of the membrane subfractions that had been kept at −70 °C were used to perform the following enzyme assays: 5′-nucleotidase (5′NT) was assayed according to the method of Rome et al. (1979) as a general plasma membrane marker; cerebroside sulfotransferase (CST) was assayed by the method of Siegrist et al. (1977) which served as Golgi membrane marker; choline acetyltransferase (ChAT) as a synaptosomal marker was assayed by the method of Fonnum (1975); acetylcholinesterase (AChE) is enriched in neuronal plasma membrane, synaptic plasma membrane, and also microsomal membranes, and this marker was assayed by the method of Ellman et al. (196]). To evaluate enrichment of these markers during subfractionation, the activities were expressed as percent of activity in the starting crude brain homogenate.

Results

Membrane Subfractions. The marker enzyme enrichment profiles of the subfractions produced from the three preparations (P3, P2, and lysed synaptosomes) are shown in Figure 1. From these, we selected subfraction M-E (1.0–1.1 M sucrose interface) as being highly enriched in Golgi complex, the P2f subfraction (11–13% ficoll interface) as most enriched in synaptosomal markers, and SPMe subfraction (1.0–1.1 M sucrose interface) as the most enriched in synaptic plasma membranes. The electron microscopic comparison of these three subfractions is shown in Figure 2, and morphological analysis of these micrographs supports the same conclusions that were reached above as to the identity of each sample. It should be noted that these micrographs are not prepared in the quality to give ultrastructural details of these subfractions. This was not the purpose of these micrographs. The purpose was to detect Golgi contamination in the synaptosomal and SPM subfractions; therefore, all subfractions were fixed overnight in osmium tetraoxide (Siegrist et al., 1979) which makes the Golgi appear dark and distinct.

Table I compares the biochemical data of the three subfractions. The SAT experiments were carried out on all subfractions, but to simplify discussion, only data for the Golgi, the synaptosomal, and the SPM-enriched subfractions are given below.

Ganglioside Analysis. The distribution of the amounts of the individual species was determined for the endogenous gangliosides extracted from each subfraction, via densitometric integration of HPTLC plates (Figure 3). The data are given for Golgi membranes, synaptosomes, and synaptic plasma membranes in Table II. In general, the distribution profiles are similar, especially for synaptosomes and SPM. The Golgi

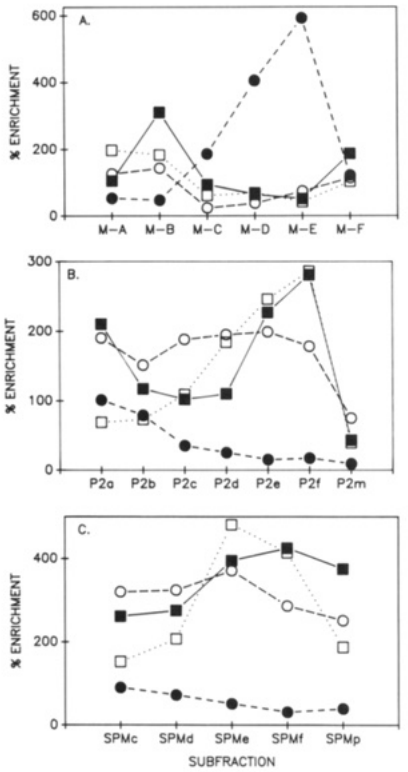

FIGURE 1: Marker enzyme enrichment profiles of the membrane subfractions produced by density gradient centrifugation of rat brain homogenate. The activities are represented as a mean percent of the activity in crude brain homogenate calculated from four experiments: (■) 5′-nucleotidase; (□) acetylcholinesterase; (○) choline acetyltransferase; (●) cerebroside sulfotransferase. (A) Microsomal subfractions (P3); (B) synaptosomal subfractions (P2); (C) synaptic plasma membrane subfractions (lysed P2e).

