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# Stage-specific expression of fuco-neolacto- (Lewis X) and ganglio-series neutral glycosphingolipids during brain development: characterization of Lewis X and related glycosphingolipids in bovine, human and rat brain

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We have purified and characterized a bovine brain pentaglycosylceramide as Lewis X and identified it in human and rat brain using anti-Lewis X (anti-SSEA 1) monoclonal antibody. Neutral glycosphingolipid expression in developing rat brain has been examined by digoxigenin immunostaining and TLC-immunostaining using anti-SSEA 1 and anti-$GgOse_4Cer$ (GA1) monoclonal antibodies. Five transient Lewis X-series bands were identified in brain at embryonic day 15 that disappear by postnatal day 5 (one disappears at embryonic day 18). Gangliotetraosylceramide (GA1) first appears at embryonic day 21 and increases in concentration with age until postnatal day 21. In addition, we have purified another minor brain neutral glycosphingolipid and tentatively identified it as a Lewis X-series glycolipid by gas chromatography-mass spectrometry analysis followed by TLC-immunostaining with anti-SSEA 1 antibody.

*Keywords*: neutral glycosphingolipid, brain development, stage-specific expression, gas chromatography-mass spectrometry

*Abbreviations*: Cer, Ceramide, GlcCer, Glc$\beta$1-1Cer; LacCer, Gal$\beta$1-4GlcCer; CTH, Gal$\alpha$1-4LacCer; $nLcOse_4Cer$, Gal$\beta$1-4GlcNAc$\beta$1-3LacCer; $nLcOse_5Cer$, Gal$\alpha$1-3$nLcOse_4Cer$; $GgOse_4Cer$, Gal$\beta$1-3GalNAc$\beta$1-4LacCer; DPA, diphenylamine-aniline-phosphoric acid; SSEA, stage-specific embryonic antigen; NGSL, neutral glycosphingolipid; TLC, thin-layer tomography; HPTLC, high performance thin-layer chromatography; GA1, gangliotetraosyleramide; SAT-2, sialytransferase-2; GalNAcT-1, galactosaminyltransferase-1; DIG-IS, digoxigenin-immunostaining; PMAAS, partially methylated alditol acetates; DCE, dichloroethane; TLC-IS, TLC-immunostaining; ($Le^x$), Lewis X; NK, murine natural killer.

## Introduction

A role for glycoconjugates in the development of the mammalian nervous system is not yet clearly defined though a high likelihood is inferred from the substantial variation of ganglioside expression and composition during vertebrate brain development [1–6]. The shift from simple to complex ganglioside synthesis observed during rat brain development is accompanied by a decrease in sialyltransferase-2 (SAT-2) activity and an increase in galactosaminyltransferase-1 (GalNAcT-1) activity [7]. Since few CNS neutral glycosphingolipids (NGSLs) other than galactosylceramide had been characterized or studied extensively [8, 9], we examined CNS NGSLs using the highly sensitive technique [10] of digoxigenin-immunostaining (DIG-IS) and found several new long chain (-CHO > 3–4) NGSLs in brain [11].

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In addition to mono-(Glc-/GalCer), di-(LacCer), tri-($GbOse_3$-$GgOse_3Cer$) and tetra-glycosylceramide ($GbOse_4$-/$nLcOse_4Cer$), we have characterized a tetraglycosyl- ($GgOse_4Cer$ or GA1; [12]) and a pentaglycosylceramide ($GbOse_5Cer$; [13, 14]) in bovine, human and rat brain. Recently we have purified another NGSL as a minor component of bovine brain with a TLC-migration close to GA1 and $nLcOse_5Cer$.

Here we describe the purification, characterization and stage-specific expression of this NGSL and GA1 during the development of rat brain. Additionally, we have purified another long chain NGSL (CHO > 5) from bovine brain and tentatively characterized it as a member of the Lewis X ($Le^x$)-series.

## Materials and methods

Bovine brain was collected from a local abattoir, human brain was obtained immediately after autopsy (within 4–10 h) following the accidental death of a healthy individual and rat brain was collected in our laboratory after decapitation. Monoclonal antibody MC 480 (anti-SSEA 1) was purchased from the Developmental Studies Hybridoma Bank (NICHHD and University of Iowa, IA). The gel materials for gas chromatography (10% DEGS-PS and 3% SE-30) were purchased from Supelco, PA, DEAE-Sephadex was from Pharmacia LKB Biotechnology (Sweden) and silicic acid and $\alpha$-fucosidase were purchased from Sigma Chemical Company (MO, USA). Precoated TLC plates were purchased from E. Merck (Germany). Standard NGSLs including $Le^x$ (kidney) and GA1, and the enzymes $\beta$-galactosidase and $\beta$-hexosaminidase from jack bean meal [15, 16] were purified and characterized in our laboratory.

