Natural Occurrence of Ganglioside Lactones

ISOLATION AND CHARACTERIZATION OF GD1b INNER ESTER FROM ADULT HUMAN BRAIN*

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Laura Riboni, Sandro Soninno, Domenico Acquotti, Anna Malesci, Riccardo Ghidoni, Heinz Egges, Salvatore Mingrino, and Guido Tettamanti

From the Study Center for the Functional Biochemistry of Brain Lipids, Department of Medical Chemistry and Biochemistry, Medical School, University of Milano, 20133 Milano, Italy, the Istitut für Physiologische Chemie, Universität Bonn, Bonn, Federal Republic of Germany, and the Division of Neurosurgery, University Hospital, Padua, Italy

A new ganglioside containing an inner ester linkage was extracted from adult brain specimens, obtained at the time of surgery on 51-70-year-old subjects, purified, and analyzed. It contains glucose, galactose, N-acetylgalactosamine, an N-acetylneuraminic acid in the molar ratio 1:2:1:2, but, on ion-exchange chromatography, behaves as a monosialoganglioside.

Structural analyses showed its basic neutral glycosphingolipid core to be ganglio-N-tetraose ceramide, carrying a disialosyl residue on the 3-position of internal galactose. Sialidase degradation and chemical analysis of the products obtained after alkaline treatments suggested one sialic acid residue to be involved in an ester linkage.

Fast atom bombardment-mass spectrometry indicated the presence of an inner ester linkage between the carbonyl group of the external sialic acid residue and a hydroxyl group of the internal one.

On these bases, the new ganglioside can be assumed to be a GD1b, in lactonic form. This ganglioside is present only in trace amounts in the brain of infants, but its content increases with age, reaching a value of 3.5% of total sialic acid in 51-70-year-old subjects.

Gangliosides are sialic acid-containing glycosphingolipids that reside in the plasma membranes of vertebrate cells (1); their oligosaccharide chains, protruding from the cell surface, are assumed to be directly involved in membrane recognition phenomena and in the process of intercellular communication (2). The sialic acid residue(s) of gangliosides may play a fundamental role in these events. In fact, the sialic acid carbonyl groups are dissociated at physiological pHs, leading to the presence of negative charges on the membrane surface. The number of negative charges provided by gangliosides may vary either due to a change in number of sialic acids/ganglioside molecule or to the formation of inner ester linkages involving the carbonyl group, with the maintenance of the sialic acid residues.

The possible esterification of the carbonyl group of a sialic acid with one of the hydroxyl groups present in the ganglioside molecule, with the concomitant formation of a ganglioside lactone, was proposed by Kuhn and Mulder in 1964 (3) and investigated in the following years. Wiegandt (4) reported that gangliosides containing a sialic acid residue in α2→8 linkage with a second sialic acid could be expected to form lactone structures. Afterwards, Evans and McCluer (5) postulated the presence of an inner ester of GM3 ganglioside in extracts from bovine adrenal glands. The same authors observed that some of the sialic acid residues present in mouse and rat brain gangliosides were labile in alkaline media and reducible with sodium borohydride (6). This led to the hypothesis of a natural occurrence of ganglioside derivatives, in which the sialic acid carbonyl group was involved in the formation of inner (lactones) or external esters. However, there are still no reports in the literature providing a definite proof of the existence of ganglioside lactones in vivo.

In this paper we produce definite evidence of the presence, in nature, of a ganglioside in lactonic form; this ganglioside, provisionally coded GD1b-L, is a derivative of GD1b and has been isolated and purified from specimens of adult human brain.

EXPERIMENTAL PROCEDURES

RESULTS AND DISCUSSION

The two-dimensional TLC analysis of gangliosides from adult human cerebral cortex is reported in Fig. 1, the relative diagrammatic representation together with the codes recently introduced (7) are also given. The methodology allows the recognition of alkali-labile gangliosides which, after intermolecular

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*The abbreviations used are: GM3, NeuAcα2→3Galα1→4Glcβ1→1′Cer; GD1, Galβ1→3GalNAcβ1→4(NeuAcα2→3)Galβ1→4Glcβ1→1′Cer; GM2, Galβ1→3GalNAcβ1→4(NeuAcα2→3)Galβ1→4Glcβ1→1′Cer; GalNAc-GM1; GalNAcβ1→4Galβ1→3GalNAcβ1→4(NeuAcα2→3)Galβ1→4Glcβ1→1′Cer; Nuca2→8-GalNAcβ1→4Galβ1→3GalNAcβ1→4(NeuAcα2→3)Galβ1→4Glcβ1→1′Cer; Cer, ceramide; HPTLC, high performance thin-layer chromatography; GC-MS, gas chromatography-mass spectrometry; FAB-MS, fast atom bombardment-mass spectrometry.

