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Isolation of three novel cholinergic neuron-specific gangliosides from bovine brain and their *in vitro* syntheses

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In the present study, three extremely minor but novel Chol-1 antigens, termed X1, X2, and X3 have been isolated from bovine brain gangliosides. Based on the results of sialidase degradation, TLC-immunostaining with anti-Chol-1 antibody and fast atom bombardment mass spectrometry, their chemical structures were identified as:

III^{6}NeuAc-GgOse4Cer	(X1: GM1α)
III^{6}NeuAc,II^{3}NeuAc-GgOse4Cer	(X2: GD1aα)
III^{6}NeuAc,II^{3}NeuAc-NeuGc-GgOse4Cer	(X3: GT1bα)

The yields of GM1α, GD1aα, and GT1bα, were approximately 150, 20, and 10 μg, respectively, from 10 g of the bovine brain ganglioside mixture. In conjunction with our previous observations, all gangliosides with anti-Chol-1 reactivity were found to contain a common sialyl α2-6 *N*-acetylgalactosamine residue, indicating that this unique sialyl linkage is the specific antigenic determinant. We subsequently examined the biosyntheses of the three novel Chol-1 gangliosides using rat liver Golgi fraction as an enzyme source. The results showed that GM1α, GD1aα, and GT1bα were synthesized from asialo-GM1, GM1a, and GD1b, respectively, by the action of a GalNAc α2-6sialyltransferase.

Keywords: ganglioside, cholinergic neuron, ganglioside biosynthesis, sialyltransferase

Abbreviations: The nomenclature used for gangliosides is based on the system of Svennerholm [37] and Hirabayashi *et al.* [8, 12]. Cer, ceramide; Gal, galactose; Glc, glucose; GalNAc, *N*-acetylgalactosamine; NeuAc, *N*-acetylneuraminic acid; NeuGc, *N*-glycolylneuraminic acid; Hex, hexose; HexNAc, *N*-acetylhexosamine; TLC, thin layer chromatography; HPTLC, high performance thin layer chromatography; ELISA, enzyme-linked immunosorbent assay; FAB-MS, fast atom bombardment-mass spectrometry; HD, Hanganutziu-Deicher.

Introduction

In order to find cholinergic neuron-specific markers, Whittaker and his colleagues have raised antisera by immunizing sheep with cholinergic synaptosomes prepared from the electric organ of *Torpedo marmorata* [1–4]. One of these antisera, anti-Chol-1, elicited the selective complement-mediated lysis of a cholinergic fraction of mammalian brain synaptosomes [3]. Immunohistochemically anti-Chol-1 stained cell bodies and nerve terminals of cholinergic neurons in the rat central and peripheral nerve systems [5]. Cytotoxity tests and immunohistochemistry demonstrated that the binding of anti-Chol-1 to nerve

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terminals was completely inhibited by gangliosides derived from the mammalian brain and *Torpedo* electric organ [3]. In addition, immunoreactive structures in rat brain with anti-Chol-1 disappeared after pre-treatment with chloroform:methanol (2:1, v/v) [3]. Thus, the chemical nature of the antigens, generally termed Chol-1, was supposed to be gangliosides. Anti-Chol-1 was found to recognize at least three gangliosides designated as Chol-1α, Chol-1β, and Chol-1γ [3].

Chol-1 gangliosides are believed to have functional importance since they are expressed in the restricted regions of the nervous systems and their expression is conserved from *Torpedo* to human. In spite of their importance, the chemical structures of Chol-1 antigens have remained obscure since they are extremely minor components of mammalian brains. Giuliani *et al.* isolated 100 µg as sialic acid of Chol-1β ganglioside from 100 kg of pig brain and characterized the partial structure of the novel antigen [6].

We developed an effective method using Q-Sepharose column chromatography for the separation of minor gangliosides [7]. Using this method, we have isolated and characterized several new species of minor gangliosides such as an α-series ganglioside [8], de-*N*-acetylated GM1 [9], and a hybrid type of ganglioside containing GM2 epitope [10]. Recently we have also identified two species of Chol-1 gangliosides as GT1aα (IV^3NeuAc,III^6NeuAc,II^3NeuAc-GgOse4Cer) [11] and GQ1bα (IV^3NeuAc,III^6NeuAc, II^3NeuAc2-GgOse4Cer) [12]. In this study, we have isolated and characterized three additional novel Chol-1 gangliosides from minor fractions of a bovine brain ganglioside mixture. Based on the structural analysis, all Chol-1 gangliosides were shown to contain a sialyl α2-6 *N*-acetylgalactosamine residue as was found in GT1aα and GQ1bα. The novel gangliosides were found to be synthesized by the action of a GalNAc α2-6sialyltransferase associated with a rat liver Golgi fraction.

Materials and methods

Materials

Gangliosides from bovine brain were prepared as described previously [7]. GT1aα and GQ1bα, Chol-1α gangliosides [12], and GD1α [8] were purified from bovine brain ganglioside mixture. Sheep anti-Chol-1 was raised as described by Richardson *et al.* [3]. Sialidase L was isolated and purified from leech as described [13, 14]. Donryu rat (female, 4 weeks old) was purchased from SLC, Inc. (Hamamatsu, Japan). CMP-NeuAc and 4-methylumbelliferyl NeuAc (4MU-NeuAc) were from Sigma Chemical Company (St Louis, USA). Anti-NeuGc-containing ganglioside antibody (anti-HD antibody) was prepared and purified as described [15].

HPTLC

Analytical HPTLC was carried out on precoated HPTLC plates (E. Merck) using the following solvent systems. Chloroform:methanol:12 mM $MgCl_2$ (5:4:1, v/v/v; solvent A) and chloroform:methanol:12 mM $MgCl_2$:15 M NH_4OH (50:40:7:3, v/v/v/v; solvent B). Gangliosides were visualized with a resorcinol/HCl reagent.