### *Purification of the NGSL*

Materials and methods for the purification and characterization of the glycolipid are as published previously [14, 18]. Briefly, lipids were solvent-extracted from the acetone dried brain and gangliosides were separated from NGSLs by ion exchange (DEAE-Sephadex) chromatography. Individual NGSLs were purified by a silicic acid column using various concentrations of chloroform: methanol:ammonia and the collected fractions were resolved by TLC. Fractions containing the NGSL were pooled and further purified by another silicic acid column (0.8 × 42 cm) using chloroform:methanol:water (120:45:6; 120:50:7; 120:60:8; v/v) as solvents and the elution was collected as fractions. Purified NGSL fractions were checked by TLC, pooled and dried. The purity of the NGSL was examined by TLC resolution in two different solvent systems (chloroform:methanol:water and chloroform:methanol:3.0 N ammonia 60:40:9, v/v). The amount of the NGSL purified was estimated as approximately 200 $\mu$g per 40 g of acetone dried brain.

### *Chemical characterization*

Approximately 50 $\mu$g of the purified NGSL was methylated and acetylated [18, 19]. The partially methylated alditol acetates (PMAAs) were analysed with a DB-1 column on a Hewlett-Packard 5980 gas chromatograph attached to a 5972 Plus mass detector with a temperature programme of 80–170 °C at the rate of 30 °C per min and then raised to 240 °C at the rate of 4 °C per min. Fatty acids and the long chain base were analysed as methyl ester and trimethylsilyl derivatives, respectively.

### *Determination of specific anomeric linkage*

The anomeric carbohydrate linkage was determined by specific exoglycosidase (200 mU) digestion of the NGSL (20 $\mu$g) in 0.2 M citrate (pH 4.5) for $\alpha$-fucosidase (20) and 50 mM citrate (pH 4.2) for $\beta$-galactosidase and $\beta$-hexosaminidase containing 0.0375% and 0.1% sodium taurodeoxycholate, respectively, to a final volume of 250 $\mu$l. The reaction was terminated with four volumes of chloroform:methanol, 2:1 (v/v), after the incubation period and the lower phase was washed twice with the theoretical upper phase, dried and resolved by TLC.

### *Immunological identification*

Lipids from bovine, human and rat brain were extracted and the NGSLs were separated from gangliosides as described earlier. The NGSLs were acetylated and purified from phospholipids and other contaminating lipids by a florisil column [21]. After deacetylation the NGSLs were resolved by HPTLC and stained with anti-SSEA 1 antibody [22].

### *Developmental profile of brain NGSLs*

Embryonic day 15 to day 21 (E15–E21), postnatal day 1 to day 30 (P1–P30) and adult rat brains were collected and the NGSLs were purified from gangliosides and other contaminating lipids as described previously. Monoglycosylceramide was removed (approximately 90–95%) after acetylation using a florisil column eluted with dichloroethane (DCE):acetone, 22:3 (v/v), as a fraction (5–10 column volumes). The purified NGSL fraction eluted with DCE:acetone 1:1 (v/v) was resolved by HPTLC and examined by DIG-IS [10]. Briefly, the coated plate (0.5% poly-isobutyl methylacrylate in hexane) was incubated with 10 mM sodium periodate in 0.1 M acetate buffer (pH 5.5), washed thoroughly and allowed to react with DIG (1:1000) in the same buffer. It was then incubated with 1% BSA in PBS (37 °C for 30 min) followed by anti-DIG antiserum (conjugated with alkaline phosphatase; 1:200, in the same buffer) at 4 °C overnight. The excess antiserum was removed by repeated washings and the colour was developed using 5-bromo-4-chloro-

indolyl-phosphate (0.5 mg ml$^{-1}$ in 0.1 M glycine buffer, pH 10.4) as sub-strate.

*Immunochemical detection of developing brain NGSLs*

The NGSL purified from developing brain was resolved by HPTLC and the Le$^x$ and GA1 profiles were examined by using anti-SSEA 1 and anti-GA1 monoclonal antibody (generous gift from Dr Pam Fredman, University of Göteborg, Sweden). The specific NGSL band was visualized by TLC-immunostaining (TLC-IS) with diaminobenzidine stain as described previously [22].

*Immunocytochemical localization of GA1 and Le$^x$-series glycolipids in adult rat brain*

Monospecific polyclonal anti-GA1 antibody was prepared according to Taki *et al.* [23] and used for the immunocytochemical study. The prepared antibody appeared very specific for GA1 by ELISA and TLC-IS when assayed with GA1, GM1, nLcOse$_4$Cer and GalNAc-GA1 [24] as substrates. The brain Le$^x$-series glycolipid was localized using anti-SSEA 1 antibody (MC-480). Immunocytochemical identification of the two glycolipid antigens was carried out according to the method previously used to localize HNK-1 antigen in mice [25]. Briefly, rats were anaesthetized and perfused intra-aortically with 10 ml of PBS containing 2000 U of heparin followed by 0.1 M phosphate buffer (pH 7.4) containing 2% glutaraldehyde and 2% paraformaldehyde. Parasagittal sections (10 $\mu$m) were postfixed in cold acetone (−20 °C) for 10 min and stained as described [25] with the exception that the detergent Triton X-100 (0.2%) was replaced with 0.25% Tween 20 during the staining procedures.

Briefly, the fixed sections were washed with PBS, blocked with 1% normal goat serum and incubated with primary antibody (neat) for 24–48 h at 4 °C. The sections were then washed and incubated with the secondary antibody (1:100 dilution) for 30 min and sites of antibody binding were visualized with diaminobenzidine.