†Portions of this paper (including "Experimental Procedures," Tables I-IV, and Figs. 1-6) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9550 Rockville Pike, Bethesda, MD 20814. Request Document No. 85M-4185, cite the authors, and include a check or money order for $3.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
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diate ammonia treatment, originate spots lying far from the diagonal where the alkali-stable gangliosides are located. Among the major components one spot, coded F1, originates after ammonia treatment from an alkali-labile ganglioside, coded GDlb.L. During the first chromatographic run GDlb.L migrates in the region between GM1 and GDls and, therefore, can be easily confused with other gangliosides (like GDlb, Fuc-GM1, or GalNAc-GM1) migrating in that area, when analyzed by conventional mono-dimensional TLC. The TLC $R_F$ of F1, originated from GDlb.L, does not correspond to any of putative parent gangliosides present in human brain, it being slightly lower than that of GDlb. Moreover, a second spot, coded I1 (present in low amounts) and showing the same $R_F$ of standard GDlb in the second run, can be detected on the vertical corresponding to GDlb.L after ammonia treatment. An identical chromatographic behavior was observed when GDlb lactone (8), prepared semisynthetically, was submitted to the same two-dimensional TLC analysis. In that case, the first spot formed by ammonolysis (and possibly corresponding to F1) was proven to be the amide derivative of GDlb (GDlb-amide), while the second one (which might correspond to I1) was the parent GDlb originated by hydrolysis. On the basis of these behavioral similarities, we posed the working hypothesis that GDlb.I might be GDlb ganglioside in lactonic form occurring naturally in animal brain.

Following this hypothesis we began collecting evidence of the natural occurrence of this compound and then determined its chemical structure. Human brain ganglioside quantification was achieved by densitometric scanning of the two-dimensional TLC plates after ammonia treatment. Since in these conditions GDlb.L is mainly (>90%) transformed into the F1 spot, GDlb.L content values were obtained from F1 ones. GDlb.L content was found to be strictly correlated with the age of the subject (see Table I) and increased from 0.16% in the 0–10-year group to 3.48% in the 51–70-year group. The differences in the age groups were statistically significant. When analyzed in parallel, GDlb ganglioside increased from 7.85% in the 0–10-year group to 20.29% in the 11–30-year group, remaining nearly constant thereafter. These data are in good agreement with previous literature reports (9–11). As shown in Table II the presence of GDlb.L (detected as F1) has already been reported in ganglioside mixtures extracted both from mammalian and avian brains (8, 38), where it ranged from 0.5 to 1.9% of total lipid-bound sialic acid. It should be noted that in all the investigated species, including human, no linear relationship between the content of GDlb.L and that of GDlb was present. In fact, in all the analyzed mixtures the ratio between GDlb.L and GDlb is quite variable (Tables I and II). All this makes unlikely the hypothesis of any artificial and spontaneous transformation (possibly lactonization) of GDlb into GDlb.L, this process should originate constant GDlb.L/GDlb ratios, not depending on the GDlb content in the tissue.

The possible artificial formation of GDlb.L throughout the procedure used for extraction and purification of gangliosides was checked. To this purpose specimens of human cerebral cortex were submitted in parallel to ganglioside extraction and purification by tetrahydrofuran/phosphate buffer or chloroform/methanol (12) as extracting solvent systems. F1 was present in the mixtures obtained by both procedures, thus suggesting the absence of the tetrahydrofuran/phosphate buffer extraction in originating GDlb.L as a non-natural ganglioside derivative. Moreover, samples of pure GDlb, a mixture of standard gangliosides, and an alkali-treated sample (0.1 M NaOH in methanol, room temperature, overnight) of human brain ganglioside mixture were kept in tetrahydrofuran/phosphate buffer solution at room temperature for 24 h and then submitted to two-dimensional TLC analysis. In all cases neither F1 nor other alkali-labile gangliosides were shown to be present.