Immunological methods

TLC-immunostaining was performed by the method of Higashi *et al.* [16]. Gangliosides were applied on a plastic TLC plate (Polygram Sil G, Nagel, Germany) and developed with solvent B and then solvent A in the same direction. In case of two-dimensional TLC-immunostaining, a plastic plate was developed with solvent B to the left (first dimension) and then with the solvent A (second dimension). Gangliosides were visualized by immunostaining with anti-Chol-1. The enzyme-linked immunosorbent assay (ELISA) was performed by the method of Higashi *et al.* [17].

Isolation of Chol-1 gangliosides

Total bovine brain gangliosides (10 g) were applied onto a Q-Sepharose column (3 × 75 cm) and fractionated into 26 fractions by the method described previously [7]. By TLC-immunostaining with anti-Chol-1, X1, X2, X3, and X4 were detected in fractions 5, 17, 20 and 25, respectively (Fig. 1). In this study, X1, X2, and X3 were isolated and characterized since the content of X4 was too low to purify. Gangliosides in each fractions were dissolved in 20 ml of chloroform:methanol:water (60:25:1, v/v/v) and applied on an Aquasil SS-552N HPLC column (2 × 30 cm, Senshu Chemicals, Tokyo) which had been equilibrated with the same solvent. The column was attached to a Jasco 880-PU HPLC pump (Japan Spectroscopic Co., Ltd.) and eluted at the rate of 5 ml min^{-1} with a linear gradient starting from the solvent system of chloroform:methanol:water (60:25:1, v/v/v) until the solvent system of chloroform:methanol:water (60:35:8, v/v/v) was attained. The elution was completed in 400 min and 15 ml fractions were collected. Five µl aliquots were analysed by TLC-immunostaining with anti-Chol-1. Fractions containing X1, X2, or X3 were collected and evaporated to dryness. The final purification of each ganglioside was achieved by a preparative TLC method as described previously [12].

Negative FAB-MS

Mass spectra were recorded on a Finnigan MAT TSQ 700 triple-stage quadrupole mass spectrometer (Finnigan MAT, Sun Jose, USA) equipped with a FAB-MS spectrometric ion source. Data were processed with a DEC Station 2100 computer. The FAB-MS spectra in the negative mode were recorded by the method of Ohashi *et al.* [18]. For FAB-MS

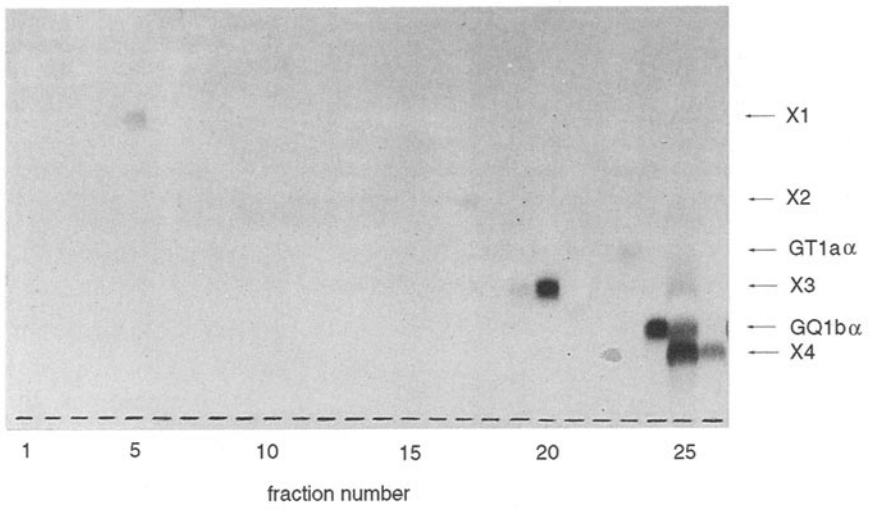


Figure 1. TLC-immunostaining of the fractionated gangliosides by Q-Sepharose column chromatography with anti-Chol-1. Plastic TLC plate was developed successively with solvent systems of B and A, and Chol-1 gangliosides were detected by immunostaining using anti-Chol-1 antiserum. Two species of Chol-1 antigens, GT1aα and GQ1bα, have been previously characterized [11, 12]. Other gangliosides recognized by anti-Chol-1 were designated as X1, X2, X3, and X4. X1, X2, and X3 were subjected to further purification. The yield of X4 was too low to be characterized in this study.

analysis, approximately 5 μg of the sample was used in a diethanolamine matrix.

Sialidase degradation

To remove the NeuAcα2-3Gal linkage at the non-reducing end of the sugar chains, GD1α, GT1aα, and GQ1bα were treated with sialidase L which selectively cleaves the α2-3 linked NeuAc residue and releases 2,7-anhydro-α-NeuAc [13, 14]. Ten μg of each ganglioside or 4MU-NeuAc were dissolved in 15 μl of 50 mM acetate buffer (pH 4.6) containing 1 U sialidase L and incubated at 37 °C for 12 h. Reaction products were analysed by HPTLC and TLC-immunostaining with anti-Chol-1.

Sialyltransferase assay using rat liver Golgi fraction

A Golgi fraction was prepared from rat liver by the method of Sandberg *et al.* [19]. Sialyltransferase activity was measured by the method of Hidari *et al.* [20] with slight modifications. In brief, the reaction mixture contained 15 nmol asialo-GM1, GM1a or GD1b, 3.2 mM CMP-NeuAc, 0.4% Triton CF-54, 10 mM $MnCl_2$, 10 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 150 mM Na cacodylate/HCl buffer (pH 6.5), and 50 μg of Golgi protein in a total volume of 50 μl. The mixture was incubated for 2 h at 37 °C. The reaction was terminated by the addition of 0.5 ml of water. After desalting by ASPEK pak tC18 CARTRIGE column chromatography (M&S Instruments Trading Inc., Tokyo, Japan), the reaction products were analysed by two-dimensional TLC-immunostaining with anti-Chol-1. The amounts of the reaction products were determined by densitometric analysis with a dual-wavelength densitometric scanner, Shimadzu CS-9000 (Shimadzu, Kyoto, Japan).

