# Anabolic sialosylation of gangliosides in situ in rat brain cortical slices

**R. Durrie and A. Rosenberg**[1]

Alcohol Research Center, Neuropsychiatric Institute, University of California, Los Angeles, CA 90024, and Division of Molecular Biology and Neural Regeneration, Nathan S. Kline Institute for Psychiatric Research, Orangeburg, NY 10962

**Abstract** Radiolabeling of the sialic acid residues of gangliosides was examined in thin slices of rat brain cerebral cortex incubated under physiologic conditions in the presence of either [$^{14}$C]N-acetyl-mannosamine (ManNAc) or cytidine 5'-monophosphoryl-[$^{14}$C]N-acetyl-neuraminic acid (CMP-NeuAc). CMP-NeuAc is the direct donor substrate in the transfer of sialic acid to gangliosides by sialosyl transferases (SATs), including ectosialosyl transferases at the cell surface. ManNAc must be internalized by the neural cells (neuronal or glial) where it serves as an obligate precursor for the biosynthesis of the NeuAc moiety of intracellular CMP-NeuAc, via multiple reactions in the cytosol and nucleus. When exogenous [$^{14}$C]ManNAc was supplied, there appeared to be a 2-h lag period before label was incorporated measurably into ganglioside sialic acid. That was followed by rapid ganglioside labeling continuing up to 6 h. There was high incorporation into ganglioside GM1. Labeling by ManNAc was inhibited by monensin, a monovalent cationophore that blocks anabolic transport in medial and trans Golgi. Extracellular CMP-NeuAc was not internalized by the cells. CMP-[$^{14}$C]NeuAc labeling of gangliosides had no lag period, reached a maximum within 2 h, and then began to level. The label distribution among gangliosides was high in GD3, but quite low in GM1. CMP-NeuAc labeling was not inhibited by $10^{-7}$ M monensin. These findings support a model in which ManNAc labels gangliosides by an intracellular route involving monesin-sensitive, Golgi-associated SATs. In this intracellular system, the major labeled products are gangliosides of the gangliotetraosyl series (GM1, GD1a, etc.). CMP-NeuAc presented to brain slices served as substrate for another set of SATs that are Golgi-independent. These latter produced highly labeled GD3 and are similar to reported outer neuronal cell surface transferases that preferentially synthesize ($\alpha$2-8) disialosyl groups in the lactosyl ceramide series of gangliosides (GD3). —**Durrie, R., and A. Rosenberg.** Anabolic sialosylation of gangliosides in situ in rat brain cortical slices. *J. Lipid Res.* 1989. **30:** 1259–1266.

**Supplementary key words** cell surface • Golgi • rat brain • sialosyltransferases • sialic acid

Gangliosides, or sialoglycosphingolipids, are a subclass of the cell surface complex carbohydrate molecules that occur at high concentrations in the central and peripheral nervous system of animals (1). Gangliosides are localized in the outer molecular leaflet of the neuronal plasma membrane and are at highest concentrations at subcellular locations specialized for cell-cell interactions (2, 3).

Although much is known about ganglioside structure (4), the biological functions of gangliosides have proved difficult to demonstrate and remain speculative. Because changes in ganglioside levels, their subcellular distribution, and composition profile are correlated to nervous tissue development, putative ganglioside function has become most closely associated with the cyto-differentiation and growth of the embryonic neuron; reviewed by Hakomori (5) and Ledeen (6).

Glycosyltransferases catalyze the sequential addition of monosaccharides from nucleotide-activated donors to the growing oligosaccharide chains of gangliosides, as well as neutral glycolipids and glycoproteins (7). The traditional de novo biosynthetic pathway of neural cell gangliosides (8) consists of *i*) the endoplasmic reticulum, where ceramide is formed and the initial glycosyltransferases are located, followed by *ii*) the Golgi complex, where the bulk of the glycosyltransferases are located. Gangliosides are actively transported intracellularly as Golgi-derived membrane components which fuse to the plasma membrane, thus supplying newly synthesized gangliosides to the cell surface.