All this indicates that GDlb.L is not artificially originated but constitutes an authentic product of brain metabolism. To date, it cannot be stated if such a compound is formed via specific enzymatic action or by the establishment of chemically favorable physiological conditions. Since GDlb.L content is higher in old rather than in young subjects, it was isolated from a pool of human brain ganglioside mixtures (8.2 mg, as lipid-bound sialic acid) from subjects aged 51–70 years. Fig. 2 shows the relative fractionation by silica gel column chromatography. After purification, 200 µg of GDlb.L (as sialic acid) corresponding to a yield of 70% were obtained. The purity of the compound, assessed as described under "Experimental Procedures," was higher than 96%. Compositional analyses revealed that GDlb.L contained glucose, galactose, N-acetylgalactosamine, N-acetyleneuraminic acid, long chain base, and fatty acid in a molar ratio (referred to glucose) of 1:0.6:2.05:9.2:1:87:1:11:9:98 (theoretical, 1:2:1:2:1:1) (see Table III). Partial acid hydrolysis, permethylation, and chromium trioxide analyses indicated for GDlb.L a structure with the neutral glycolipid core Galβ1–3GalNAcβ1–4Galα1–4Glcβ1–1 Cer, in which the hydroxyl group at position 3 of the inner galactose unit is linked to a NeuAca2–5NeuAca2→ disialosyl residue. This structure corresponds to that of GDlb ganglioside. Therefore, the structural difference between GDlb and GDlb.L has been cancelled by both the acidic conditions required for sugar and lipid analyses and the alkaline conditions used for permethylation analysis. In addition, when GDlb.L was treated with NaOH, it changed its chromatographic behavior and migrated exactly as GDlb ganglioside (Fig. 3); no difference was found in the chemical composition between GDlb.L/NaOH and GDlb (see Table III). Instead, when GDlb.L was submitted to ammonia treatment, a compound with a TLC $R_F$ intermediate between GDlb.L and GDlb was formed (Fig. 3). The lipid and neutral saccharide composition of GDlb.L/NH₄ was the same as that of GDlb (see Table III) while one of the two sialic acid residues had a chemical structure different from that of N-acetyleneuraminic acid. In fact, when released by mild acid methanalysis and derivatized as Me₃Si, it displayed a GLC retention time (1.75) different from that corresponding to standard N-acetyleneuraminic acid (stated as 1.00). The mass spectrometry spectrum of this particular sialic acid derivative is reported in Fig. 4. The spectrum, which shows a molecular ion $M^+$ minus CH₃ at $m/z$ 586, and a series of ions as those at $m/z$ 327, 405, and 283 differing 15 units from those obtained from the standard derivative of sialic acid (13) can be correlated to the amide derivative of N-acetyleneuraminic acid, as proposed in the same figure. The only possibility for the formation of an amide derivative after ammonia treatment of GDlb.L is the involvement of a sialic acid carboxyl group in an ester linkage. In fact, in the presence of ammonia, tais linkage undergoes a process of ammonolysis with the concurrent formation of the amide derivative. Of course, these results are consistent with the presence of either an inner or an external ester.

The behavior of GDlb.L on DEAE-Sepharose column chromatography was the following. GDlb.L was retained by the column in the course of the methanol-washing step, as expected for all gangliosides, and was eluted with 4 mM sodium acetate, the molarity being used to elute monosialogangliosides from the resin. Conversely, GDlb as well as other disialogangliosides processed in parallel were eluted from the column using 50 mM sodium acetate (Fig. 5). This means that, in spite
of the presence of two sialic acid residues in the molecule, GD
\textsubscript{lb-L} behaves as a monosialoganglioside indicating that only one sialic acid carboxyl group is actually available for interacting with the resin.

Upon sialidase treatment, GD\textsubscript{lb-L} was resistant to the enzyme action, in contrast with GD\textsubscript{lb} and GD\textsubscript{lb-L}/NaOH which were completely converted to GM\textsubscript{3} under the same conditions. It is worth remembering that a prerequisite for sialidase action is the presence of a free carboxyl group on the sialic acid residue (14). Therefore, the resistance of GD\textsubscript{lb-L} to sialidase, as opposed to the susceptibility of GD\textsubscript{lb}, suggests that the chemical difference within the two substances concerns the external sialic acid residue.