Results

Chemical structure of ganglioside X1

As shown in Fig. 1, ganglioside X1 was enriched in fraction 5 eluted from Q-Sepharose column chromatography. Ganglioside X1 was purified as a single species for structural characterization (Fig. 2). The yield of X1 was approximately 150 μg from 10 g bovine brain ganglioside mixture. Purified X1 migrated between GM1a and GD1a with solvent A, and between GD1a and GD1b with solvent B. X1 could be readily converted to asialo GM1 by *clostridial* sialidase in the absence of a detergent (data not shown). The product, asialo-GM1, was confirmed by TLC-immunostaining using anti-asialo-GM1 antibody. This result indicated that X1 was different from regular ganglioside GM1a.

The negative FAB-MS spectrum of the purified ganglioside is shown in Fig. 3. Major molecular ion species, $(M-H)^-$, were seen at *m/z* 1572 and 1544 which corresponded to the structure composed of three hexoses, one *N*-acetylhexosamine, one *N*-acetylneuraminic acid, and a ceramide comprised of sphingosine d20:1/fatty acid C18:0 and sphingosine d18:1/fatty acid C18:0. The presence of ions at *m/z* 1410, 1119, 916, 754, and 592 indicated that the ganglioside possessed the following carbohydrate sequence which was identical to GM1α: Hex-(NeuAc-)HexNAc-Hex-Hex-Cer.

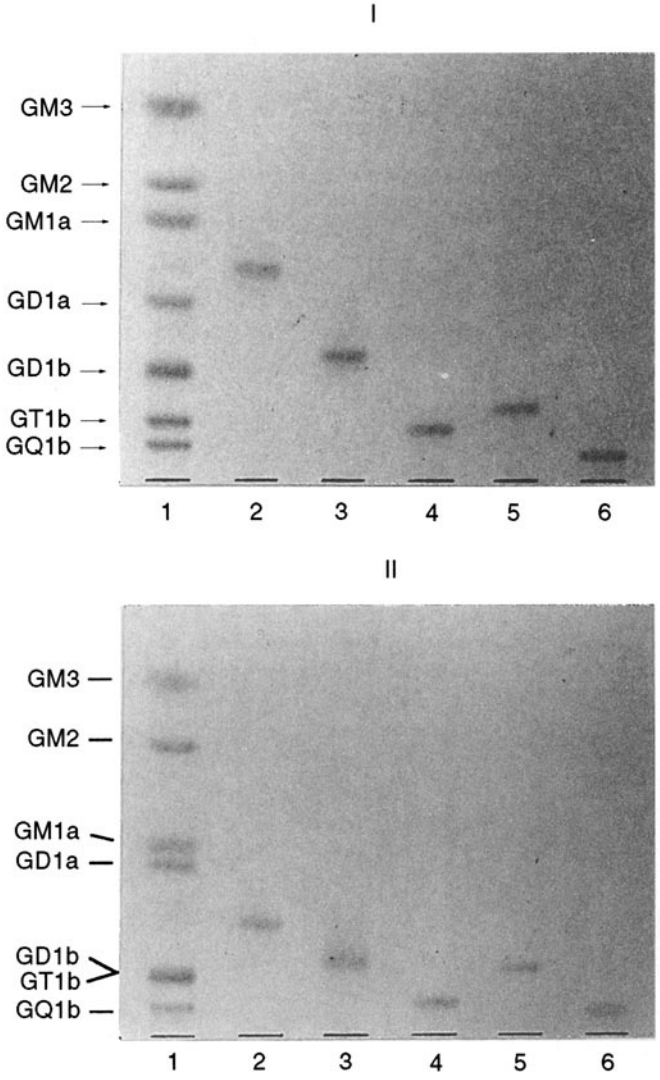


Figure 2. HPTLC of Chol-1 gangliosides. The purified Chol-1 gangliosides, 0.2 μg each, were analysed by HPTLC using solvent A (panel I) and solvent B (panel II). Lane 1, standard ganglioside mixture (GM3, GM2, GM1a, GD1a, GD1b, GT1b, and GQ1b); lane 2, X1; lane 3, X2; lane 4, X3; lane 5, GT1aα; Lane 6, GQ1bα. Gangliosides were visualized with a resorcinol/HCl reagent.

In order to confirm the identity of ganglioside X1 as GM1α, standard GM1α was prepared from the putative substrate, GD1α, using sialidase L which selectively hydrolyses the sialic acid attached to Gal of the non-reducing terminal through an α2-3 linkage (Fig. 4) [13, 14]. Treatment of GD1α with sialidase L gave a single resorcinol-positive spot, GM1α (lane 2 in Fig. 5-I). The TLC mobility of GM1α was found to be identical to that of X1, and GM1α was shown to be recognized by anti-Chol-1 (Fig. 5-II, lanes 2 and 10). By FAB-MS, the mass spectrum of GM1α derived from GD1α was found to be identical to that of X1 isolated from bovine brain.

Based on these results, the chemical structure of X1 was determined to be:

NeuAcα2
\
6
Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1′Cer

(III^{6}NeuAc-GgOse^{4}Cer: GM1α)

The name GM1α is proposed for ganglioside X1 in accordance with our system of the nomenclature [8] because of the presence of the NeuAcα2-6GalNAc structure.