More recently glycosyltransferases, sialosyltransferases (SATs) in particular, have been reported to be located on the outer neuronal cell surface (ecto), specifically in the synaptic region (9–12), and in brain coated vesicles (13). SATs associated with distal plasma membrane compartments are hypothesized to function in concert with ecto-

Abbreviations: CMP-NeuAc, CMP-N-acetyl neuraminic acid; ManNAc, N-acetyl-mannosamine; SAT, sialosyl transferase; C-M, chloroform-methanol; HPTLC, high performance thin-layer chromatography.

[1]To whom reprint requests should be addressed at: Neuropsychiatric Institute, University of California School of Medicine, 760 Westwood Plaza, Low Angeles, CA 90024-1759.

sialidase to modulate the ganglioside sialosylation pattern in situ (9, 14).

Much of the evidence for the existence of ecto-SATs thus far has been derived from transferase activity studies in subcellular fractions. These experiments reinvestigate the cellular location and properties of putative ecto-SATs using a paradigm independent of subcellular fractionation, thus avoiding the uncertainty due to fraction cross-contamination. For this purpose, we borrowed a brain slice procedure and monensin protocol used to demonstrate an extracellular location for cerebroside:galactosyltransferase versus a Golgi location for cerebroside: sulfotransferase (15). We used cerebral cortex thin tissue slices that were washed and then incubated under physiological conditions. This provides for the study of large numbers of intact neural cells, although cell fragments exist on the cut surface.

To label the sialic acid residues of gangliosides, we used two different radiolabeled precursors: *i* ) the immediate sialic acid donor for the SAT reaction, cytidine-monophosphoryl-N-acetyl-[$^{14}$C]neuraminic acid (CMP-NeuAc); and *ii* ) N-acetyl-[$^{14}$C]mannosamine (ManNAc), an obligate intracellular precursor for the formation of the NeuAc moiety of CMP-NeuAc (16, 17).

ManNAc labeling of sialic acid residues of gangliosides is inherently dependent on intracellular anabolic routes, and therefore predisposing to Golgi SATs. In contrast, labeling of gangliosides directly with extracellular CMP-NeuAc could label gangliosides via putative cell surface SATs. The purpose of these experiments was to compare radiolabeling of ganglioside sialic acid in neural cells under conditions which distinguish between intracellular SATs that function in an anabolic pathway versus putative cell surface ganglioside modulation SATs.

## MATERIALS AND METHODS

### Materials

Universally labeled N-acetyl-[$^{14}$C]mannosamine (ManNAc, 254 mCi/mmol) was obtained from Amersham International (Amersham, UK) and universally labeled cytidine-5'-monophosphoryl-N-acetyl-[$^{14}$C]neuraminic acid (CMP-NeuAc, 249.9 mCi/mmol) was obtained from New England Nuclear (Boston, MA). Both were diluted with the corresponding unlabeled compound (Sigma, St. Louis, MO) to a specific activity of 100 mCi/mmol. Krebs-Henseleit buffer was made freshly each day from distilled-deionized-redistilled water and reagent grade chemicals were from Fisher (Fairlawn, NJ) and Sigma (St. Louis, MO) in the following concentrations: 117 mM NaCl; 4.7 mM KCl; 0.9 mM $MgSO_4$; 1.9 mM $CaCl_2$; 1.17 mM $NaH_2PO_4$; 0.9 mM $KH_2PO_4$; 25.0 mM $NaHCO_3$; and 11.1 mM *D*-glucose. This buffer solution was bubbled for several h with 95% $O_2$-5% $CO_2$ to oxygenate and establish the pH at 7.4.

### Animals and tissue preparation

Sprague-Dawley rat pups of both sexes were used to provide cortical brain slices. At 28 days of age, the rats were killed by decapitation, and the skin and occipital region of the skulls were quickly removed. After the meninges were carefully peeled away, one slice of gray matter (1 mm thick, approximately 50 mg cell protein) was removed from each cerebral hemisphere using an unused Bard Parker no. 15 blade. The slices never were handled with forceps, so as not to crush the tissue. Using the wooden shaft of a cotton tipped applicator as a spatula, the slices were placed immediately into individual 20-ml injection port vials containing 5 ml of oxygenated incubation medium (Krebs-Henseleit, above). After 5 min at 37°C, the buffer was removed and replaced with fresh buffer. The vials were sealed, and 95% $O_2$-5% $CO_2$ was bubbled through each vial with hypodermic needles inserted through the septa for 1 min. The slices were then preincubated for 1 h at 37°C in a shaking water bath before the radioactive substrates were added.