Table I: Marker Enzyme Analysis[a]

	Golgi membrane	synaptosomes	synaptic plasma membranes
5′NT	0.14 (0.02)	1.54 (0.05)	3.46 (0.19)
ChAT	0.01 (0.01)	0.49 (0.04)	0.92 (0.17)
AChE	0.40 (0.07)	3.34 (0.45)	5.43 (0.22)
CST	7.17 (1.92)	0.17 (0.01)	0.08 (0.01)
STase	2.64 (0.22)	0.66 (0.12)	1.41 (0.32)
sp gang concn	94.21 (8.40)	52.51 (3.90)	112.01 (1.55)

[a] All subfractions were assayed, and the results of the Golgi membrane, synaptosomes, and SPM-enriched subfractions are given as the means of four experiments with the standard errors of the mean in parentheses. The units of each assay are the following: 5′-nucleotidase (5′NT), micromoles of AMP hydrolyzed per milligram of protein per hour; choline acetyltransferase (ChAT), micromoles of choline transferred per milligram of protein per minute; acetylcholinesterase (AChE) is in arbitrary units (see Materials and Methods); cerebroside sulfotransferase (CST), picomoles of sulfate transferred per milligram of protein per hour; total sialosyltransferase (STase), picomoles of NeuAc transferred per milligram of protein per hour; specific ganglioside concentration (sp Gang concn), nanomoles of lipid-bound NeuAc per milligram of protein.

membranes show some differences, which were mainly confined to GM4, GM3, GM2, and GD3, which were all increased in quantity in Golgi over the synapse-related membranes (Figure 3). GD1a may have been slightly decreased in Golgi membranes.

Total Sialosyltransferase Activity and Ganglioside Specificity. After SAT incubation of each subfraction, the endogenous gangliosides were extracted and counted, and also analyzed by thin-layer chromatography (TLC). The Golgi membranes were far richer in total glycolipid SAT activity than were synaptosomes and SPM, although the latter two

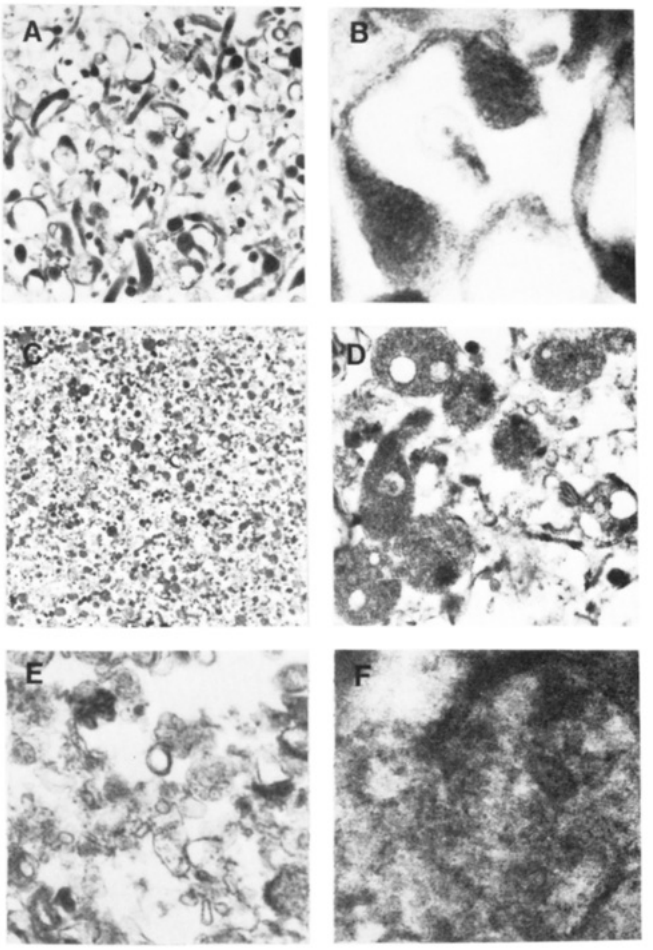

FIGURE 2: Electron micrographs of the membrane subfractions deemed by marker enzymes to be most enriched in Golgi complex [(A) 26400×; (B) 17050×; synaptosomes [(C) 3905×; (D) 42955×]; and SPM [(E) 10890×; (F) 60500×]. These preparations were substantially overfixed (overnight in osmium tetraoxide) in order to detect even trace amounts of Golgi in the synaptosomal and SPM samples.