Chemical structure of ganglioside X2

Ganglioside X2 was detected in fraction 17 separated by Q-Sepharose column chromatography (Fig. 1). Preparative TLC on silica gel HPTLC plate yielded approximately 20 μg of ganglioside X2. Figure 2 shows that X2 was purified to homogeneity and migrated between GD1a and GD1b with both solvent systems of A and B. Treatment of X2 with clostridual sialidase produced a compound reactive with the B-subunit of cholera toxin (data not shown), showing that the backbone structure of X1 was GM1a.

Intact ganglioside X2 was analysed by negative FAB-MS (Fig. 3). The detection of a major molecular ion peak, $(M-H+Na-H)^-$ at *m/z* 1858, indicated that this ganglioside had three hexoses, one *N*-acetylhexosamine, two *N*-acetylneuraminic acids, and a ceramide consisting of sphingosine d18:1/fatty acid C18:0. The fragment ions at 1696, 1392, 1091, 888, 726, and 564 indicated that the ganglioside X2 has the carbohydrate sequence of Hex-(NeuAc-)HexNAc-(NeuAc-)Hex-Hex-Cer.

For comparison, we prepared standard ganglioside GD1aα from GT1aα using sialidase L (Fig. 4), and analysed GD1aα and X2 in parallel by HPTLC (Fig. 5-I) and TLC-immunostaining with anti-Chol-1 (Fig. 5-II). GD1aα and X2 were found to have the same TLC-mobility and react with anti-Chol-1. By FAB-MS analysis, the mass spectrum of GD1aα was also found to be identical to that of X2.

Judging from these results, the chemical structure of X2 was identified as GD1aα:

NeuAcα2
\
6
Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1′Cer
3
/
NeuAcα2

(III^{6}NeuAc,II^{3}NeuAc-GgOse4Cer:GD1aα).

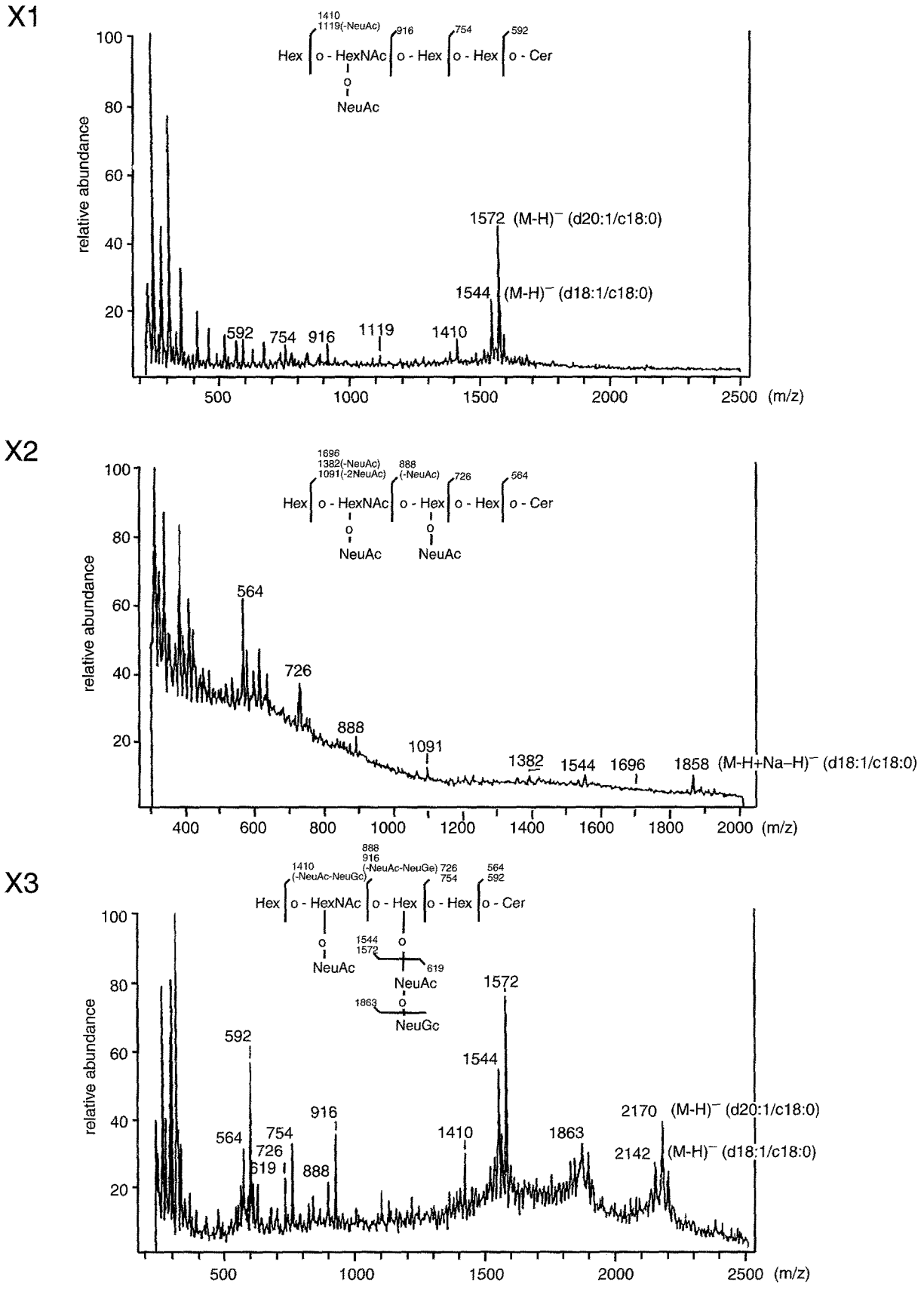


Figure 3. Negative FAB-MS spectra of purified X1, X2, and X3.