### Ganglioside SAT activity assay

After the preincubation period, 0.1 μCi of [$^{14}$C]ManNAc or CMP-[$^{14}$C]NeuAc was added to each vial (2.0 μM final concentration of either compound). The vials were re-bubbled with 95% $O_2$-5% $CO_2$ after addition of substrates. As controls, some slices were heat-denatured in boiling water for 15 min before addition of substrate. When monensin (Calbiochem, San Diego, CA) and/or unlabeled NeuAc were added to slice incubations, this was done at the start of the preincubation period described above. The slices were allowed to incubate at 37°C in a shaking water bath for various time intervals (1 to 6 h). The reactions were stopped by placing the vials on ice, then transferring the brain slices into ice-cold phosphate-buffered saline (PBS) for 5 min. Rinsing was repeated twice with fresh cold PBS. The slices were then placed in test tubes, quickly frozen at −70°C, and the frozen slices were lyophilized. The gangliosides were extracted and analyzed as follows.

### Ganglioside isolation

Our procedure was that of Irwin and Irwin (18) as modified by Durrie, Saito, and Rosenberg (11). To each lyophilized brain slice was added 1.0 ml of chloroform-methanol 2:1 (v/v) (C-M), and the lipids were extracted at room temperature for 1 h with brief intermittent sonication. The test tubes were centrifuged at high speed in a table-top clinical centrifuge for 15 min, and the C-M was removed and transferred to separate tubes. The extraction was repeated with C-M 1:2, and again with C-M 2:1, and the extracts were pooled and dried under $N_2$ gas. The C-M-insoluble residues were used for protein determinations (19). The dry lipid extracts were reconstituted with 1.0 ml of C-M 2:1 and applied to 0.5-ml columns of

Unisil, a brand of activated silica (Clarkson Co., Williamsport, PA), and the columns were washed with 3.0 ml of C-M 2:1. The gangliosides were eluted from the columns with 2 ml of chloroform-methanol-water 50:50:15, and these eluates were dried under $N_2$. Phospholipids in the eluates were hydrolyzed with 1.0 ml of 0.4 N KOH in methanol for 1 h at room temperature. After adjusting the pH of the hydrolysate to 9.0 with conc. HCl and pelleting by centrifugation the precipitates that formed, the supernatants were collected and dried under $N_2$. The gangliosides were redissolved and desalted by a reversed phase chromatography procedure using Sep Pak C18 cartridges (Waters Associates, Milford, MA) as described by Williams and McCluer (20). Total ganglioside SAT activity was measured by counting the radioactivity in aliquots of these samples by liquid scintillation in Aquasol (New England Nuclear, Boston, MA). The gangliosides were then separated into fractions by HPTLC.

### Ganglioside separation

The purified gangliosides were dissolved in 50 μl of C-M 2:1 and were spotted in 1-cm-wide streaks on activated silica gel 60 HPTLC plates (Merck, Darmstadt, FGR). The plates were developed with chloroform-methanol-0.25% aqueous $CaCl_2$ 50:40:10 (v/v/v) for 1 h. The ganglioside bands were visualized by resorcinol spray (21). The ganglioside species were identified by co-migration with ganglioside standards from bovine brain (Calbiochem, San Diego, CA) and were quantitated by densitometric analysis (22) compared against co-chromatographed 10 μg pure GM3 standard (23). After quantitation by densitometric analysis, 2-mm consecutive sections of the HPTLC lanes were scraped into scintillation vials. Water was added (0.5 ml) and the vials were sonicated; then, Aquasol was added and $^{14}C$ in the vials was counted. In this way the radioactivity in each ganglioside species was determined (11). As a visual record of ganglioside labeling, representative HPTLC lanes were autoradiographed.