*Brain fucosyltransferase and α-fucosidase activity in relation to NGSL expression*

We have determined the fucosyltransferase (FucT3) and α-fucosidase activity in developing brain (E15–P5). Brain homogenate was used as the enzyme source. FucT3 was assayed using nLcOse$_4$Cer/LcOse$_3$Cer as the acceptor and $^{14}$[C]GDP-Fuc as the donor in the presence of HEPES buffer (pH 7.8), $MnCl_2$ (metal ion) and G3634A detergent [26]. After incubation (2 h at 37 °C), the activity was assayed by double chromatography or by Sep-Pak C-18 cartridge as described previously [27, 28]. Paranitrophenol-α-fucose (4 mM) in 0.2 M acetate buffer (pH 5.5) was used as the substrate for the α-fucosidase assay [29].

## Results

*Purification and characterization*

We have purified a bovine brain NGSL that appears to be homogeneous by TLC in two different solvent systems. The NGSL comigrates with the standard GA1 and nLcOse$_5$Cer in solvent system 'a' (containing water) but has a lower $R_f$ in an ammoniacal solvent system 'b' (Fig. 1a,b) indicating configurational dissimilarity with either of the standards. The carbohydrate molar composition determined as alditol acetates indicates the NGSL is a pentasaccharide (Glc:Gal:GlcNAc:Fuc, 1.0:1.8:0.82:0.89).

The GC-MS of the PMAAs revealed the presence of the following peaks: 2,3,4,6-tetra-*O*-methylgalactitol-1,5-diacetates; 2,3,4-tri-*O*-methylfucitol-1,5-diacetates; 2,4,6-tri-*O*-methylgalactitol-1,3,5-triacetates; 2,3,6-tri-*O*-methylglucitol-1,4,5-triacetates and 6-*O*-methyl-2-acetamido-2-deoxyglucitol-1,3,4,5-tetraacetates (Fig. 2), thus indicating that the fucose and galactose are two terminal carbohydrates attached to the GlcNAc by 1-3 and 1-4 linkage, a typical Le$^x$-series ligand. Several minor non-carbohydrate peaks were also observed which appear to be due to background noise as well as reagent contaminants. The parent NGSL was resistant to β-galactosidase but

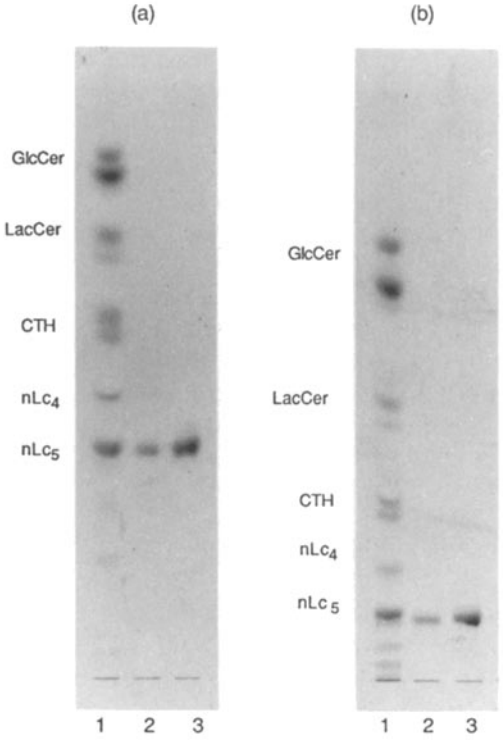
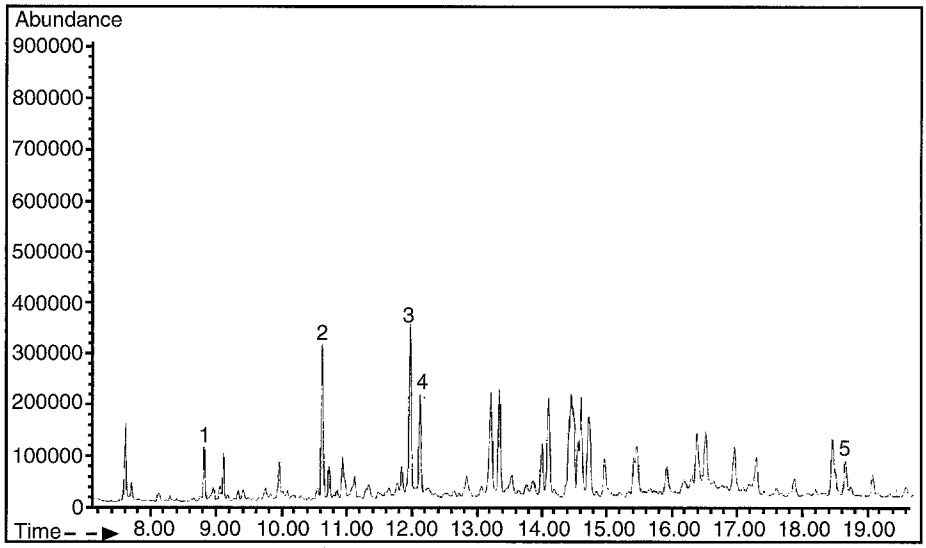



**Figure 1.** TLC of purified bovine brain NGSL (Putative Le$^x$). The plates were developed in (a) chloroform:methanol:water and (b) chloroform:methanol:3.0 N ammonia, 60:40:9 (v/v), and visualized by DPA-spray [14]. Lane 1, standard NGSLs; lane 2, putative Le$^x$; lane 3, GgOse$_4$Cer.