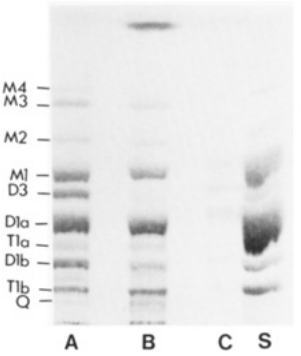

FIGURE 3: Ganglioside profiles of the subfractions of interest are shown on this HPTLC silica gel plate developed in chloroform–methanol–0.25% $CaCl_2$ (50:40:11) and stained by resorcinol spray. Lane A, gangliosides extracted from the Golgi-enriched subfraction (M-E). Lane B, synaptosomal-enriched subfraction (P2e). Lane C, SPM-enriched subfraction (SPMf) which is only faintly stained because of the low yield of SPM preparation. Lane S, bovine brain ganglioside standards.

were enriched over crude homogenate (Table I). SPM was enriched in total ganglioside SAT activity over the synaptosomes. However, when the synaptosomes were simply lysed in hypoosmotic medium and not subfractionated, there was no significant increase in SAT activity over intact synaptosomes (intact, 0.66 ± 0.12 SAT unit/mg of protein; lysed, 0.64 ± 0.20 SAT unit/mg of protein). These data suggest that synaptosomal SAT does have a cell-surface orientation.

Table II: Distribution of Gangliosides[a]

ganglioside species	Golgi membranes	synaptosomes	synaptic plasma membranes
GQ	7.20 (1.10)	7.02 (0.80)	5.65 (0.62)
GT1b	10.23 (1.97)	13.30 (2.30)	10.75 (3.23)
GD1b	6.33 (0.33)	11.98 (1.50)	10.83 (3.23)
GT1a	5.75 (0.59)	4.20 (1.00)	5.13 (1.45)
GD1a	14.28 (1.07)	32.32 (2.80)	27.39 (0.70)
GD3	12.16 (2.25)	1.78 (0.60)	1.50 (0.45)
GM1	18.80 (2.25)	15.05 (3.10)	12.60 (5.09)
GM2	12.53 (0.42)	2.42 (0.60)	6.75 (1.14)
GM3	13.13 (1.18)	4.18 (0.60)	11.50 (1.43)
GM4	7.18 (1.08)	0.63 (0.30)	0.80 (0.21)

[a] The gangliosides from Golgi membranes, intact synaptosomes, and synaptic plasma membranes were extracted and separated by HPTLC as described under Materials and Methods. The amount of each ganglioside is expressed as a percent of the total gangliosides which was determined by the method of Saito and Rosenberg (1982) utilizing densitometric analysis of the HPTLC plates after visualization of the bands with resorcinol.

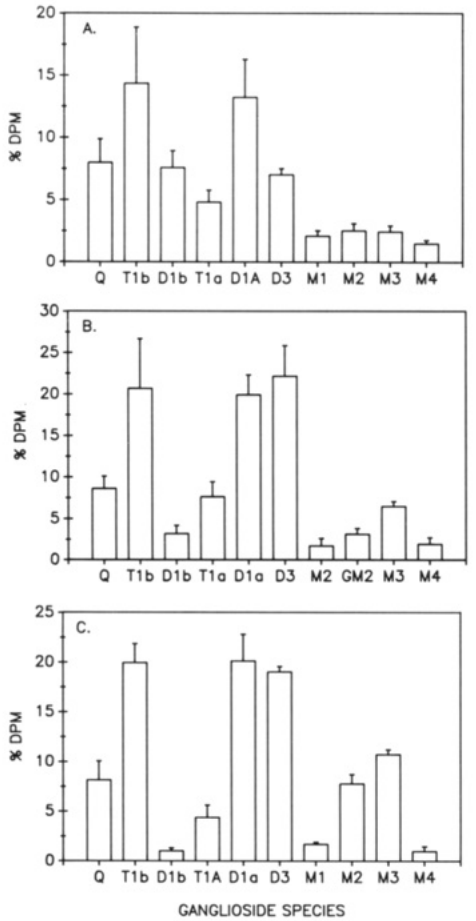

FIGURE 4: Percent distribution of radioactivity among the individual gangliosides as labeled by SAT. The values are the mean and standard error of four experiments. (A) Golgi-enriched subfraction; (B) synaptosomes; (C) synaptic plasma membranes.