### Analysis of sialic acid derivatives in the media

After the brain slices were removed from the incubation media in which CMP-[$^{14}$C]NeuAc was the sialic acid donor, 20μl of the incubation media was spotted in streaks directly on silica gel 60 HPTLC plates. On the same lanes were co-spotted free NeuAc and CMP-NeuAc standards obtained from Sigma (St. Louis, MO). The plates were developed in 2-propanol-methyl acetate-0.25% aqueous $CaCl_2$ 2:2:1 (v/v/v). The developed chromatograms were sprayed with resorcinol and visualized. The bands corresponding to free NeuAc and CMP-NeuAc were scraped and counted.

## RESULTS

### Effects of substrate on total ganglioside labeling

Radioactivities incorporated into the total ganglioside fractions of the brain slices were measured over a 6-h time course for both ManNAc and CMP-NeuAc substrates. The results are shown in **Fig. 1**. ManNAc labeling proceeded slowly for 2 h, then proceeded rapidly and quasi-linearly to the last time point (6 h). CMP-NeuAc labeling did not show a lag period, and was at its maximum rate for the first 2 h. Little additional label was incorporated from exogenous CMP-NeuAc after the initial 2 h.

### Ganglioside separation by HPTLC

A resorcinol pattern of rat brain gangliosides as separated by HPTLC is shown in **Fig. 2**, lane 3. Radioactivity found on the plates was assigned to the ganglioside species accordingly. Radiolabeling is visualized by the autoradiography in Fig. 2, lanes 1 and 2. Quantitation of ganglioside species by densitometric analysis showed that the actual amounts of individual gangliosides did not change substantially over the time course nor between the two substrates (data not shown). The resorcinol-densitometric method of analysis is unable to detect the small changes in ganglioside levels predicted from the radiolabeling data (picomole range). The quantitative ganglioside profile of the rat brain slices is given in **Table 1.**

There were several minor bands on the plates that remained unidentified. One such band is located between the origin and GQ (labeled "Un"). It could be a polysialosyl glycosphingolipid (GPX?) or a glycopeptide. There were certain gangliosides that were not separated well from other major bands. For instance, GD2 migrates with GT1a, which we demonstrated by the appearance of a band in this area in both the di-sialo and the tri-sialo gangliosides upon gradient elution of total gangliosides

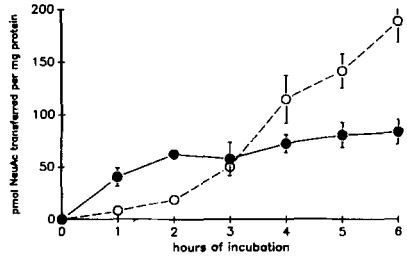


**Fig. 1.** The radioactivity incorporated into total gangliosides was converted into pmoles NeuAc transferred per mg protein (SAT activity). Radioactivity incorporated into the heat-denatured controls (background) was subtracted before calculating SAT activity. The means of three experiments are plotted with error bars showing one standard deviation (some symbols are larger than the error bars). The means are connected by a straight line. ManNAc-labeled brain slices are given by (○), and CPM-NeuAc labeled by (●).