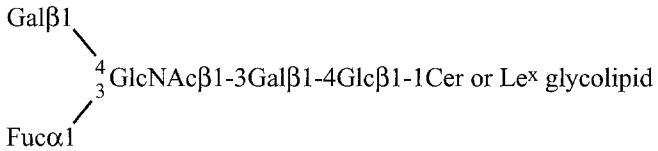



| Peak No. | E.I.-M.S. fragment ions | Linkage | RT* | Nature of carbohydrate |
|---|---|---|---|---|
| 1 | 89,131,175 | terminal | 8.813 | Fucose |
| 2 | 118,161,205 | terminal | 10.642 | Galactose |
| 3 | 118,161,233 | 4 | 11.992 | Glucose |
| 4 | 118,161,234 | 3 | 12.114 | Galactose |
| 5 | 117,159,261,346 | 3,4 | 18.662 | NAcGlucosamine |

*Retention time in minutes

**Figure 2.** GC-MS of PMAAs prepared from NGSL. Conditions are as mentioned in text under Materials and methods. Individual peaks are marked and their retention time along with fragment ions identified by EI-MS are listed as above.

susceptible to α-fucosidase digestion. The hydrolysed product (tetraglycosylceramide) was converted to tri-, di- and GlcCer by sequential digestion with β-galactosidase, β-hexosaminidase and β-galactosidase (Fig. 3). The GC-MS of the fucosidase-hydrolysed product showed the disappearance of the fucose (terminal) and 6-*O*-methyl-glucosaminyl peaks and the appearance of the 3,6-di-*O*-methyl-2-acetamido-2-deoxyglucitol-1,4,5-triacetates peak (results not shown). indicating a $nLcOse_4Cer$ structure. This evidence clarifies that fucose is directly attached to the glucosamine of $nLcOse_4Cer$ by α1-3 linkage. The major fatty acyl groups were stearic (32.5%), oleic (25%) and eicosenoic (23.3%) with less palmitic (7.4%), arachidic (6.2%) and behenic (5.6%) acid. Sphingenine was the major (92%) base identified by GC together with sphinganine as the minor (8%). Our results suggest the following structure for the NGSL:




The NGSL has also been identified in human and rat brain by TLC-IS using anti-SSEA 1 antibody (Fig. 4).

*Developmental studies*

The NGSL developmental profile in rat brain examined by DIG-IS (Fig. 5) indicates the following changes: (1) the appearance of several long chain NGSLs in early embryonic stages that disappear at P1 and onwards (marked with an arrow): they are identified as $Le^x$ and its related glycolipids (Fig. 6) by TLC-IS with anti-SSEA 1 antibody; (2) a transient band near triglycosylceramide (identified by TLC-$R_f$ compared to standard) in early embryonic stages that reappears at P10 and increases steadily until P21 (marked with an arrow); (3) a band near pentaglycosylceramide that has been identified as GA1 (marked by an arrow) by TLC-IS with anti-GA1 antibody (Fig. 7) that increases until P21; and (4) the appearance of a NGSL with a TLC-$R_f$ higher than Glc/GalCer (marked with an arrow). Those bands with higher TLC-$R_f$ appeared to be GalCer related NGSLs when characterized by GC (after purification; Dasgupta and Hogan, unpublished).

Immunocytochemical examination of GA1 and $Le^x$ in adult rat brain localized GA1 primarily in myelin.

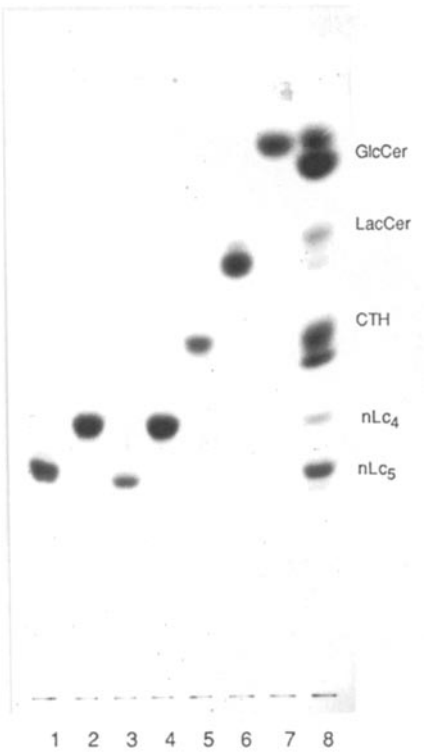



**Figure 3.** Stepwise exoglycosidase hydrolysis of NGSL. The plate was developed in chloroform:methanol:water, 60:40:9 (v/v), and visualized by DPA-spray [14]. Lane 1, $GgOse_4Cer$; lane 2, $nLcOse_4Cer$; lane 3, purified bovine brain NGSL; lane 4, lane 3 + $\alpha$-fucosidase; lane 5, lane 4 + $\beta$-galactosidase; lane 6, lane 5 + $\beta$-hexosaminidase; lane 7, lane 6; + $\beta$-galactosidase; lane 8, standard NGSLs.