The percent distribution of ganglioside species (Table II) and the percent distribution of radioactivity (Figure 4) for all the gangliosides species are given for the Golgi, the synaptosomal, and the SPM-enriched fractions. The calculated relative specific radioactivity (RSRA) for each ganglioside is shown in Figure 5. This is the quotient of percent radioactivity over the percent distribution, for each ganglioside species (Harzer et al., 1969). When the RSRA value is near unity for all the gangliosides in a given profile, the sialic acid residues were all labeled equally. Such was the case with the Golgi-

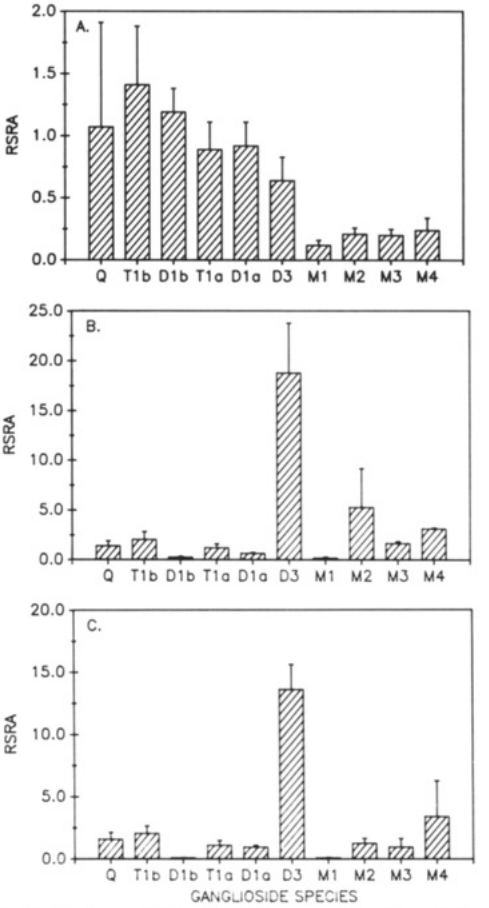

FIGURE 5: Product specificity of SAT expressed as the relative specific radioactivity (RSRA) for each ganglioside species. RSRA is calculated by percent distribution divided by percent ganglioside distribution, and the values are the mean and standard error of four experiments. (A) Golgi complex; (B) synaptosomes; (C) synaptic plasma membranes.

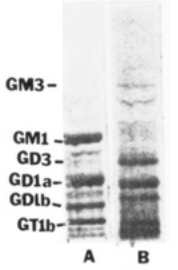

FIGURE 6: SAT-labeled synaptosomal gangliosides were separated by thin-layer chromatography as described under Materials and Methods. The developed plate was placed in a light-tight cassette with Kodak X-Omat AR autoradiographic film. After 3 weeks, the film was developed, and the TLC was sprayed with resorcinol reagent. Lane A is a photocopy of the ganglioside resorcinol pattern of one SAT assay, and lane B is the matching autoradiograph of the sample.

enriched fraction (Figure 5A). Each sialic residue on any given ganglioside was transferred by the Golgi SAT with the same net rate. The gangliosides with more than one sialic acid residue (di-, tri-, and tetrasialosyl) received proportionally more radiolabel than did the monosialosyl gangliosides in the Golgi membranes.

In the synaptosomal and the SPM subfractions, the RSRA patterns were similar to one another, but the values varied greatly from ganglioside to ganglioside, which is a major difference from Golgi SAT (Figure 5B,C). An RSRA value of greater than 1 indicates that the sialic acid residues were labeled more frequently on that ganglioside than those residues on a ganglioside with an RSRA value of less than 1. The

SATs of synaptosomes and SPM both show a high RSRA value for ganglioside GD3. This GD3 product specificity for ganglioside SAT was reported previously (Durrie et al., 1987) in metabolically active, intact synaptosomes, where the identity of GD3 was confirmed by several independent experimental procedures (i.e., chromatography in different solvent systems, analysis of the sialidase products, etc.). In Figure 6, a single lane of sialosyltransferase-labeled synaptosomal gangliosides is shown both as the resorcinol pattern and as the autoradiographic pattern. Here the highly radioactive GD3 band can be seen. This GD3-specific SAT fits the classification of Basu et al. (1987) as "SAT-2" which denotes the disialosyl linkage. Here we see that this GD3 specificity also persists in the synaptic plasma membrane. Because the total SAT activity of SPM was enriched over the synaptosomes from which they were derived, this GD3-specific SAT-2 labeling should be located in the SPM.