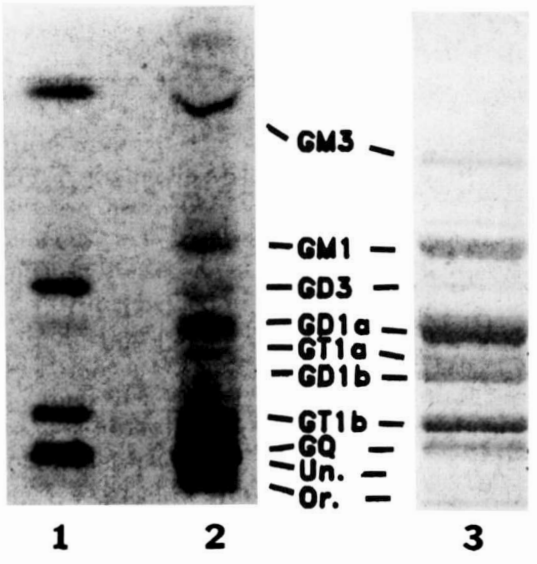


**Fig. 2.** Samples containing approximately 100 dpm radiolabeled gangliosides were separated on silica gel 60 HPTLC plates in chloroform-methanol-0.25% aqueous $CaCl_2$ 50:50:15 (v/v/v), and then were placed in contact with autoradiographic film in a light-tight cassette for 2 weeks. After autoradiography, the ganglioside bands were visualized on the HPTLC plate with resorcinol and identified using bovine brain ganglioside standards. The samples shown are from a 1-h incubation. Lanes: 1) autoradiograph of gangliosides labeled by CMP[$^{14}$C]NeuAc; 2) labeled by [$^{14}$C]ManNAc; 3) resorcinol staining pattern on a separate but comparable HPTLC plate of rat brain slice gangliosides.

from DEAE-Sephadex ion exchange chromatography. Microbial sialidase digestion of this area of a preparative TLC plate produced both GM1 and GM2 as products (data not shown), proving the occurrence of GD2 as well as GT1a. GT3 is also overlooked because it migrates with or near GD1b. GD1a, GT1a, and GD1b all are close together on standard TLC plates; therefore, this is a complex area of the plate and our interpretations of the radioactivity distribution could well neglect labeling of minor but important gangliosides which are hidden (e. g., GD2 and GT3).

### Effect of substrate on distribution of label

The radioactivity distribution among gangliosides is shown in Table 1 and Fig. 2 and **Fig. 3**. In the ManNAc-labeled gangliosides (Fig. 3A), the pattern shows labeling of most of the ganglioside species, and is notable for high levels of incorporation of label into GM1. This is not an artifact due to accumulation of GM1 from the action in situ of sialidase (GM1 is resistant to sailidase), because the percent of the total ganglioside radioactivity in GM1 slowly decreased with time: 25.0 ± 8.5% at 2 h; 24.2 ± 8.1 at 3 h, and 19.6 ± 4.8 at 4 h.

CMP-NeuAc labeling had a different radioactivity distribution (Figs. 2 and 3B). The amount of radioactivity in GM1 was low (Table 1). There was a pronounced labeling of GD3, which represents a quite high specificity because of the small pool of GD3 as well as its precursor, GM3 (Table 1). Other major locations of label were in GD1a, GT1b, and GQ.

### Analysis of the incubation media for the fate of extracellular CMP-NeuAc

In **Fig. 4**, the levels of residual radioactivity in the incubation media, which correspond to free NeuAc and CMP-NeuAc standards on HPTLC, are shown over a CMP-[$^{14}$C]NeuAc labeling time course. It is clear that the major reaction involving exogenous CMP-NeuAc is hydrolysis to produce labeled free NeuAc. We determined that approximately 2% of the label derived from the ex-

TABLE 1. Content and anabolic radioactive labeling of ganglioside sialic acid in rat brain cortical slices

| Species of Ganglioside | NeuAC per mg Protein | Percent of Total | Radioactivity per mg Protein | |
|---|---|---|---|---|
| | | | ManNAc[a] | CPM-NeuAc[b] |
| | *μg* | *mol* | *dpm* | |
| GM3 | 0.3 | 0.4 ± 0.2 | 970 ± 249 | 1385 ± 318 |
| GM2 | 0.1 | <0.1 | 644 ± 136 | 859 ± 232 |
| GM1 | 10.0 | 13.1 ± 0.8 | 5956 ± 1496 | 105 ± 46 |
| GD3 | 1.9 | 1.9 ± 0.2 | 1957 ± 297 | 2774 ± 467 |
| GD1a | 28.0 | 35.7 ± 3.1 | 5878 ± 1377 | 1394 ± 274 |
| GD2/GT1a | 4.0 | 5.0 ± 1.2 | 2526 ± 235 | 1076 ± 337 |
| GT3/GD1b | 12.1 | 15.1 ± 1.6 | 2544 ± 929 | 426 ± 44 |
| GT1b | 14.3 | 17.8 ± 2.1 | 3974 ± 1069 | 1153 ± 447 |
| GQ | 8.0 | 10.1 ± 0.9 | 3915 ± 441 | 958 ± 271 |
| Un | 0.1 | <0.1 | 125 ± 21 | 1479 ± 315 |

Gangliosides were separated on HPTLC plates, visualized with resorcinol, and quantitated by densitometry. HPTLC lanes were scraped in consecutive 2-mm segments, and radioactivity was counted in a liquid scintillation spectrometer.
[a]Label incorporated into ganglioside sialic acid from the precursor substrate [$^{14}$C]ManNAc at maximum (4 h).
[b]Label incorporated from the precursor substrate CMP-[$^{14}$C]NeuAc at maximum (2 h).