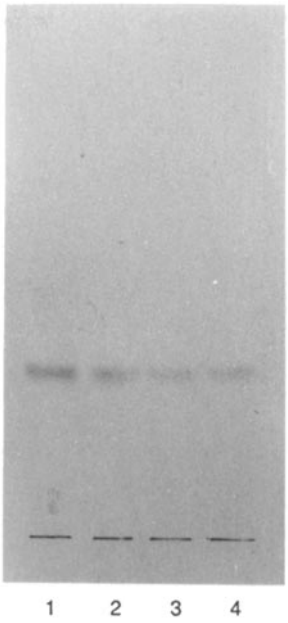



**Figure 4.** TLC-IS of bovine, human and rat brain NGSL with anti-SSEA 1 antibody. The plate was developed in chloroform:methanol:water, 60:40:9 (v/v), and stained with anti-SSEA 1 antibody. Lane 1, standard $Le^x$; lane 2, bovine brain NGSLs; lane 3, human brain NGSLs; lane 4, rat brain NGSLs; lanes 2–4. The samples were dissolved in 100 $\mu$l of solvent per g of (wet) brain and 15 $\mu$l was spotted on the individual lane.

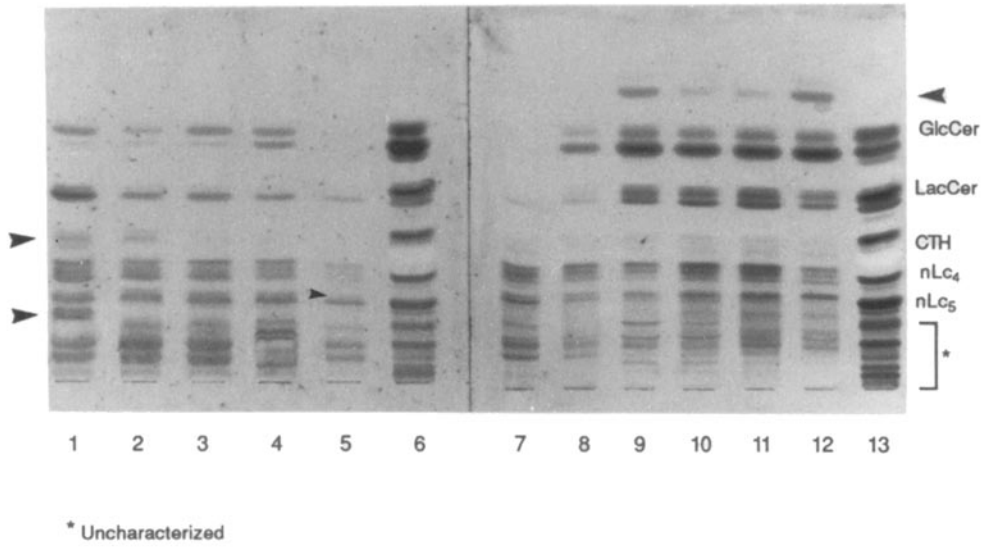



**Figure 5.** DIG-IS of developing rat brain NGSLs. The plates were developed in chloroform:methanol:water, 60:40:9 (v/v), and stained by DIG-IS as described in the text. The samples were dissolved in 100 $\mu$l of solvent per g (wet) brain and spotted as follows: Lanes 1–5, 30 $\mu$l and lanes 7–12, 15 $\mu$l. Lane 1, E15; lane 2, E18; lane 3, E21; lane 4, P1; lane 5, P5; lanes 6 and 13, standard NGSLs; lane 7, P10; lane 8, P15; lane 9, P21; lane 10, P25; lane 11, P30; lane 12, adult.

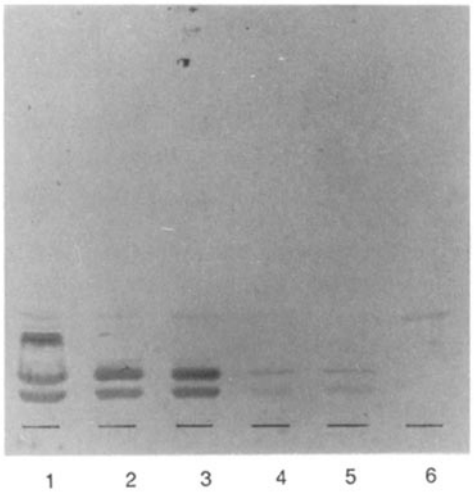



**Figure 6.** TLC-IS of developing rat brain NGSLs with anti-SSEA 1 antibody. The plate was developed in chloroform:methanol:water, 60:40:9 (v/v), and stained with anti-SSEA 1 antibody. The samples were dissolved in 100 $\mu$l solvent per g (wet) brain and 30 $\mu$l was spotted in each lane. Lane 1, E15; lane 2, E18; lane 3, E21; lane 4, P1; lane 5, P5; lane 6, standard $Le^x$ (kidney).