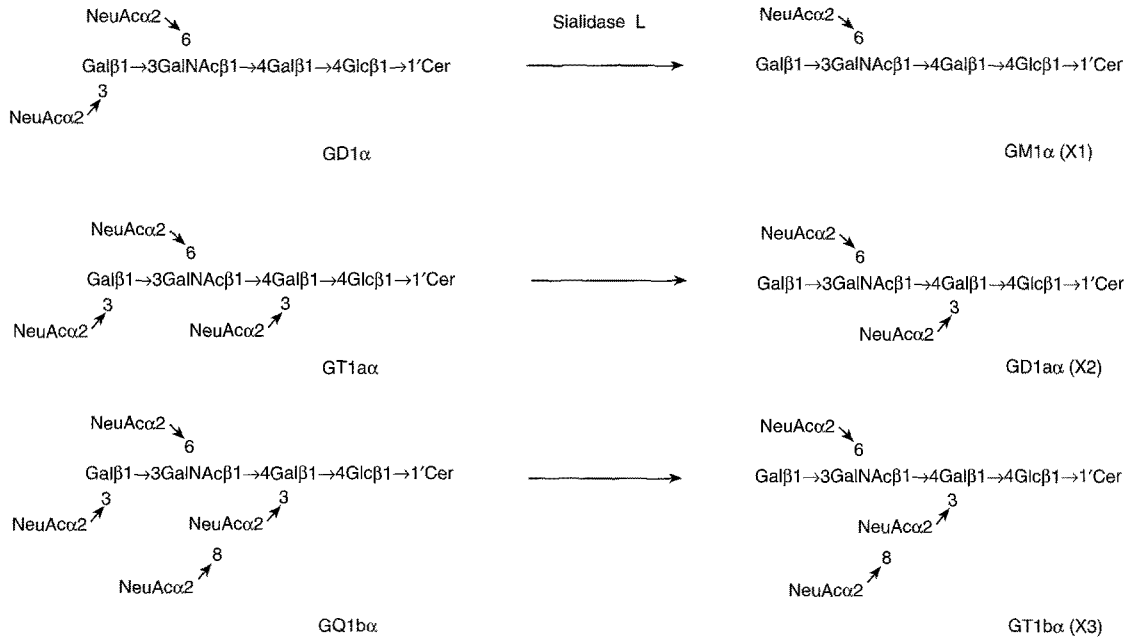


Figure 4. Reactions showing the hydrolysis of GD1α, GT1aα, and GQ1bα with sialidase L. Sialidase L selectively hydrolyses the terminal NeuAcα2-3Gal linkage [13, 14].

Since this disialoganglioside can be considered as a hybrid of a-series (GM1a) and α-series (GM1aα), we named X2, GD1aα according to our nomenclature system [8, 12].

Chemical structure of ganglioside X3

Ganglioside X3 was detected in fraction 20 obtained by Q-Sepharose column chromatography (Fig. 1). Ganglio-

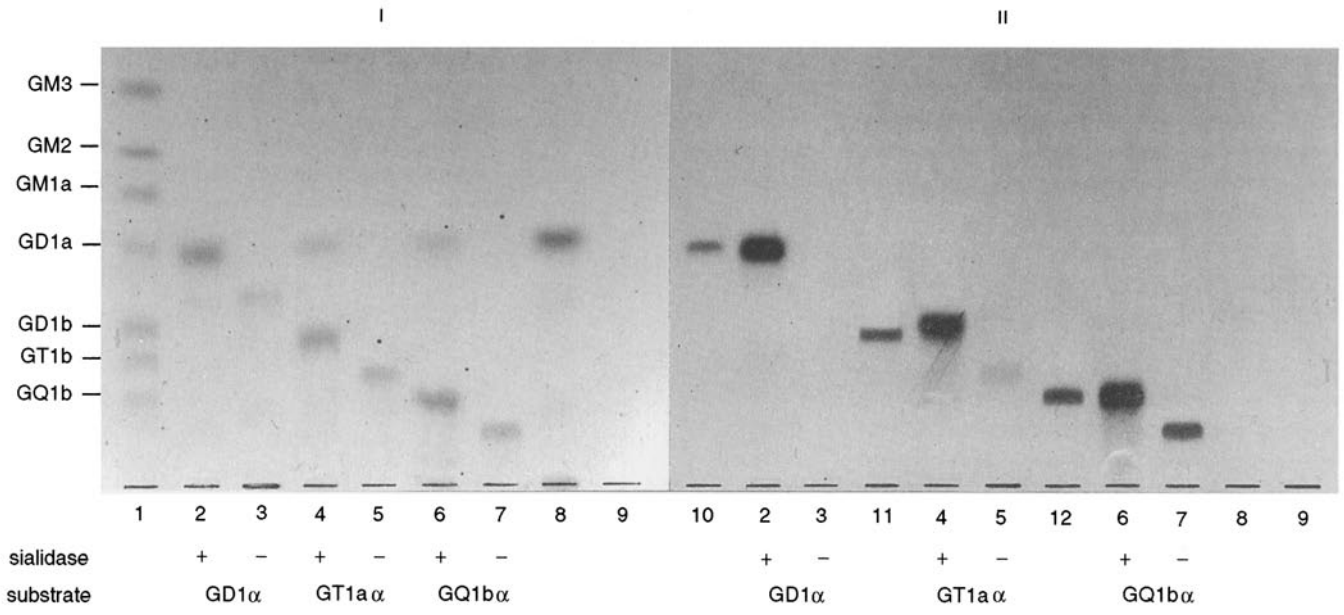
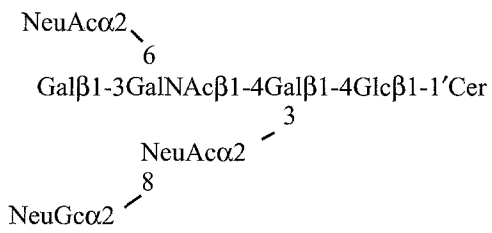