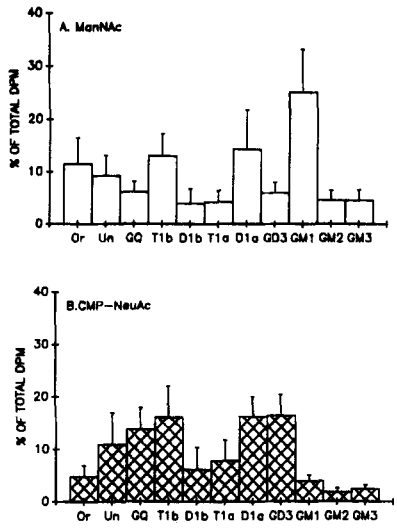


**Fig. 3.** The lanes of HPTLC-separated gangliosides were scraped in sequential 2-mm segments, and the radioactivity was measured in each. After the radioactivity was assigned to each ganglioside as identified according to Fig. 2, the distribution of radioactivity was calculated as a percent of the total radioactivity in a lane for each ganglioside. A) [$^{14}$C]ManNAc-labeled brain slices. B) CMP-[$^{14}$C]NeuAc-labeled brain slices. The data are for a 3-h incubation and represent the means of three experiments. The error bars are one standard deviation.

ogenous CMP-[$^{14}$C]NeuAc had been incorporated into the ganglioside fraction after 6 h of incubation. The remainder of the radioactivity was accounted for by CMP-NeuAc and free NeuAc in the media (94%), and by the radioactivity in the C-M-insoluble residue (i. e., in the sialoglycoprotein fraction, 4%). There is still sufficient donor substrate remaining at 6 h incubation, so that its concentration should not be a rate limiting factor in these studies.

### Effects of monensin and unlabeled free NeuAc on ganglioside labeling

Rat brain slices were preincubated with 2.0 mM unlabeled NeuAc or 0.1 $\mu$M monensin or both for 1 h prior to the addition of labeled substrates. The labeling of the total ganglioside fraction (4 h) is given in **Fig. 5.** Preincubation with unlabeled NeuAc had little effect on labeling with either substrate. Monensin had little effect on labeling of gangliosides with CMP-NeuAc (Fig. 5A), but when labeling was with ManNAc, there was an inhibition of approximately 30% (Fig. 5B).

With ManNAc-labeled slices, inhibition was proportional to the concentration of monensin, and inhibition was enhanced by preincubation with monensin (**Table 2**). Labeling with CMP-NeuAc was not inhibited by monensin except at high concentration and then only when preincubated (Table 2).

Inclusion of high concentrations of unlabeled free NeuAc in the medium to saturate uptake systems and thus inhibit reutilization of labeled free NeuAc liberated from hydrolyzed CMP-NeuAc did not diminish the degree of labeling by CMP-NeuAc.

## DISCUSSION

By itself, the brain slice model cannot be considered to represent purely intact cells, as many cells will be transected. Therefore, the basis for interpretation of our findings rests in observing differences in labeling characteristics between two sources for ganglioside sialic acid.

### [$^{14}$C]ManNAc-labeling of brain slice gangliosides

[$^{14}$C]ManNAc must be internalized and converted intracellularly to the ultimate sialic acid donor, CMP-NeuAc. We noted a 2-h lag period before radiolabel was rapidly incorporated into gangliosides, which reflects the time required for intracellular synthesis of CMP-NeuAc from ManNAc. After this lag period, the labeling proceeded rapidly and all species of gangliosides were labeled. Ganglioside GM1 had relatively high label incorporation. The observed GM1 labeling could reflect its central role as precursor acceptor for sialosylation to produce GD1a and other major gangliosides of the gangliotetrahexosyl series. At later time points, GM1 became proportionately less labeled than other gangliotetrahexosyl gangliosides.