The results hitherto reported unequivocally postulate the presence in the GD\textsubscript{lb-L} molecule of an esterification of the sialic acid carboxyl group. The direct proof that GD\textsubscript{lb-L} contains an ester linkage and that the ester linkage involves an intramolecular alcoholic function was provided by fast atom bombardment-mass spectrometry analysis. The fast atom bombardment-mass spectrometry spectrum of GD\textsubscript{lb-L} is reported in Fig. 6, together with the scheme indicating the major pathways of fragmentation. The most significant ions were the pseudomolecular ions M-1, corresponding to the molecular species that contain C18 and C20 sphingosine, observed at \( m/z \) 1817 and 1845, 18 units below those of GD\textsubscript{lb} measured under identical conditions (15) and the ions at \( m/z \) 581 and 537, deriving from the disialosyl residue minus 18 (H\textsubscript{2}O) and to 581 minus CO\textsubscript{2}, respectively. The loss of 18 units, corresponding to a molecule of water, in the GD\textsubscript{lb-L} molecule with respect to GD\textsubscript{lb} indicates that the ester linkage involves one of the ganglioside hydroxyl groups. Moreover, the presence of ions corresponding to a sialosyl residue still lacking 18 units indicating that the lacton ring resides in the same disialosyl residue. As in the spectrum there are hardly any significant peaks at 308 and 290 \( m/z \), corresponding to sialic acid, we can postulate that the ester linkage involves the carboxyl group of the external sialic acid and one of the hydroxyl groups of the internal sialic acid residue. By the use of molecular models it could be hypothesized that the hydroxyl groups in positions 7 or 9 are involved. On the basis of all the provided evidence it can be suggested that GD\textsubscript{lb-L} is a GD\textsubscript{lb} carrying an inner ester linkage between the carboxyl group of the external sialic acid residue and a hydroxyl group of the internal sialic acid residue.

The composition of human brain gangliosides has been extensively studied; however, to the best of our knowledge, the present study is the first to report the presence, in human brain ganglioside composition, together with the conditions employed for ganglioside extraction and analysis, are reported in Table IV. In nine of these (11, 17, 18, 20, 22, 24, 26, 28, 29) alkaline (NaOH and KOH) methanolysis or hydrolysis was used to get rid of contaminant phospholipids in the course of the ganglioside purification; under these conditions GD\textsubscript{lb-L} is completely converted into GD\textsubscript{lb}. In other reports (16, 19, 23, 27), the analysis of the ganglioside mixture was performed on TLC by the use of an ammonia-containing solvent system. This system converts the esterified carboxyl group partly into an amide and partly into a free carboxyl group, the ratio between the two derivatives depending on the ammonia molarity. GD\textsubscript{lb-}
amide migrates on TLC similarly to GD\textsubscript{lb}, thus becoming indistinguishable. Vanier et al. (9) did not employ alkaline conditions; however, they missed the detection of GD\textsubscript{lb-L} because of the use of a TLC solvent system (propanol/water), in which GD\textsubscript{lb-L} co-migrates with GM\textsubscript{3}. Finally, Fredman et al. (25) did not find GD\textsubscript{lb-L} in their investigation on the brain of an infant (3 months) because of the very low GD\textsubscript{lb-L} content at this age (Table I). According to the present survey, only Iwamori and Nagai (21) and Svennerholm and Fredman (10) could have detected the presence of lactonized ganglioside derivatives as the experimental conditions and the starting tissues were optimal for this purpose. In both papers the possible presence of ganglioside lactones was mentioned; however, the authors did not focus their attention on the natural occurrence of these compounds or their artifactual formation.

In conclusion, the present report provides conclusive evidence of the natural occurrence, in human brain, of a GD\textsubscript{lb} ganglioside carrying an inner ester linkage. This ganglioside is also present in the brain of other animal species, and it is most likely that other ganglioside lactones occur in human as well as in other vertebrate brain tissue.

The biological significance of lactonized gangliosides is open to inspection. Of course the possibility of reducing the number of negative charges without changing the number of sialic acid residues exposed on the cell surface may be specifically important in some membrane recognition phenomena and intercellular events. It has been postulated, for instance, that there is involvement of the sialic acid negative charge with the binding of Ca\textsuperscript{2+} ions and the process of neurotransmitters release at nerve terminals (30, 31). In this regard it would be important to ascertain the existence of any enzymatically assisted process of both formation and hydrolysis of the lactone ring as a mechanism for prompt and reversible control of the negative charges provided by gangliosides on the cell surface.