Figure 5. TLC-immunostaining of the three Chol-1 gangliosides and the gangliosides derived from GD1α, GT1aα and GQ1bα by sialidase L treatment. Approximately 0.1–0.5 μg of each ganglioside was applied to the plastic plates and developed with solvent B and then solvent A in same direction. Gangliosides were detected by resorcinol/HCl reagent (panel I) and by immunostaining with anti-Chol-1 (panel II). Lane 1, standard ganglioside mixture (GM3, GM2, GM1a, GD1a, GD1b, GT1b, and GQ1b); lanes 2 and 3, reaction products from GD1α; lanes 4 and 5, reaction products from GT1aα; lanes 6 and 7, reaction products from GQ1bα; lane 8, 2,7-anhydro-α-NeuAc (reaction products from 4MU-NeuAc); lane 9, sialidase only; lane 10, X1; lane 11, X2; lane 12, X3. Lanes 2, 4, and 6, reaction products with sialidase L; lanes 3, 5, and 7, reaction products without enzyme.

side X3 was purified as a single species when examined by HPTLC (Fig. 2). The yield of X3 was approximately 10 μg from 10 g of bovine brain ganglioside mixture. With the neutral solvent system A, the TLC-mobility of X3 was slightly slower than that of GT1b; with the alkaline solvent system B it migrated slightly faster than GQ1b. Treatment of X3 with clostridual sialidase produced a monosialoganglioside which was recognized by the cholera toxin-B subunit, indicating that ganglioside X3 had the backbone structure of GM1a (data not shown).

Intact ganglioside X3 was analysed by negative FAB-MS (Fig. 3). Two major molecular ion species, $(M-H)^-$, were detected at *m/z* 2170 and 2142 which corresponded to a structure composed of three hexoses, one *N*-acetylhexosamine, two *N*-acetylneuraminic acids, one *N*-glycolylneuraminic acid, and a ceramide comprised of sphingosine d20:1/fatty acid C18:0 and sphingosine d18:1/fatty acid C18:0. The presence of ions at *m/z* 564 and 592 also indicated that the major ceramide species are sphingosine d18:1/fatty acid C18:0 and sphingosine d20:1/fatty acid C18:0. A characteristic ion, $(NeuAc-NeuGc-H)^-$ was observed at *m/z* 619. Interestingly, ganglioside X3 has two different species of sialic acid, NeuAc and NeuGc. The presence of the latter species was proven by TLC-immunostaining: X3 showed strong immunoreactivity with anti-NeuGc-containing ganglioside antibody (anti-HD-antibody) on a TLC plate (data not shown). Similar to X1 and X2, ganglioside X3 was sensitive to jack bean β-galactosidase (data not shown), indicating that in X3 the galactose at the non-reducing terminal was not substituted.

Based on the above observations, we concluded that the chemical structure of X3 was identical or similar to Chol-1β antigen as characterized by Giuliani *et al.* [6]. We subsequently prepared the standard GT1bα (Chol-1β) from GQ1bα using sialidase L and analysed GT1bα and X3 in parallel by TLC-immunostaining with anti-Chol-1. As shown in Fig. 5, GT1bα derived from GQ1bα by the sialidase L treatment was recognized by anti-Chol-1 and migrated at the same position as purified X3 ganglioside. Taken together, the chemical structure of ganglioside X3 was identified to be:



(III^6NeuAc,II^3NeuAc-NeuGc-GgOse4Cer: GT1bα).

Ganglioside X3 was novel hybrid type of ganglioside containing b-series ganglioside (GD1b) and α-series (GM1α) sugar chains. Accordingly, X3 is termed GT1bα.

Immunological reactivity of Chol-1 gangliosides with anti-Chol-1

The binding specificity of anti-Chol-1 was examined by ELISA (Fig. 6). Among three gangliosides, GM1α and GD1aα were found to react more strongly than GQ1bα. Immunoreactivities of purified GM1α (Panel A) and GD1aα (Panel B) were almost identical with those products derived from GD1α and GT1aα by the sialidase L treatment. This result further supported X1 and X2 being identical to GM1α and GD1aα, respectively. However, the immunoreactivity of X3 (Panel C) was much less than that of the standard GT1bα obtained from GQ1bα. This is most probably due to the difference in the molecular species of the sialic acid residue; in contrast to the standard GT1bα, X3 contained a NeuGc-NeuAc residue at the internal galactose residue.

Biosynthetic pathways of Chol-1 gangliosides

As shown in Fig. 7, GM1α, GD1aα, and GT1bα were also synthesized from asialo-GM1, GM1a, and GD1b, respectively, by the GalNAc α2-6sialyltransferase in the Golgi fraction of rat liver. Each product was shown to react with anti-Chol-1 and migrated to the positions corresponding to standard GM1α, GD1aα, and GT1bα (Fig. 6). The amounts of GM1α, GD1aα, and GT1bα detected were approximately 12.65, 0.38, and 1.12 pmol per assay (0.127, 0.004, and 0.011 pmol h^{-1} mg^{-1}), respectively. Under similar assay conditions, 2.6 pmol of GT1aα and 2.9 pmol of GQ1bα were synthesized [21]. No reaction products from GM1α, GD1aα, and GT1bα could be detected [21]. Combined with the previous work [21], it may be concluded that gangliosides containing a NeuAcα2-6GalNAc residue are the final products of ganglioside metabolism, and therefore the GalNAc α2-6sialyltransferase is the enzyme responsible for the termination of ganglioside synthesis. Fig. 8 summarizes biosynthetic pathways of Chol-1 gangliosides.