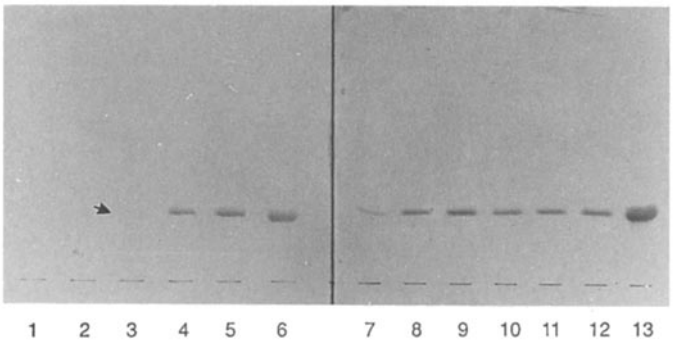



**Figure 7.** TLC-IS of developing rat brain NGSLs with anti-GA1 antibody. The plates were developed in chloroform:methanol:water, 60:40:9 (v/v), and stained with anti-GA1 antibody (monoclonal). The samples were dissolved in 100 $\mu$l solvent per g (wet) brain and in order to visualize the band at E21 they were spotted as follows: Lanes 1–5, 60 $\mu$l and lanes 7–12, 15 $\mu$l. Lane 1, E15; lane 2, E18; lane 3, E21; lane 4, P1; lane 5, P5; lanes 6 and 13, standard GA1; lane 7, P10; lane 8, P15; lane 9, P21; lane 10, P25; lane 11, P30; lane 12, adult.

Specific linear filamentous staining of white matter in both cerebellum and cerebral cortex (Fig. 8a,b) was observed with anti-GA1 antibody. Antibody to SSEA 1 stained presumed dendritic processes in the molecular layer of the cerebellum (Fig. 9a). This antibody, in contrast to GA1, failed to react with myelin in white matter (Fig. 9a). Anti-SSEA 1 also failed to bind to cerebellar Purkinje cells and yielded only faint coloration of some neurons in cortical grey matter (Fig. 9b).

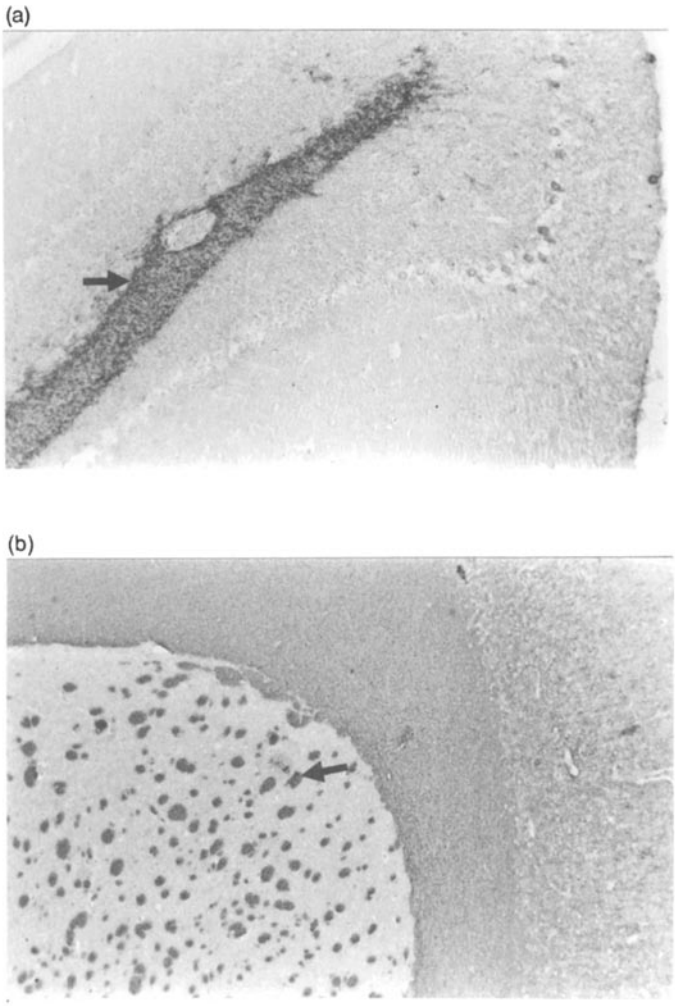



**Figure 8.** Immunohistochemical staining of adult rat brain (a) cerebellum and (b) cerebral cortex with anti-GA1 antibody. Brain tissues (a) cerebellum and (b) cerebral cortex from an adult rat were stained with anti-GA1 (monospecific/polyclonal) antibody as described in the text.

*Fucosyltransferase and α-fucosidase activity in early brain development*

We have examined the FucT3 and $\alpha$-fucosidase activity in E15 to P5 brain in relation to the transient expression of the $Le^x$-series NGSLs. The FucT3 ($nLc_4$) showed a high initial activity in the embryonic brain (E18 and E21) with optimum (140 pmol) activity at E21 and a rapid decrease (25 pmol) immediately after birth (P1). An intermediate (80 pmol) activity was observed in P15- to P27-day brain with a decrease at the adult stage. Only one intermediate active peak (80 pmol) at E21 was obtained for $nLc_3$. The activity declined (30 pmol) at P1 and remained steady thereafter (Fig. 10a). There were two identical activity peaks for $\alpha$-fucosidase at E21 and P5: they gradually decreased with increasing age (Fig. 10b). The activity curve is consistent with the rapid disappearance of the