REFERENCES
17. Vanier, M. T., Holm, M., Månsson, J. E. & Svennerholm, L.
Natural occurrence of ganglioside lactones: isolation and characterization of a volatil oil from adult human brain


EXPERIMENTAL PROCEDURES

1. Preparation of brain material

Samples of normal, cortex weighing 0.1-1.0 g were obtained at the time of autopsy from 44 patients aged from 3 months to 90 years, and operated for different intracranial pathologies. The brain material sampled were those parts that had to be removed for that particular operation. In each case, a small specimen was taken from the area located near the pathologically involved tissue and composed to be free of either necroscopic or histological abnormalities.

2. Isolation and purification of GSL

Extraction and purification of the crude ganglioside mixture from each sample of human cerebral cortex was achieved as described by Tettamanti et al. (3). The ganglioside distribution was assessed in each individual sample by two-dimension TLC with intermediate separative treatment (see below). Furthermore, in order to isolate and purify the GD3-like gangliosides, the gangliosides eluted from the cerebral cortex of 20 rats aged 18-20 g were eluted a total of 0.2 g of lipid bound stachic acid was obtained from 10 g of fresh tissue. The isolation was achieved on column chromatography over a column bed of a 2.5 x 100 cm column (50% o-4 g) and eluted and in the solvent system chloroform/methanol/water, 60/38/2 by vol in 10% increments and at a flow rate of 0.05 ml/min. The total volume for a complete elution was about 2000 ml, 2 ml fractions were biochemically analyzed. The elution pattern was monitored as GD3 as indicated below. The fractions containing GG2 were collected, eluted under N2 flow at 4°C and rechromatographed under the above conditions. The compound was then obtained as glycolic acid under 5°C, 1:1 by vol, at 25°C. The purity of the isolated ganglioside was assessed by chemical analysis (see below).

3. Chemical composition and structural analyses

The following compositions and structural analyses were performed: a) determination of the carbohydrate and lipid composition, b) partial acid hydrolysis followed by TLC recognition of the formed neutral glycolipids, c) perem-disc analysis for the determination of sugar linkage positions d) enzymatic analysis. The experimental details of the above analytical methods have been given in previous papers (32,34,35).

4. Final treatment

GD3-like was submitted to alkaline treatment according to the following procedure: a) addition of NaOH aqueous, successively dialyzed through dialyze with water, was added to a 0.1% solution (125 μg/ml) of demethylated chloroform/methanol. 1:1 by vol for 1 h. At the end of the incubation, the solution was dried under a stream of nitrogen and the reaction products were assayed by TLC and GLC and TLC analyses (see below). The product obtained after ammonium treatment of GD3-like was then dissolved in 0.1 N NaOH (10 μg/ml) and kept overnight at room temperature. At the end of the reaction the solution was dialyzed in UF was filtered through a 0.2 ml of distilled water and the reaction products were then analyzed by TLC (see below). The product obtained after NaOH treatment of GD3-like was then submitted to 0.2 M HCl.

Gas chromatography - mass spectrometry (GC/MS)

GC/MS analysis of the GD3-like, obtained by mild acid hydrolysis (32) from GD3-like with 0.1 N NaOH for 1 h, was performed on a Perkin Elmer 8000 (USA) gas chromatograph-mass spectrometer coupled with a PEP 84 mass data system. The operating conditions were as follows: The GC column was 200 cm x 0.2 mm, packed with 3% on 100-150 mesh Gas Chrom P (Supelco, Bellefonte, PA); temperature: 27°C; helium flow: 20 ml/min, transfer line and ion source temperature: 270°C; filament current: 40 μA; electron energy: 70 eV; accelerating voltage: 3.5 kV resolution 1000.

5. Post column two-dimensional spectrometry (TLC-GC)

An eluted mixture from TLC was then run was separated using the spectrometric system of chloroform/methanol/20% aqueous NaCl (1:1:4) by vol (2) (2°C, run time 40 min). Gangliosides were eluted in the peaks by quenching it with a sodium-methanolepidermalgrease derivative followed by heating of 100°C for 10 min and quantitated by densitometry according to Chigorno et al. (19), with the improvement that the beam was 0.2 m and consequently the scan shift were reduced to 0.2 m, thus providing better resolution.