Discussion

We have previously characterized the chemical structures of two species of Chol-1 gangliosides from bovine brain as GT1aα ($IV^3NeuAc,III^6NeuAc,II^3NeuAc$-GgOse4Cer) [11] and GQ1bα ($IV^3NeuAc,III^6NeuAc,II^3NeuAc2$-GgOse4Cer) from bovine brain [12]. In this study, we have purified and characterized three novel antigens detected by anti-Chol-1 antiserum in the minor ganglioside fractions derived from the bovine brain ganglioside mixture. All antigens including GM1α, GD1aα and GT1bα, are novel and extremely minor components possessing a sialyl α2-6 *N*-acetylgalactosamine moiety. Interestingly, GT1bα contained two species of sialic acid, NeuAc and NeuGc. The presence of NeuGc in bovine brain ganglioside is not

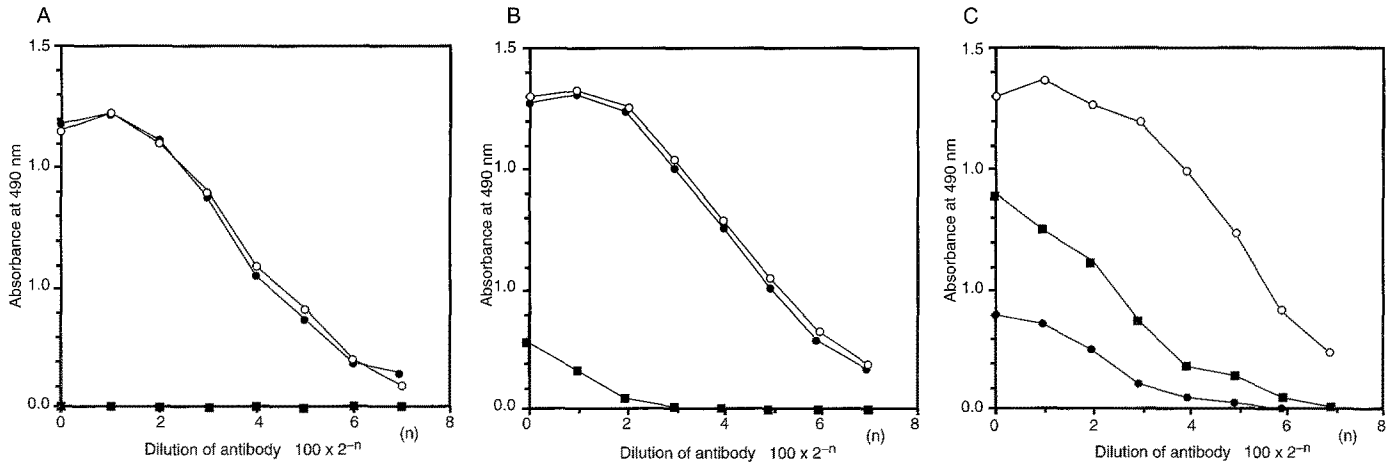


Figure 6. Reactivity of Chol-1 gangliosides with anti-Chol-1. A plastic 96-well plate was coated with antigens, 10 pmol. In *panel* (I), ●, purified X1; ○, GD1α treated with sialidase L; ■, GD1α. In panel (II), ●, purified X2; ○, GT1aα treated with sialidase L; ■, GT1aα. In panel (III), ●, purified X3; ○, GQ1bα treated with sialidase L; ■, GQ1bα.

unexpected since we have previously detected GT1b containing NeuGc in bovine brain [22].

Since all gangliosides with anti-Chol-1 reactivity possess a common NeuAcα2-6GalNAc saccharide structure, we can conclude that the NeuAcα2-6GalNAc structure is the epitope which is recognized by anti-Chol-1 polyclonal antibodies. For unknown reasons, GD1α ganglioside (IV^{3}NeuAc,III^{6}NeuAc-GgOse4Cer) was not reactive with anti-Chol-1 (Figs 5 and 6).

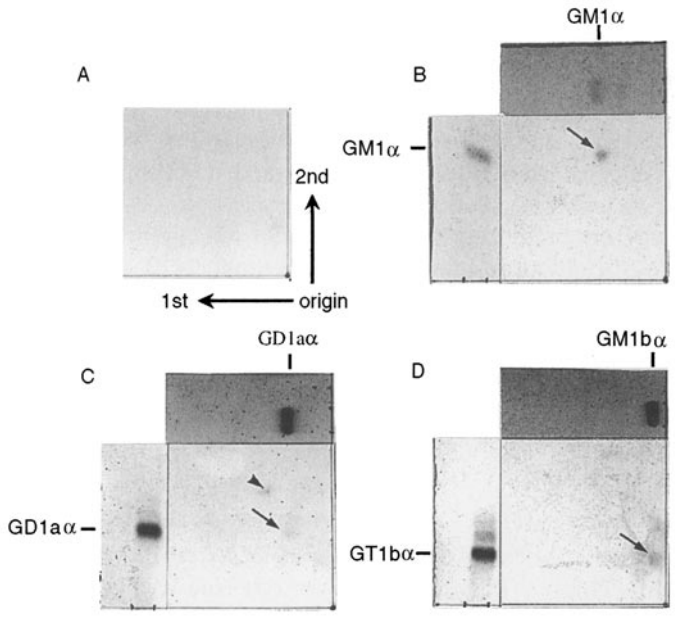


Figure 7. Identification of sialyltransferase products by two-dimensional TLC-immunostaining with anti-Chol-1. Reaction mixtures were applied to plastic plates and developed with solvent B in the first dimension and then solvent B in the second dimension. Panel A, product without any acceptor; panel B, product from asialo-GM1; panel C, product from GM1a; panel D, product from GD1b. Reaction products were stained with anti-Chol-1. The arrows in panel B, C, and D indicate the reaction product. The arrowhead in panel C shows the GM1α produced from GD1aα by sialidase L treatment.