Monensin inhibited the labeling of gangliosides by ManNAc The sodium ionophore, monensin, causes the Golgi saccules to swell hydrostatically, when incubated with intact cells, and serves to antagonize Golgi function by inhibiting *cis* to *trans* movement of vesicular membrane-bound molecules (24, 25). Our monensin results

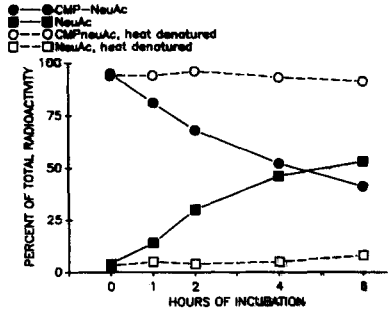


**Fig. 4.** The media of brain slices incubated with CMP-[$^{14}$C]NeuAc were analyzed by HPTLC (see Materials and Methods), and the resorcinol-positive bands corresponding to free NeuAc and CMP-NeuAc standards were scraped and counted for radioactivity. The radioactivity at each time point is expressed as the percent of the total radioactivity in an aliquot of the media. The circles represent CMP-NeuAc, and the squares are free NeuAc. The broken lines with open symbols are the corresponding values from heat-denatured control brain slices.

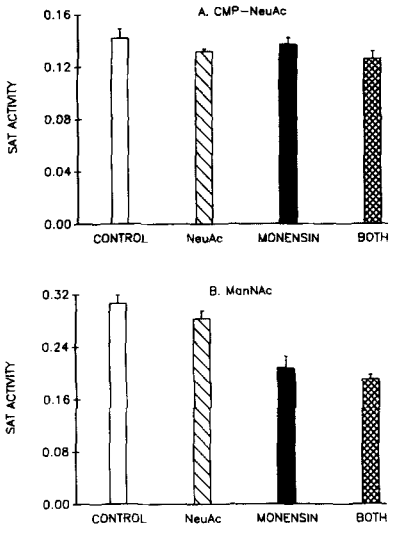


**Fig. 5.** Ganglioside radiolabeling experiments were carried out in which the rat brain slices were preincubated for 1 h in the presence of 2.0 mM unlabeled NeuAc and/or 0.1 μM monensin at 37°C, before the addition of labeled precursor. The radioactivity incorporated into the purified total ganglioside fraction after a total of 4 h of incubation at 37°C was measured and is reported as SAT units (pmol NeuAc transferred to gangliosides per mg protein per h). The control samples were preincubated as before with no added unlabeled NeuAc or monensin. The bars are the means of four slices and the error bars are the standard error of the mean. A) CMP-NeuAc-labeled brain slices. B) ManNAc-labeled slices.

with ManNAc labeling indicate the involvement of Golgi, which is an accepted site for biosynthetic SATs. The monensin inhibition was sensitive and concentration-dependent. When the slices were not preincubated with monensin, the inhibition of labeling was only slightly less than when slices were preincubated. Because sialic acid labeling with ManNAc has a lag period required for conversion metabolically to CMP-NeuAc, monensin is effective when added at the same time as the label. These results support the generally accepted model that ganglioside biosynthetic SATs reside in the Golgi.

### CMP-[$^{14}$C]NeuAc labeling of brain slice gangliosides

Cell surface SATs have been described in numerous reports; for instance: in neural tissue by subfractionation enrichment studies (9, 11, 26), by identification of major differences between Golgi SAT preparations versus synaptosomal and SPM SAT preparations (12), and by the transfer of sialic acid from CMP-NeuAc to non-internalizable acceptors by intact cells such as lymphocytes (27) and neurons (28).