6. Gas chromatography and densitometry quantification of gangliosides

Alkaline binding of GD3-like was achieved by the reverse-TLC method (6) as modified by Mattissen and Takki-Luukkainen (2).

Table I. Content of GD3-like in normal adult human brain

<table>
<thead>
<tr>
<th>Age Group</th>
<th>0-14</th>
<th>15-29</th>
<th>30-69</th>
<th>70+</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD3-like</td>
<td>0.15±0.04</td>
<td>0.49±0.16</td>
<td>1.09±0.07</td>
<td>3.48±0.36</td>
</tr>
<tr>
<td>GD3-like</td>
<td>7.89±0.34</td>
<td>20.39±0.43</td>
<td>20.39±0.41</td>
<td>30.84±0.47</td>
</tr>
</tbody>
</table>

*Number of cases in parentheses.

*GD3-like has been extracted in PI spot (see Fig. 1), the main derivative of GD3-like after ammonium treatment.

*Ganglioside content was measured as a 0.2 M of total lipid-bound stachic acid. Each sample was analyzed in duplicate.

Table II. Content of GD3-like and GD3 in normal adult human brain

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>GD3-like</th>
<th>GD3-like</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain cortex</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Blood plasma</td>
<td>1.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Serum</td>
<td>0.5</td>
<td>1.1</td>
</tr>
<tr>
<td>GD3-like</td>
<td>10.5</td>
<td>12.3</td>
</tr>
<tr>
<td>GD3-like</td>
<td>0.048</td>
<td>0.056</td>
</tr>
<tr>
<td>GD3-like</td>
<td>0.002</td>
<td>0.052</td>
</tr>
<tr>
<td>GD3-like</td>
<td>0.016</td>
<td>0.016</td>
</tr>
</tbody>
</table>

*Ganglioside content was measured as a percentage of total lipid-bound stachic acid.
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TABLE III. Chemical composition of G01b-L and of its derivatives after sodium hydride (G01b-L/NaH) and aminolevulinic acid (G01b-L/Mel) treatments.

<table>
<thead>
<tr>
<th>Glycinate</th>
<th>Ganglioside</th>
<th>Neuraminic acid</th>
<th>Lactone</th>
<th>Fatty acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>G01b-L</td>
<td>1.00</td>
<td>2.05</td>
<td>0.92</td>
<td>1.87</td>
</tr>
<tr>
<td>G01b-L/HM</td>
<td>1.00</td>
<td>1.98</td>
<td>0.91</td>
<td>1.94</td>
</tr>
<tr>
<td>G01b-L/Mel</td>
<td>1.00</td>
<td>1.94</td>
<td>0.99</td>
<td>1.92</td>
</tr>
</tbody>
</table>

Long chain base composition [%] Fatty acid composition [%]

<table>
<thead>
<tr>
<th>Column Chromatographic Fractions</th>
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Fig. 1. Two-dimensional TLC analysis of gangliosides from adult human cerebral cortex and relative diagnostic representation.

The sample refers to the temporal lobe of a 66-year-old subject. 5 μL of total lipid-bound sialic acid were spotted. At the plate edges, twodimensional TLC of standard gangliosides are displayed. In both directions, from the lowest polar gangliosides, the sequence corresponds for GM2, GM1, GM0, GD1a, GD1b, Fuc-GD1a, GT1b, and GD1b. For experimental details see the text.

Fig. 2. Fractionation of human brain cerebral cortex on silica gel columns.

In the first two layers, standard ganglioside mixture (a) and human brain ganglioside mixture (b) were spotted.
Fig. 3. Alkaline treatment of gangliosides. Lanes 1 and 2, standard ganglioside mixture; lane 3, purified GD1b; lane 4, GD1b after acidic treatment (GD1b-L/N); lane 5, GD1b-L after sodium hydroxide treatment (GD1b-NaOH); lane 6, standard GD1b. For experimental details, see the text.

Fig. 4. Mass spectrum of the sialic acid derivative, released by acid hydrolysis from GD1b-L/N, and derivatized on MS MDI.

Fig. 5. Behavior of GD1b-L (a), standard GM1 (b) and standard GD1b (c) on an ion-exchange HPLC detector column chromatography. For experimental details, see the text.

Fig. 6. Negative ion FAB-MS analysis of native standard GM1 (a) and GD1b-L (b). Schemes indicating the major pathways of fragmentations are also given.