Discussion

It is generally believed that in de novo biosynthesis of glycosphingolipids the precursor lipids are membrane components that pass through the Golgi complex before they reach their final destination in the plasma membrane. Here most of the sugars in the oligosaccharide chains are added to ceramide (Landa et al., 1977a,b). It is thought that the glycosyltransferases required to synthesize a specific glycosphingolipid are bound together into multi-transferase complexes. The Golgi should contain many such complexes which would contain the sialosyltransferases needed to synthesize the various ganglioside species. Basu et al. (1984) have produced data which demonstrate that there are several ganglioside sialosyltransferases that exist in brain and each is specific for the glycosidic linkage being formed. Thus, the purpose of the Golgi SATs would be for the de novo biosynthesis of all types of gangliosides for use throughout the cell. The total SAT we measured in Golgi membranes was very active but showed no acceptor–product specificity for sialic acid transfer to individual gangliosides. This lack of specificity is not inconsistent with the function of the Golgi complex.

One observation worth noting was the high degree of radiolabel found in ganglioside GD1b by the Golgi-enriched subfraction. This may seem confusing because it has been shown in solubilized transferase systems in vitro that GD1b is not formed via sialosylation of GM1 but via addition of galactosamine and galactose to GD3 (Cumar et al., 1971; Keenan et al., 1974). In contrast, it has been demonstrated in intact microsomal transferase systems that GD1b can be formed by the direct sialosylation of GM1 (Caputto et al., 1974). Our data support the existence of the latter pathway in the Golgi.

We measured the endogenous activities of synaptosomal and SPM SATs and found them to be less active than Golgi-associated SATs. The notion that the observed SAT activity in synaptosomal and SPM preparations is artifactual, due to Golgi membrane contamination, is still considered by some and is not entirely obviated by the results of this experiment. On the other hand, we can calculate the percentage Golgi contamination in the synaptosomal preparation by using the specific activity of CST (Golgi marker), which we measured in our most purified Golgi subfraction, and then calculating the amount of Golgi that must be present in the synaptosomal subfraction to produce the CST activity measured there (Table I). The result is 2.4% contamination of the synaptosomal subfraction by Golgi membranes. By the same method, if we assume that the SAT activity in synaptosomal subfractions is due to Golgi, we calculate that the synaptosomes are 25% contaminated by Golgi; therefore, we must conclude that this level of SAT activity in synaptosomes could not be due to the 2.4% contamination of Golgi. Although this argument seems convincing, it should be noted that the enzyme–substrate saturation levels and other kinetic factors are unknown in this endogenous acceptor, membrane-bound assay system which could nullify the preceding calculations.

However, on a totally different line of reasoning, the acceptor–product specificity of synaptosomal SAT is highly pronounced under these assay conditions (physiologic buffer without detergents). This is clearly different than Golgi under identical conditions. The most preferred transfer involved the sialic acid residues that are found in ganglioside GD3. A putative SPM ecto-SAT probably would function to maintain or modify the in situ endogenous cell-surface gangliosides rather than being involved in the major cellular anabolic machinery for gangliosides. Therefore, the highly specific labeling pattern we see for synaptosomal and SPM SATs may reflect a specific biological function of individual gangliosides such as the minor GD3 fraction in the synaptic region. A previous report (Higashi et al., 1975) has described a transferase in crude synaptosomal preparations from stage-specific embryonic chick brain that sialosylates LM1 (NeuGcα2→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer) to produce LD1c by the formation of an α2→8disialosyl linkage and produces GD3 from GM3 by the same linkage. The SAT-2 we are describing on the surface of the synaptic membrane is very similar to this developmentally important transferase in chick brain.