We tested whether ganglioside labeling by CMP-[$^{14}$C]NeuAc in our system was related to internalization of the substrate, or free [$^{14}$C]NeuAc released from it. We added great excesses of unlabeled NeuAc to saturate uptake systems and dilute internalized label, and found no effect on incorporation of label. We also analyzed the fate of the label in the incubation media. We found that the bulk of the exogenous CMP-NeuAc was cleaved by an active cell surface CMP-NeuAc hydrolase (29) to produce free labeled NeuAc. The amount of label available for any SAT reaction could only be that portion of radioactivity in the media not accounted for as free NeuAc, which was about 6% of the total radioactivity after 6 h incubation. The approximate percentage of the total radioactivity incorporated into the ganglioside fraction was 2%. The remaining radioactivity was accounted for in the C-M-insoluble residue (glycoprotein). This glycolipid/glycoprotein labeling ratio by neuronal ecto-sialosyltransferase agrees with previous reports (10).

TABLE 2. Effects of exposure of rat brain slices to monensin on the labeling of ganglioside sialic acid by cellular sialosyltransferase (SAT) activity

| | Precursor Substrate | |
|---|---|---|
| Monensin Treatment | [$^{14}$C]ManNAc | CPM-[$^{14}$C]NeuAc |
| 1. Control, no monensin | 43,925 ± 7,760 (100)[a] | 19,474 ± 948 (100)[a] |
| 2. 0.1 μM monensin | 33,856 ± 1,146 (77.1 ± 2.6) | 19,519 ± 741 (100.5 ± 3.9) |
| 3. 0.1 μM monensin, pre-incubated 1 h | 27,990 ± 2,444 (67.7 ± 3.4) | 18,465 ± 531 (95.0 ± 2.8) |
| 4. 0.3 μM monensin | 24,804 ± 2,092 (56.6 ± 4.8) | 16,618 ± 1,004 (85.4 ± 5.2) |
| 5. 0.3 μM monensin, pre-incubated 1 h | 9,295 ± 2,413 (21.2 ± 5.5) | 10,872 ± 1,172 (55.9 ± 2.9) |

Brain slices were incubated with radiolabeled ManNAc that serves as an obligate intracellular precursor for labeled CMP-NeuAc or, alternatively, with extracellular radiolabeled CMP-NeuAc that serves as a direct donor of labeled NeuAc to cell surface gangliosides. Radioactivity incorporated into the total ganglioside fraction in the brain slices is presented as dpm per mg of protein. The results listed are the mean of four samples ± standard error of the mean.

[a]Values in parentheses represent percent of radioactivity incorporated as compared with that in control slices, taken as 100%.

Incorporation of label into gangliosides was immediate when the cells were incubated with extracellular CMP-NeuAc. The incorporation rate was at a maximum well before labeling by ManNAc had reached significant levels. The rate of incorporation markedly decreased after 2 h, an indication that acceptors in the microenvironment of the cell surface SAT may have become saturated with label.

The ganglioside labeling pattern of brain slices with CMP-NeuAc showed low specific incorporation of label into GM1 and high incorporation into GD3. This pattern is consistent with the labeling patterns reported for synaptosomes and SPM ecto-sialosyltransferases (12).

CMP-NeuAc labeling was not inhibited by monensin at a concentration that markedly inhibited ManNAc labeling, indicating that Golgi was not involved in labeling of gangliosides by exogenous CMP-NeuAc. At threefold higher dosages of monensin, there was inhibition of CMP-NeuAc labeling, but this was dependent upon preincubation with the Golgi inhibitor. Since monensin is a powerful sodium ionophore, its continuous action could alter the ionic environment of all intracellular compartments, thus inhibiting enzyme reactions nonspecifically.

These results suggest a model in which there exists more than one SAT system for anabolism of gangliosides. The classical system is involved in the bulk de novo biosynthesis of gangliosides for vesicular transport and delivery to the various membrane compartments of the cell. The ManNAc labeling experiments demonstrated the operation of this system.

The other systems(s) are proposed to be involved in modulation and modification of existing gangliosides for the purpose of controlling ganglioside profiles in situ. Since a major location of gangliosides is the ecto surface of the plasma membrane, especially in the synapse (30, 31), this would be a prime location for such modulating transferases. Our results with CMP-NeuAc labeling of intact neural cells are consistent with this hypothesized ganglioside modulation system on the cell surface.

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