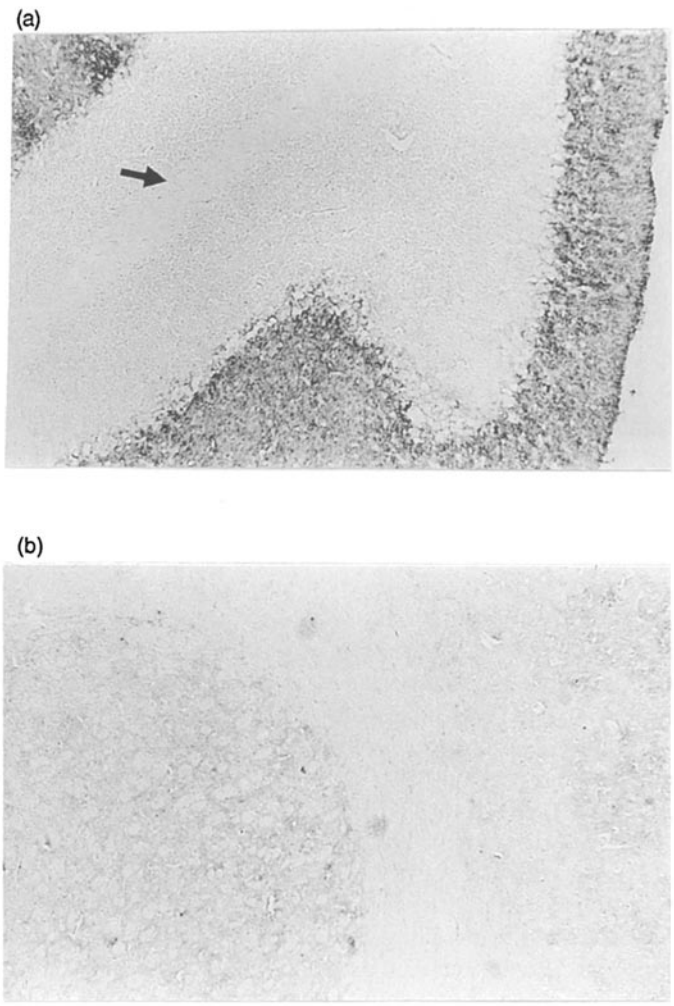



**Figure 9.** Immunohistochemical staining of adult rat brain (a) cerebellum and (b) cerebral cortex with anti-SSEA 1 antibody. Brain tissues (a) cerebellum and (b) cerebral cortex from an adult rat were stained with anti-SSEA 1 antibody as described in the text.

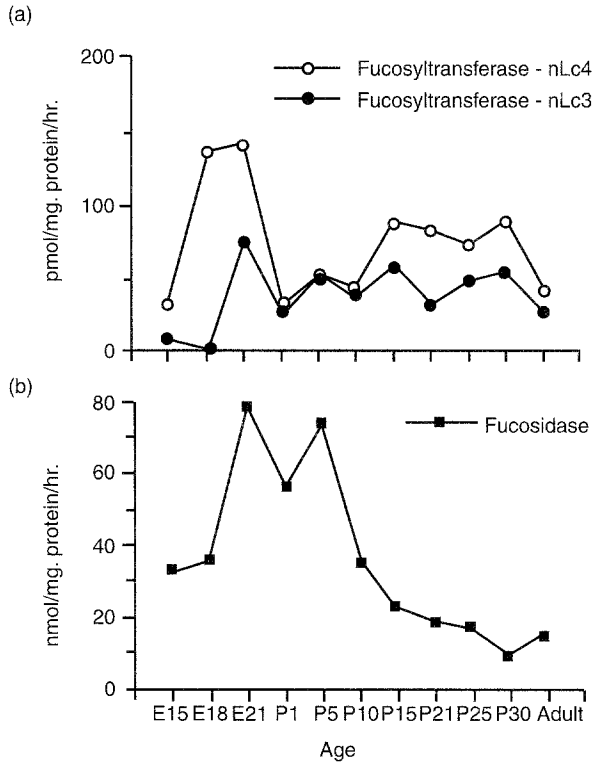



**Figure 10.** Fucosyltransferase (a) and fucosidase (b) activity in developing rat brain. (a) fucosyltransferase (FucT3) and (b) $\alpha$-fucosidase activity in developing brain were assayed from E15 to P30 and adult using whole brain homogenate as the enzyme source. The method of assay is described in the text.

Le$^x$-series NGSLs in developing brain (P1) with less FucT activity detected at these stages as well. The abundance of Le$^x$-series NGSLs (as observed by immunostaining) at E15 may be attributed to a strong brain FucT3 activity during early stages of embryonic development. The technical difficulties in obtaining the tissues (prior to E15) prevent us from determining the related enzyme activity.