We used a rat liver Golgi fraction to study the biosynthetic pathways of GM1α, GD1aα, and GT1bα gangliosides. It has been shown that the biosynthesis of gangliosides takes place in the Golgi by sequential addition of Gal, GalNAc and NeuAc to the growing oligosaccharide chains of the glycolipids [23]. Sandhoff and his colleagues characterized the biosynthetic pathways of a-, b-, c-, and α-series gangliosides in rat liver [24–27]. Our present and previous results showed that the five species of Chol-1 gangliosides are synthesized by the action of a GalNAcα2-6sialyltransferase as summarized in Fig. 8. It should be pointed out that all Chol-1 gangliosides including GM1α, GD1aα and GT1bα are the final products of ganglioside metabolism and the GalNAc α2-6sialyltransferase may be responsible for the termination of ganglioside synthesis. At present, however, it is not clear as to whether the syntheses of Chol-1 gangliosides are carried out by a single α2-6sialyltransferase enzyme or multiple α2-6sialyltransferases.

An immunohistochemical study with anti-Chol-1 showed that Chol-1 gangliosides were expressed on the cell bodies and nerve terminals of the cholinergic neurons in the rat central nervous system [3]. In contrast, using the monoclonal antibody GGR-41, specific for GT1aα and GQ1bα [28], GT1aα and GQ1bα were shown to be distributed at the cholinergic nerve endings [29, 30]. These observations suggested that distributed regions of GT1aα and GQ1bα were different from those of GM1α, GD1aα, and GT1bα. Each ganglioside may have characteristic distribution patterns and different biological

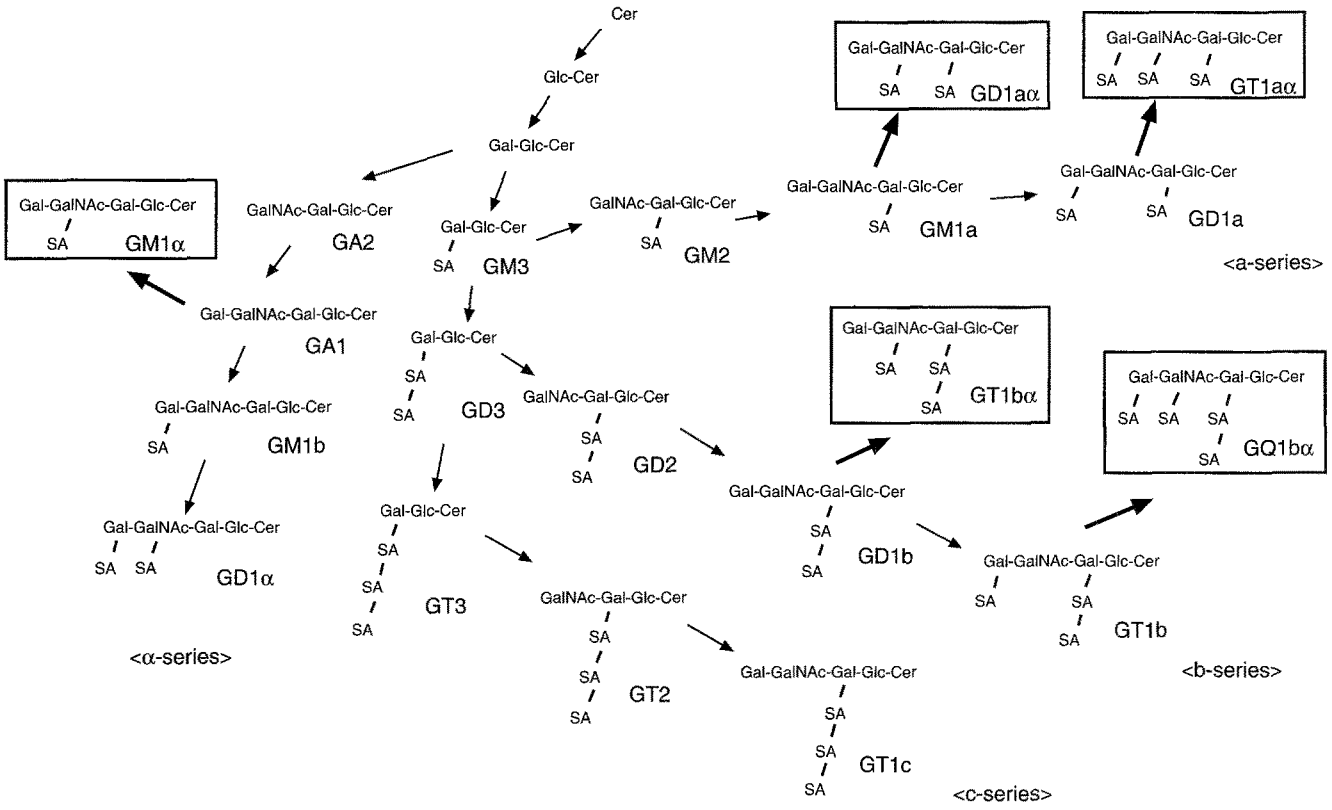


Figure 8. Biosynthetic pathways of Chol-1 gangliosides.

functions in the cholinergic neurons. Gangliosides have been shown to modulate the various enzyme activities including Ca^{2+}/calmodulin-dependent kinase [31], calmodulin-dependent nucleotide phosphodiesterase [32, 33], and the kinase associated with the epidermal growth factor and platelet-derived growth factor receptors [34, 35]. Very recently we have found that GQ1bα has the strongest activity for inhibition of ADP-ribosyltransferases and NAD^+ glycohydrolase of pertussis toxin [36]. The inhibition of ADP-ribosyltransferase activity by GQ1bα was very specific when compared to other structurally related gangliosides, suggesting that a new class of gangliosides, Chol-1, may have novel functions in the central nervous system.

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