It should be pointed out that sialidase coexists in SPM (Schengrund & Rosenberg, 1970) along with the above-described SAT and the endogenous gangliosides. It is possible that the specific ganglioside labeling we have noted in SPM is not due to SAT specificity but to sialidase action on the labeled products of SAT. If this were the case, the highly labeled GD3 in SPM would be resistant to sialidase hydrolysis. Such resistance has not been reported for GD3. Kuhn and Wiegandt (1964) have reported that specific hydrolysis of GD3 by mammalian brain sialidase exists at a level not unlike other gangliosides, but other reports involving GD3 are scarce. Also, the effects of sialidase should be minimized because the SAT assays were carried out at a pH range (7.2) that is far above the pH optimum of sialidase (Schengrund & Rosenberg, 1970), and lastly, if the GD3–SAT-2 labeling were due to sialidase action, then it should follow that upon extended incubation of the membranes the overall ganglioside pattern would also reflect the GD3 sialidase resistance. The ganglioside profiles did not change even at our longest incubation period (6 h, 37 °C), which also supports the notion that the GD3-specific labeling is not due to sialidase action.

The SAT activity we measured in SPM was increased in specific activity over the synaptosomal preparation from which the SPMs were isolated. This supports the idea that the SAT is located somewhere within the synapse. Since the gangliosides are on the outer membrane surface for the most part, we are inclined to believe that SAT is an ectoactivity in SPM.

In our continuing study of membrane-bound SATs of rat brain, we are analyzing SAT acceptor substrate specificities under simplified assay conditions similar to those reported to maximally activate, solubilize, and saturate the SAT enzyme [cacodylate buffer, pH 6.5, Triton CF-54 detergent, and exogenous acceptor substrates; reviewed by Basu et al. (1987)]. When we analyzed the labeled ganglioside products of these reactions by the same thin-layer chromatographic methods used in this report, we found that the effects of detergents and

exogenous acceptors on labeling specificity of brain SAT systems are extremely complex and beyond the scope of this report. However, to support our current findings, we report the following preliminary findings in qualitative terms. When exogenous lactosylceramide (LacCer) was included in SAT radiolabeling assays, rat brain synaptosomes showed a great increase in labeled GD3, while microsomes (crude source of Golgi SAT) produced large amounts of labeled GM3. When exogenous GM3 was included, synaptosomes produced high levels of labeled GD3 and some labeled GM3. Microsomes with exogenous GM3 produced large amounts of labeled GM3 (very little labeled GD3). The increased labeling of GM3 when GM3 was added exogenously is very confusing. Some possible explanations include LacCer contamination of the GM3 acceptor, a NeuAc "exchange" reaction of some sort, or a concerted action of a sialadase followed by SAT. At any rate, the "GD3 synthetase"–SAT-2 activity is much more prevalent in synaptosomes than in microsomes. When exogenous GM1 was added to these assays, both synaptosomes and microsomes produced labeled GD1a, but the microsomes were much more pronounced in producing GD1a and the higher gangliosides (tri- and tetrasialosyl) than were synaptosomes. This represents very preliminary data on a complex subject, which at this time is being analyzed for future comprehensive publication.

It has proven difficult to unequivocally establish specific functions for gangliosides on the surface of cells. Because of the complexities involved in their ubiquitous occurrence as plasma membrane components, experimental systems have not been devised that clearly define the function of gangliosides. The concept that individual gangliosides have very specific biological functions has slowly been gaining experimental support. For example, it has been noted that ganglioside GD3 is specifically enriched in cells that absorb great quantities of metabolites (Seyfried & Yu, 1985) and GD3 has specific gene-regulated expression on the surface of certain transformed cells (Nakakuma et al., 1984); ganglioside GQ1b has been reported to specifically induce differentiation in neuroblastoma cells and modulate a protein kinase (Tsuji et al., 1985); GM1 may potentiate the effects of nerve growth factor [reviewed by Ledeen (1984)]; it is recognized that individual gangliosides serve as specific receptors for bacterial endotoxins [reviewed by Fishman (1982)].

The findings in this study identify gross differences between the behavior of Golgi and synaptosomal SPM SATs when assayed under identical conditions. This supports the existence of a cell-surface SAT-2 in the neuron that could function in part to regulate the ganglioside population of the functional synapse in situ.

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