*Tentative characterization of a minor brain NGSL*

We have purified a minor brain NGSL and tentatively characterized it as a Le$^x$-series glycolipid. GC-MS of the PMAAs prepared from the NGSL indicated that the terminal fucose (as 2,3,4-OMe$_3$Fuc) and galactose (2,3,4,6-OMe$_4$Gal) are attached to the GlcNAc (6-

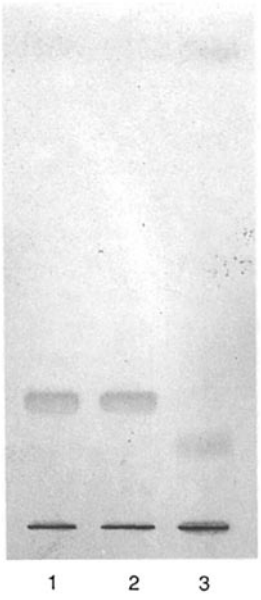



**Figure 11.** TLC-IS of bovine brain Le$^x$ and the purified NGSL with anti-SSEA 1 antibody. The plate was developed in chloroform:methanol:water, 60:40:9 (v/v), and stained with anti-SSEA 1 antibody. Lane 1, standard kidney Le$^x$; lane 2, putative Le$^x$ from bovine brain; lane 3, purified unknown brain NGSL.

OMeGlcNAcMe) along with other carbohydrates (results not shown). We further confirmed the terminal $Le^x$-group of the NGSL by TLC-IS with anti-SSEA 1 antibody (Fig. 11).

## Discussion

Fucolipids are found in rapidly proliferating tissues (e.g. salivary glands, bone marrow, testis, etc.) and are not usually considered in such tissues with low rates of cell division as brain, myocardium and kidney [30]. We have purified a minor fucolipid from bovine brain and characterized it as a $Le^x$ glycolipid by GC-MS and stepwise exoglycosidase hydrolysis. Using anti-SSEA 1 antibody, we have also identified the NGSL in adult rat and human brain. $Le^x$ glycolipid has previously been characterized in human brain and has been considered as a minor (1.3–6.5 nmol $g^{-1}$) and a major (32–40 nmol $g^{-1}$) NGSL in adult [31] and fetal [32] human brain, respectively. The $Le^x$ determinant is highly expressed during early mammalian development [33] suggesting a role for this cell surface carbohydrate in the cell interactions occurring in embryogenesis [33–35]. The early specific expression of $Le^x$ in the preimplantation embryo (8–32 cell stages), its disappearance after cell compaction and the inhibition of compaction by fuconeolacto-lysyllysine conjugate strongly suggest that it is important in embryogenesis [36]. We have observed the expression of $Le^x$-series (X-series) glycolipids in embryonic rat brain and their disappearance at P5. This is consistent with the previous developmental study of Yamamoto *et al.* [37] carried out upon pooled embryonic (E15–E17) brain. Our systematic examination of NGSL expression revealed a transient band ($R_f$ near hexaglycosylceramide) at E15 (both by DIG-IS and TLC-IS) that had not been previously reported. Hence, we pursued the study of $Le^x$ in early neuroembryogenesis. Although the X-series glycolipids have also been detected in embryonic brain of other vertebrates (e.g. calf, chicken, mouse, rabbit and human [37]), the sialo-derivative of the fucoglycolipid has not been identified in brain. Brain fucolipid expression is regulated by two key enzymes, FucT and $\alpha$-fucosidase, whose developmental profiles coincide with the expression of X-series glycolipid.

Additionally, a long chain NGSL (CHO $>$ 5) has been purified and tentatively characterized as X-series. This NGSL could be a stage-specific antigen that has been expressed in the embryo(s) and exists as a minor component in adult brain or it may be expressed during later developmental stages (late postnatal and adult) in a minor concentration (beyond the detection limit) and/or its expression is obscured by other long chain brain NGSLs. Precise structural elucidation of this NGSL and other embryonic X-series glycolipids will resolve their expression specificity.

The details of the evolution of brain NGSLs are further revealed by DIG-IS of developing brain as follows: (a) several new long chain NGSLs have been identified; (b) a transient band appears near triglycosylceramide at early embryonic stages, E15–E21, and a band in this $R_f$ region reappears at P10; and (c) a NGSL with a higher $R_f$ than Glc/GalCer has been detected that appears to be related to GalCer. The identity of these NGSLs, stage-specifically expressed during development, and may well be critically important effectors, is currently being studied.

A band migrating near pentaglycosylceramide (identified as GA1 by TLC-IS) appears at E21 and increases until P21. GA1, previously reported in mouse myelin by immunohistology [22], has also been localized in adult rat myelin. Since GA1 is a marker for murine natural killer (NK) cells [38, 39] and neuropathies mediated by anti-GA1 antibody have been reported [40–44], the appearance of GA1 in E21 brain is intriguing. We previously characterized GA1 in normal human brain and identified it in postnatal brain [11]. Expression of GA1 in E21 brain and its localization in myelin strongly supports our previous hypothesis that a regulatory mechanism of GA1-mediated myelinogenesis exists [11]. Alternatively, GA1 may be a major NGSL in oligodendrocytes that synthesize myelin or an important antigen/marker for microglial cells (since GA1 and related glycolipids are considered as lymphocyte specific antigens) that might actively participate in myelin compaction.

In summary, we have characterized several NGSLs in vertebrate brain including $Le^x$ and GA1 and established a stage-specific expression of these and other NGSLs during brain development. Even though $Le^x$ and GA1 are expressed during critical stages of brain development and their localization in brain appears to be site-specific, their precise regulatory function as brain molecules in relation to brain development and disorders has not been defined. A complete characterization of NGSLs and delineation of their regulation in brain development and disorders will eventually clarify the function(s) of these amphipathic cell surface molecules.

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